UNIVERSIDADE FEDERAL DO RIO GRANDE

INSTITUTO DE OCEANOGRAFIA

LABORATÓRIO DE CIANOBACTERIAS E FICOTOXINAS - LCF

PROGRAMA DE PÓS-GRADUAÇÃO EM OCEANOGRAFIA FÍSICA, QUÍMICA E GEOLÓGICA

Influência de parâmetros abióticos e bióticos na ocorrência da cianobactéria *Trichodesmium* no Talude sudoeste do Atlântico Sul

Mariana Bernardi Bif

Dissertação apresentada ao programa de Pós-graduação em Oceanografia Física, Química e Geológica, como parte dos requisitos para a obtenção do título de Mestre.

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2015

APRESENTAÇÃO

Para a redação desta dissertação, optou-se por escrevê-la na forma de artigos científicos a serem submetidos à literatura especializada. A primeira parte do texto contém, no entanto, uma apresentação geral do conteúdo: resumo em português e inglês (abstract); uma introdução apontando as motivações e objetivos do presente estudo; materiais e métodos caracterizando o ambiente bem como as condições de coletas e metodologia utilizada para a geração dos resultados. A segunda parte do texto está dividida em capítulos, sendo estes os artigos científicos: O **Capítulo I** contém uma revisão bibliográfica acerca do assunto principal e os **Capítulos II e III** contêm dois manuscritos na forma de artigo original, frutos das saídas de campo. A terceira e última parte da dissertação contém um sumário dos resultados obtidos, além de sugestões para trabalhos futuros.

SUMÁRIO

RESUMO

O gênero *Trichodesmium* ocorre nos oceanos tropicais e subtropicais do planeta e atua diretamente no ciclo do nitrogênio e do carbono. Por ser diazotrófico, *Trichodesmium* consegue estabelecer populações em regiões oligotróficas, onde outros organismos não se desenvolvem por carência de nitrogênio dissolvido. Apesar da grande biomassa, pouco se sabe sobre a distribuição e ecologia do gênero na costa Brasileira, principalmente nas regiões Sul e Sudeste do país. Neste trabalho, foram realizadas coletas e identificação, em nível de espécie, do gênero *Trichodesmium* no talude sul-sudeste do Brasil, durante o período de Outono (2013 e 2014). Os principais grupos do microplâncton que coocorreram com o organismo foram identificados e relacionados com variáveis físicas e químicas do ambiente. Como se trata de um ambiente com baixas concentrações de nutrientes, apontamos fontes alternativas de nutrientes não dissolvidos, possíveis de serem assimilados por *Trichodesmium*. Os resultados mostraram que as agregações do gênero se concentravam nas estações mais ao norte da região amostrada (> 157.000 tricomas L^{-1}), em condições de baixa velocidade de vento (< 8 nós) e profundidade da camada de mistura relativamente rasa (< 40 m). Quatro novas espécies de *Trichodesmium* foram identificadas para a região de um total de cinco espécies, além de outros organismos do plâncton típicos de regiões oligotróficas. A área de estudo apresentou concentrações baixas de fosfato, nitrogenados e silicatos, o que caracterizou a região como oligotrófica. As imagens de microscopia eletrônica e óptica mostraram concentrações de sedimento das colônias de *Trichodesmium*. Análises por raios-X realizadas no Microscópio Eletrônico revelaram que os grãos eram ricos em Fe (10-20 % de contribuição total) e outros nutrientes, incluindo P e Zn. Os tricomas, ainda, apresentaram associações com bactérias que podem estar envolvidas no processo de dissolução dos elementos mineralizados, tornando-os biodisponíveis para assimilação.

PALAVRAS-CHAVE: Cianobactéria; Diazotróficos; Ferro; Talude Brasileiro; *Trichodesmium*.

ABSTRACT

The genus *Trichodesmium* occurs in tropical and subtropical oceans through the world and contributes to the nitrogen and carbon cycle. As a diazotrophic organism, it establishes populations in oligotrophic oceans, where other planktonic organisms cannot develop because of dissolved nitrogen limitation. In spite of their large biomass, they are poorly studied in Brazilian waters, especially among South and Southeast regions. In this work we collected and identified the genus in the level of specie, in the South-Southeast Brazilian slope during 2013 and 2014 autumns. Thus, we identified the main groups of microplankton co-occurring with the cyanobacterium, as well as some physical and chemical environmental conditions related to their occurrence. As the region of study was characterized as being poor in nutrients, we also searched for alternative sources of non-dissolved nutrients for *Trichodesmium* assimilation. The results showed that the genus aggregations were concentrated in the northernmost stations (> 157,000 trichomes L^{-1}), during low wind speed conditions (< 8 knots) and shallow mixed layers (< 40 m). From the five species identified in the samples, four are first reported as occurring in Brazilian coast. Also, other typical organisms from oligotrophic regions were identified co-occurring with *Trichodesmium*. Finally, the study area presented low concentrations of phosphate, nitrogenates and silicon. The electron and inverted microscopy images showed concentrations of sediment into the colonies. The X-ray analysis coupled with Electron Microscopy revealed that the grains were not just rich in Iron (10 - 20% of total contribution) but also contained other elements as phosphorus and zinc. Also, the trichomes were in association with other bacteria that could be involved in dissolution processes by using the mineralized elements, transforming them into bioavailable forms.

KEYWORDS: Cyanobacteria; Diazotrophics; Iron; Brazilian Continental Slope; *Trichodesmium*.

1. INTRODUÇÃO

A cianobactéria do gênero *Trichodesmium* (Ehrenberg 1830), Ordem Oscillatoriales, pode ser vastamente encontrada no ambiente marinho e tem como característica morfológica células distribuídas em tricomas que podem atingir milímetros de comprimento (Figura 1). Estes tricomas, por sua vez, são formadores de colônias fusiformes (tufts) ou esféricas (puffs), conformações não exclusivas de uma espécie. É um organismo diazotrófico, isto é, capaz de fixar nitrogênio atmosférico (N_2) e transformá-lo em nutriente para o seu metabolismo. No entanto, diferentemente de outras cianobactérias diazotróficas, *Trichodesmium* é desprovido de células não clorofiladas especializadas para a fixação, chamadas heterocistos. Mesmo assim, o organismo consegue fixar N_2 em suas células ao mesmo tempo em que faz a fotossíntese (Carpenter and Price, 1976).

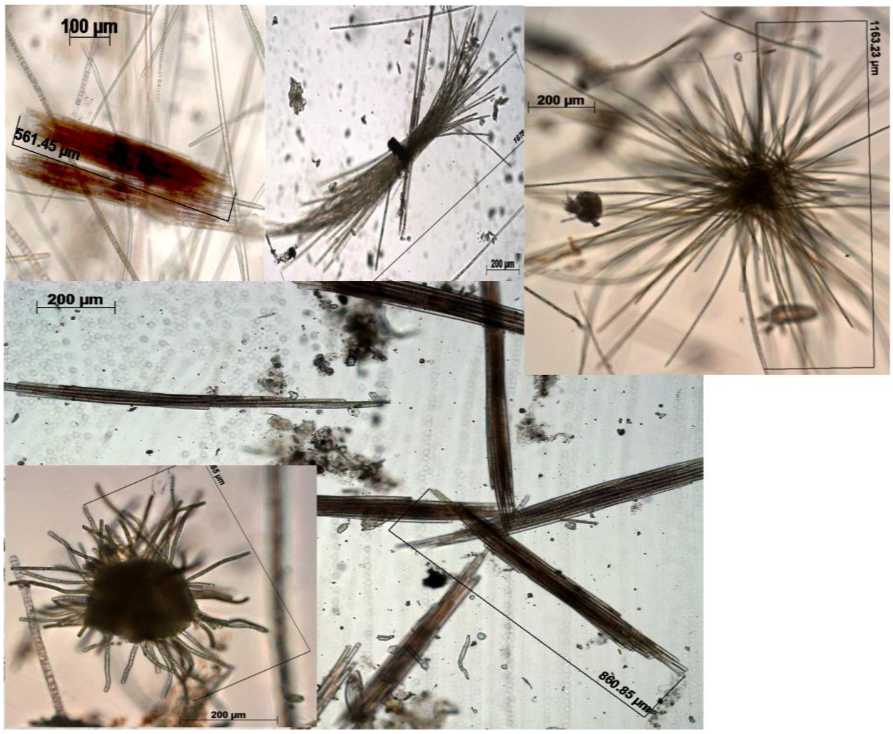

Fig. 1: Diferentes formas coloniais de *Trichodesmium* encontradas no Talude Sul-Sudeste do Brasil.

As agregações de *Trichodesmium* na superfície da água podem ser encontradas ao longo das regiões tropicais e subtropicais dos oceanos (Karl *et al*, 2002). As condições físico-químicas do ambiente parecem delimitar a biogeografia do *Trichodesmium*, que normalmente se encontram associadas a águas estratificadas e salinas com baixa concentração de nutrientes dissolvidos, ventos fracos e uma alta intensidade luminosa (Letelier and Karl 1996; Hood e*t al*, 2001; Hegde *et al*, 2008).

Os eventos de grandes manchas de *Trichodesmium* foram descritos com maior frequência, até o momento, nas regiões oligotróficas do Atlântico Norte (Carpenter and Price, 1976; Capone e*t al*, 2005; Agawin *et al*, 2013) e costa Australiana (Villareal and Carpenter, 2003; Cropp *et al*, 2013). Estas ocorrências estão associadas tanto a feições oceânicas de larga-escala, como os grandes giros oceânicos do Atlântico e Pacífico, quanto a feições de pequena e média-escala, como Células de Langmuir e vórtices (Capone *et al*, 1997; Tyrrell *et al*, 2003).

No entanto, o número de ocorrências de *Trichodesmium* em novos locais é crescente, e mostram a real ocorrência do organismo nos oceanos (Figura 2). Como exemplos de novas áreas reveladas podem ser citados o Mar de Andaman (Arun Kumar *et al*, 2012) e a costa da Índia (Srinivas and Sarin, 2013), ambos localizados no Oceano Índico, além da costa Brasileira em toda a sua extensão (Siqueira *et al*, 2006; Carvalho *et al*, 2008; Monteiro e*t al*, 2012).

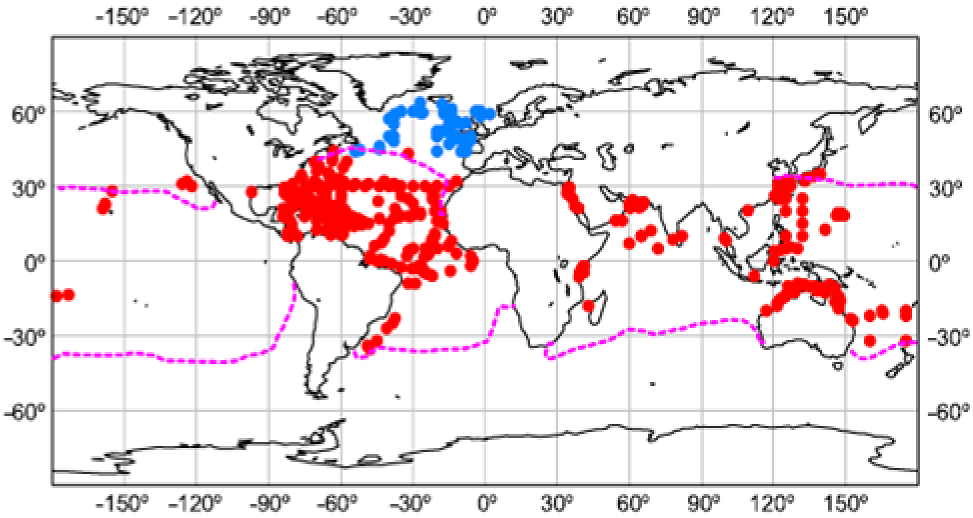

Fig. 2: Distribuição de *Trichodesmium* no planeta. Pontos vermelhos significam organismos com atividade de fixação de nitrogênio, pontos azuis significam ausência de fixação. A linha tracejada em rosa representa a isoterma superficial média anual de 20°C. Fonte: LaRoche and Breitbarth, 2005.

O aumento das avistagens de *Trichodesmium* reforça a ideia de que sua biomassa é de grande importância para a produtividade primária dos oceanos, especialmente em regiões pobres em nutrientes que impossibilitam o estabelecimento de outros organismos (Capone *et al*, 2005). A estimativa da fixação anual de nitrogênio por organismos diazotróficos é de 110 Tg N. Ano^{-1}, e tem como principal contribuinte *Trichodesmium*. Este montante ultrapassa, inclusive, o aporte de rios e a deposição atmosférica suprindo a necessidade do organismo pelo elemento (Gruber and Sarmiento, 1997).

Como o processo de fixação é muito dispendioso energeticamente, quando há concentrações suficientes de compostos nitrogenados dissolvidos, o organismo opta por esta fonte direta (Agawin *et al*, 2013). A especialização em colonizar ambientes oligotróficos, no entanto, faz com que o *Trichodesmium* seja dependente, na maior parte do tempo, do N_2 para o seu metabolismo. Por isso, o gênero apresenta uma baixa taxa de crescimento, sendo sua máxima (μ) estimada em 0,14 d^{-1} (LaRoche and Breitbarth, 2005).

Recentemente, muitos esforços estão sendo feitos para se estimar a assimilação de CO_2 pelos organismos aquáticos, bem como o impacto dos mesmos nos ciclos biogeoquímicos dos oceanos (Metz *et al*, 2005). O carbono assimilado pelos fotossintetizantes, após sua morte e sedimentação, sofre um importante processo de afundamento, que faz com que o elemento acabe sendo “sequestrado” para os oceanos profundos. O processo, que ocorre principalmente na zona fótica, é um dos que controla a temperatura do nosso planeta, evitando oscilações abruptas ao balancear as trocas de CO_2 do oceano com a atmosfera (Metz *et al*, 2005). Como o gênero *Trichodesmium* ocorre em grande biomassa, é considerado parte do grupo de organismos que atuam significativamente no ciclo do carbono. A capacidade de assimilação de N_2, além de contribuir no ciclo do nitrogênio, também contribui para o *input* do elemento, como nutriente, na coluna d’água (LaRoche and Breitbarth, 2005). O aumento de nitrogênio no sistema aquático, ainda, confere à coluna d’água uma maior capacidade de remover o CO_2 atmosférico ao rebalancear suas proporções entre superfície e profundidade, contribuindo com o fluxo vertical de nutrientes nos oceanos (Peterson, 1979).

A contribuição do novo nitrogênio para o oceano como produto metabólico do *Trichodesmium* tem chamado a atenção dos cientistas por décadas. Modelos matemáticos vêm sendo empregados para tentar estimar a contribuição do gênero em termos de biomassa e, consequentemente, seu papel no ciclo biogeoquímico. Estes modelos utilizam como variáveis os elementos considerados limitantes para o crescimento do organismo, como concentrações de ferro (Fe) e de fósforo (P) do ambiente (Ward e*t al*, 2013).

O Fe é um dos nutrientes mais importantes para o crescimento de *Trichodesmium*. É um metal-traço essencial para todos os organismos do fitoplâncton porque compõe muitas proteínas do aparato fotossintético. No entanto, o gênero requer concentrações extras do elemento para compor, principalmente, os complexos proteicos da enzima nitrogenase, intermediadora na fixação de N_2 (Raven, 1988; Sañudo-Wilhemy *et al*, 2001). Durante altas taxas de assimilação, estima-se que o organismo utilize mais da metade do Fe intracelular para este processo, associando-o prioritariamente às enzimas em vez de investir em pigmentos fotossintéticos (Kutska *et al*, 2003).

A maior fonte de Fe para o oceano aberto é limitada à poeira atmosférica, que contém o elemento na forma mineralizada. Esta fertilização parece manter populações permanentes de *Trichodesmium* em regiões do Atlântico Central (Sañudo-Wilhemy *et al*, 2001), Austrália (Cropp *et al*, 2013) e norte do Oceano Índico (Srinivas and Sarin, 2013). Destas três regiões, a contribuição atmosférica mais estudada é, sem dúvidas, a do Atlântico Equatorial, vinda principalmente do deserto do Saara (Tyrrell *et al*, 2003; Agawin *et al*, 2013; Fernández e*t al*, 2013). Apesar do Fe estar biodisponível para o fitoplâncton apenas na forma orgânica ou dissolvida, e não mineralizada, isto não parece estar limitando a assimilação pelo *Trichodesmium*.

Um estudo realizado em laboratório adicionou sedimentos manipulados artificialmente, enriquecidos com óxido de ferro, a culturas de *Trichodesmium*. O que se observou foi que os tricomas agregados em colônias conseguiam transportar os grânulos para o seu interior. Este processo aumentou a aquisição de Fe na região interna das colônias, mais anóxica e redutora. Ainda, tricomas solitários também conseguiram aumentar a aquisição do elemento contido nos sedimentos (Rubin *et al*, 2011). Outro comportamento observado, também utilizado para maximizar a aquisição de Fe pelo organismo, é a associação com outras bactérias (Roe e*t al*, 2012). Estas, por sua vez heterotróficas, conseguem transformar as formas mineralizadas de Fe e liberá-los em formas dissolvidas.

Outro importante nutriente que controla o crescimento do *Trichodesmium* é o P. Como o gênero conhecidamente possui reservas do elemento, a análise *in situ* de P dissolvido normalmente não reflete na biomassa da cianobactéria (Moutin *et al*, 2005). Ainda, a variação do P na água do mar é sazonal, e depende da ascensão de massas d’água, por exemplo. Por isso, a relação direta entre a concentração de P e a variação de biomassa de *Trichodesmium* no ambiente pode ser explicada apenas parcialmente, e deve estar sempre associada a outros fatores de dinâmica físico-químicos (Villareal and Carpenter, 2003).

Geralmente, o fitoplâncton consegue assimilar o P apenas quando na forma de fosfato inorgânico dissolvido (DIP), ou ortofosfato. No entanto, colônias de *Trichodesmium* conseguem suprir as necessidades de P através da aquisição suplementar de fósforo orgânico dissolvido (DOP) via fosfatase alcalina. A utilização do DOP como fonte alternativa parece ser muito importante para o crescimento do gênero em regiões com baixa concentração de DIP (Sohm and Capone, 2006). Como adaptação à limitação de P superficial, alguns estudos mostraram que o gênero consegue adquirir o elemento de outras profundidades da coluna d’água. A migração vertical, neste caso, é fundamental para as colônias atingirem regiões mais profundas em locais que possuem uma fosfoclina (Villareal and Carpenter, 2003).

Para atingir profundidades com maiores concentrações de P, o gênero utiliza a conformação colonial somada à regulação na concentração de carboidratos da vesícula de gás, que funciona como um lastro (Villareal and Carpenter, 2003). A resistência das vesículas a migrações de dezenas de metros de profundidade está associada ao pequeno diâmetro, permitindo com que o *Trichodesmium* atinja regiões afóticas. O organismo, inclusive, consegue sobreviver por dias na ausência de luz (White *et al*, 2006). Uma adaptação importante, que maximiza a absorção de luminosidade nessas regiões, está na composição de pigmentos acessórios contidos em seus tricomas. Assim como outras cianobactérias, o *Trichodesmium* possui ficobiliproteínas no aparato fotossintético, especialmente da antena II (Lawrenz e*t al*, 2011) (Figura 3). Estes pigmentos requerem, no entanto, grandes quantidades de Fe em sua composição, fazendo com que o gênero aumente ainda mais sua dependência pelo elemento.

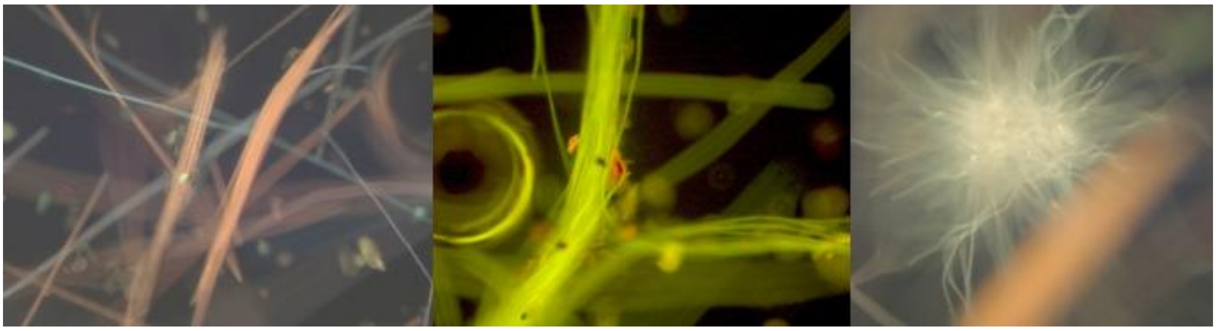

Fig. 3: Colônias de *Trichodesmium* sob luz ultravioleta (UV fs09 ph1-04) em microscópio invertido. Podem-se observar os diferentes comprimentos de onda que são emitidos pelos pigmentos acessórios contidos nas células.

As concentrações de Fe e P, quando combinadas, também limitam o crescimento de *Trichodesmium* no meio ambiente. Um estudo feito com a adição de Fe e DIP mostrou que, quando estão em alta concentração, estimulam uma maior taxa de fixação de N_2 em culturas, o que reforça a importância de ambos para o metabolismo (Mills *et al*, 2004). Ainda, os oceanos parecem limitar o crescimento do organismo de acordo com concentrações diferentes destes mesmos elementos. No Atlântico, por exemplo, o *Trichodesmium* tende a ser limitado pelas baixas concentrações de P, segundo a relação de Redfield (Sañudo-Wilhemy *et al*, 2001; Fernández *et al*, 2013). Já o Pacífico Norte possui baixas concentrações de Fe, sendo este o controlador de biomassa (Landing e*t al*, 1987).

1.1. ANTECEDENTES

Na costa brasileira, poucos trabalhos envolvendo *Trichodesmium* foram realizados até hoje, e muito pouco se conhece sobre a composição de espécies e ecologia do organismo em nossas águas. O primeiro trabalho reportando o aparecimento do gênero no Brasil foi feito por Satô (1963), na cidade de Tamandaré – Pernambuco. O estudo atribuiu sintomas inflamatórios que apareceram na população ao acúmulo da cianobactéria na região praial. Este evento ficou conhecido como febre de Tamandaré, atribuindo ao gênero, inclusive, a produção de toxinas. Na década de 1990, foram relatadas ocorrências de manchas de *Trichodesmium erythraeum* na região de Ubatuba-SP (Gianesella-Galvão *et al*, 1995) e seu aparecimento nas estações de maricultura do litoral norte de Santa Catarina (Rorig *et al*, 1998b).

Na primeira década dos anos 2000, o gênero foi relatado em coocorrência com outros organismos dentro de um estuário no Rio Grande do Norte (Naithirithi e*t al*, 2005). Em 2004, uma grande concentração de *Trichodesmium* atingiu o litoral sul do Rio Grande do Sul, no balneário Cassino, associado com uma mortalidade massiva de hidrozoários (Silva *et al*, 2005). Em 2006, um estudo realizado no litoral do Paraná encontrou *Trichodesmium erythraeum* coocorrendo com o fito e bacterioplâncton na região (Siqueira *et al*, 2006). No ano de 2008 houve o primeiro relato de manchas do gênero na região *offshore* do Atlântico Sul (Carvalho *et al*, 2008). Este trabalho relacionou a agregação de *Trichodesmium erythraeum* com alta salinidade, temperatura e estratificação da coluna d'água, além de baixas concentrações de nutrientes.

Outro caso de doença associada ao gênero foi relatado no ano de 2007 (Proença *et al*, 2009) na Baía de Camamú- Bahia, mas a baixa toxicidade encontrada nas amostras não pôde ser correlacionada com os sintomas da população. Ainda na região nordeste, agregações permanentes de *T. thiebautii* foram identificadas (Monteiro *et al*, 2012) e correlacionadas às condições físico-químicas do oceano no litoral de Pernambuco (Monteiro *et al*, 2010).

1.2. JUSTIFICATIVA E MOTIVAÇÃO

Apesar do grande volume de estudos sobre *Trichodesmium* ao redor do mundo, muito pouco se sabe sobre sua ocorrência e ecologia no Atlântico Sul e costa Brasileira. Os estudos antecedentes no Brasil, além de terem focado em áreas costeiras, limitaram-se a coletar apenas em superfície, e coletaram poucas informações sobre os parâmetros físicos e químicos do ambiente. A insuficiência destes dados impede análises mais aprofundadas sobre a sua distribuição vertical, estimativa de biomassa e adaptações do gênero.

Alguns destes trabalhos, ainda, identificaram os organismos em nível de espécie, mas não utilizam técnicas de morfometria, características morfológicas e fotos anexas em microscopia invertida ou eletrônica que pudessem confirmar a taxonomia propriamente.

Sendo assim, a região oceânica de contorno oeste do Atlântico Sul ainda carece de estudos das populações de *Trichodesmium*. Dada a importância destes organismos para os ciclos biogeoquímicos marinhos e produtividade primária, o estudo ecológico é de extrema relevância não apenas regionalmente, como a nível global. A estimativa de biomassa do gênero somado ao entendimento sobre a sua ocorrência no Brasil contribuiria significativamente para os cálculos de sequestro de CO_2 e *input* de N_2 do Atlântico Sul.

O talude sul-sudeste do Brasil ainda não possui relatos publicados de *Trichodesmium* e, tampouco, a sua identificação em nível de espécie. Sendo esta uma região bastante influenciada por águas quentes e correntes pobres em nutrientes, como a corrente do Brasil (Möller *et al*, 2008), também é de grande importância estudar as adaptações que permitem que as populações prosperem nesta região. Neste caso, o melhor entendimento das possíveis fontes de nutrientes ajudaria a explicar o desenvolvimento das comunidades de *Trichodesmium*. Ainda, a associação com outros organismos do fitoplâncton e zooplâncton, também adaptados a condições oligotróficas, complementa o estudo sobre a ecologia da cianobactéria. Portanto, este trabalho pode ser considerado pioneiro no estudo do gênero na região e é o primeiro passo para o entendimento da sua distribuição.

2. OBJETIVOS

2.1.OBJETIVO GERAL

Determinar parâmetros biológicos, físicos, químicos e geoquímicos que influenciam na ocorrência das populações de *Trichodesmium* spp. no talude sul-sudeste do Brasil.

2.2. OBJETIVOS ESPECÍFICOS

- Identificar as espécies de *Trichodesmium* da região do Talude sul-sudeste;
- Identificar organismos do microplâncton e zooplâncton que coocorrem com *Trichodesmium*, assim como a abundância de espécies;
- Estimar a abundância e distribuição vertical de *Trichodesmium;*
- Relacionar a ocorrência e abundância de *Trichodesmium* com fatores físicos e características das massas d'água;
- Relacionar a ocorrência e abundância de *Trichodesmium* com nutrientes dissolvidos da água do mar;
- Avaliar geoquimicamente o conteúdo dos sedimentos agregados no interior das colônias de *Trichodesmium* como fontes alternativas de nutrientes para o organismo, além de possíveis adaptações que maximizem a aquisição dos elementos.

3. MATERIAL E MÉTODOS

3.1. ÁREA DE ESTUDO

A região amostral compreende o talude da região sul e sudeste da costa brasileira, localizado entre as latitudes de 24° e 34° S do Oceano Atlântico (Figura 4). As amostras foram coletadas durante as expedições de outono VI e VII do projeto TALUDE, a bordo do Noc Atlântico Sul, durante os meses de maio e junho de 2013 e 2014.

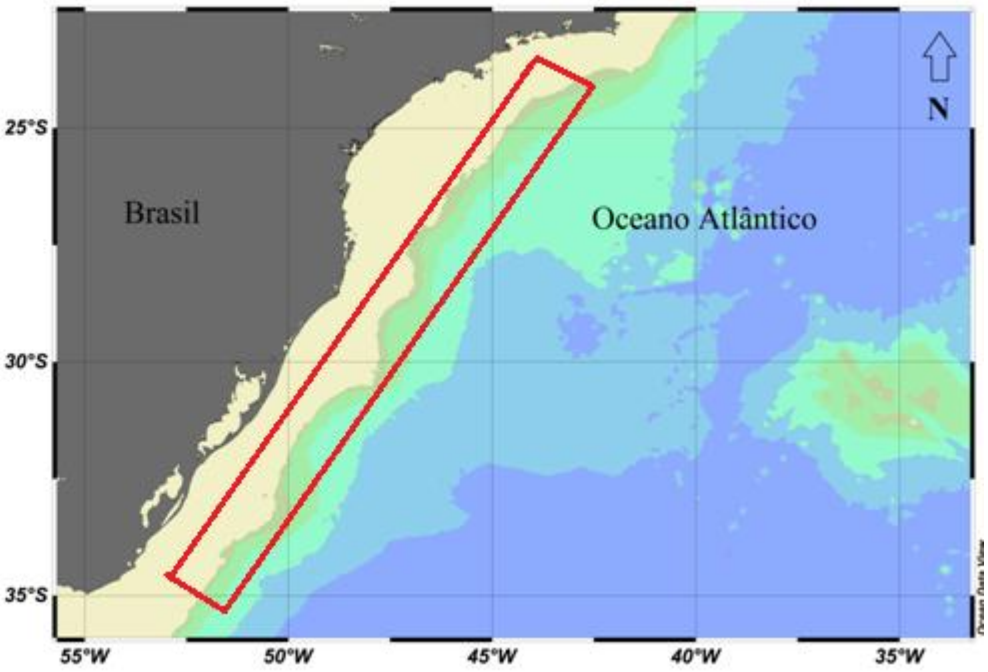

Fig. 4: Mapa do contorno oeste do oceano Atlântico Sul, região Sul-Sudeste do Brasil. A área de estudo compreendeu a região do polígono vermelho.

As coletas realizadas na expedição de 2013, que cobriram a parte norte da área de estudo, se limitaram a coletas apenas quando as agregações de *Trichodesmium* eram visíveis a olho nu na superfície da água. Durante a expedição de 2014, na parte central e sul da área de estudo, também foram realizadas coletas na ausência macroscópica de agregações. Em ambas as expedições, as coletas foram realizadas apenas quando as condições meteorológicas permitiam. Como as coletas eram direcionadas às agregações da cianobactéria, não houve um padrão espacial amostral, e elas dependeram da frequência de avistagem e condições meteorológicas.

3.2.AMOSTRAGEM

3.2.1. PARÂMETROS FÍSICOS

Os parâmetros físicos de velocidade e direção do vento foram adquiridos através do próprio equipamento do navio, para todos os pontos coletados. As características meteorológicas foram anotadas como observações na planilha de coleta, para posterior consulta, auxiliando na interpretação dos dados ambientais.

Para as medidas de temperatura e salinidade em relação à profundidade, foi utilizado um CTD (Condutivity, Temperature and Density) (SeaBird® 911). Dados de temperatura e salinidade foram utilizados para construir perfis verticais, diagrama TS e profundidade da camada de mistura (MLD) (MatLab® 9.0). A identificação e a classificação das massas d'água basearam-se nos índices termo-halinos de Möller et al. (2008).

3.2.2 PARÂMETROS QUÍMICOS

Amostras de água foram coletadas para análise de nutrientes utilizando-se garrafas de Niskin. Elas foram presas ao cabo do navio, acopladas a mensageiros, para coleta de amostras pontuais a partir de 15 m de profundidade, até o limite de 140 m, dependendo da profundidade local. As amostras, depois de coletadas, foram filtradas em filtro de éster de celulose 0,45 µM, armazenadas em tubos falcon, rotulados e congelados.

Cada tubo tipo falcon continha o volume necessário de amostra para as análises de nitrogênio amoniacal total (NAT - NH_4^+), nitrato (NO_3^-), nitrito (NO_2^-), fosfato (PO_4^{-2}) e silicato (Si).

3.2.3. PARÂMETROS BIOLÓGICOS

As amostras biológicas foram coletadas com o auxílio de uma rede com dispositivo de fechamento, malha de 50 µm (Figura 5), em amostragem vertical dos diferentes estratos da coluna d’água. A máxima profundidade de coleta dos arrastos verticais foi de 200 m. Os primeiros estratos que continham grande biomassa de organismos foram amostrados em intervalos de 15 ou 30 m, enquanto que os estratos mais profundos foram coletados em perfis de 40 ou 50 m.

Além das coletas verticais, coletas restritas à superficie foram realizadas no intuito de quantificar e identificar a biomassa superficial de *Trichodesmium* e outros organismos do microplâncton. Por causa da micragem da malha, apenas os organismos pertencentes ao microplâncton menores do que 20 µm foram coletados. Esta porosidade foi escolhida para evitar a colmatação da rede em situações de grande biomassa do organismo, já que o foco principal das coletas eram as agregações de *Trichodesmium*.

Fig. 5: Coleta de parâmetros biológicos utilizando a rede de fitoplâncton com de malha de 50 µM.

O armazenamento do material biológico foi feito em frascos âmbar com formol 4%, para posterior quantificação de densidade quanto à tricomas (filamentos de *Trichodesmium*); colônias do tipo puffs (tricomas distribuídos radialmente) e tufts (tricomas distribuídos paralelamente); tricomas totais (tricomas solitários + colônias) e identificação do gênero em nível de espécie nos estratos superficiais (até 30 m de profundidade). Os parâmetros de abundância de espécies e identificação dos outros organismos do microplâncton foram avaliados apenas nos estratos superficiais. As amostras fixadas em formol também foram utilizadas para posterior análise geoquímica em MEV (Microscopia Eletrônica de Varredura) acoplado ao EDS (Energy-Dispersive X-ray Spectroscopy).

3.3. METODOLOGIA

3.3.1. PARÂMETROS FÍSICOS

Os resultados do CTD foram analisados através do software Matlab® (versão 2009b). Com estes dados, foi possível gerar os gráficos que relacionaram os perfis de temperatura e salinidade em relação à profundidade e os TS para análise de massas d’água (Möller e*t al*, 2008). A partir destas variáveis físicas, também foi possível calcular a camada de mistura através da rotina MLD, que relaciona o intervalo fixo de variação da densidade (variação de 0,8 do sigma-T) como o limite inferior dessa camada, ou seja, a sua profundidade máxima. Os dados de direção e intensidade de vento foram organizados em tabelas, servindo de suporte para a discussão dos resultados.

3.3.2. PARÂMETROS QUÍMICOS E BIOGEOQUÍMICOS

As análises de nutrientes foram realizadas a partir das amostras de água trazidas do navio e descongeladas em geladeira. Em todos os métodos, utilizou-se a leitura das concentrações dos nutrientes por espectrofotometria, com comprimento de onda ajustado para cada composto. As metodologias estão contidas no manual de análises químicas, que serviu como guia (Baumgarten e*t al*, 2010), e foram realizadas no núcleo da Hidroquímica do Instituto de Oceanografia da FURG.

A análise geoquímica dos sedimentos concentrados dentro das colônias foi realizada por MEV (JEOL, Modelo 6060 LV) (Figura 06) acoplado a um EDS com detector termo-científico de raios-X, sob voltagem fixa de 15 kV. O equipamento encontra-se no Centro de Microscopia Eletrônica da Zona Sul do Estado (CEME-Sul), pertencente à FURG, no campus Carreiros. Para a análise, os pontos de coleta foram escolhidos de acordo com a localização (sul, centro e norte da área de estudo). Foram analisados, aleatoriamente, grânulos de sedimento de cada ponto, gerando um banco de dados para cada região representativa.

Fig. 6: Modelo do microscópio eletrônico utilizado para as análises geoquímicas do presente estudo.

Antes da análise por MEV, as amostras biológicas seguiram um preparo especial para serem visualizadas. Inicialmente, alguns mililitros de cada réplica foram filtrados em filtro nucleopore 0,05 µM de porosidade por filtração natural por fluxo, até que os tricomas atingissem uma concentração ideal de observação. Após, o filtro foi coberto por outro filtro e as amostras foram lavadas três vezes com água Milli-Q para a remoção dos sais marinhos. Posteriormente, houve sucessivas lavagens lentas, utilizando etanol em concentrações crescentes, para a desidratação celular gradual. Estas concentrações seguiram: 5%, 25%, 50%, 75%, 95% e 100% (Association of European Marine Biological Laboratories - ASSEMBLE).

Após concentrar as células nas membranas, estas foram levadas até o núcleo CEME-SUL para a realização do ponto crítico, ou CPD (Critical Point Drying) de CO_2 (Tousimis Autosamdri®, modelo 815). Este método consiste na substituição do etanol da amostra por gás carbônico. Condiciona-se a amostra sob uma pressão e temperatura tais que as densidades líquidas e gasosas são igualadas, ocorrendo sua desidratação completa. O método é utilizado para evitar uma possível tensão superficial da amostra, que poderia ser ocasionada pelas diferentes densidades de seus compostos líquidos e gasosos. A última parte do preparo, que consiste no recobrimento metálico das amostras já posicionadas sobre os stubs, foi feita com ouro (Denton Vacuum, modelo Dorsk V).

3.3.3. PARÂMETROS BIOLÓGICOS

Para a análise de concentração de organismos, tanto do *Trichodesmium* quanto dos organismos do microplâncton, foi utilizado o método de Utermöhl (1958) em microscópio invertido (Zeiss Axio Vert. A1) acoplado a uma câmera (Axio Cam MRc).

Gênero *Trichodesmium*: A densidade de tricomas em relação à profundidade foi estimada de através da contagem de tricomas solitários, total de colônias e tricomas totais. Para a última, foi utilizada, por convenção, uma média de 200 tricomas por colônias (Carpenter, 1983). A identificação das espécies de *Trichodesmium* seguiu Komarék and Anagostidis (2005).

Organismos do microplâncton: Estimou-se a abundancia de espécies fitoplanctônicas para todas as estações de coleta, considerando a superficie até 30 m. A identificação destes organismos foi realizada, quando possível, até o nível de espécie (Round *et al.*, 1990; Tomas, 1997).

CAPÍTULO I

Trichodesmium: chemical, physical and adaptive conditions that support its biogeography

A Trichodesmium review

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Abstract

Species of the genus *Trichodesmium* are an important component of phytoplankton in terms of their biogeography, biomass and roles in geochemical processes. *Trichodesmium* spp. can be found throughout tropical and subtropical regions but prefer hot and calm waters with oligotrophic characteristics. How physical and chemical variables appear to delimit the occurrence of *Trichodesmium* has been well investigated during recent decades. Analyses of the relationships among these external patterns and the unique physiological adaptations that this genus has developed can help to inform our understanding of the ecology and distribution of *Trichodesmium* across the oceans. Recent researches, thus, combine *in situ* observations with remote sensing observations to evaluate the distribution of *Trichodesmium* because they emit unique pigment signals.

Keywords: Biogeography, cyanobacteria, diazotrophic, nitrogen fixation, *Trichodesmium.*

Introduction

The genus *Trichodesmium* (Ehrenberg 1830), within the order Oscillatoriales, includes species of marine cyanobacteria. This genus is composed of a few species that have cells that are morphologically differentiated as filaments, or trichomes, that can reach a few millimeters in length. Research on *Trichodesmium* does not usually focus on individual species and often reports the organism results by the genus (Capone et al. 2005). Because of this current transition period in terms of identification, the number of species and their nomenclature are not yet well established. In addition, *Trichodesmium* spp. are able to form colonies with fusiform (tuffs) or spherical (puffs) morphologies and are well-known diazotrophs, organisms that have the ability to fix atmospheric nitrogen. Although diazotrophs generally contain modified cells called heterocysts in which they exclusively fix atmospheric N_2, *Trichodesmium* spp. lacks heterocysts. Even without specific cells for this activity, they are able to fix nitrogen at the same time that they perform photosynthesis (Carpenter and Price 1977).

Trichodesmium spp. often form blooms and surface aggregations in tropical and subtropical oceans and are spread throughout oceans across the world (Karl et al. 2002). The main bloom events reported to date have been located in the North Atlantic (Carpenter and Price 1977, Capone et al. 2005; Agawin et al. 2013, Fernández et al. 2013), North Pacific (Letelier and Karl 1996, Chen et al. 2003) and off the Australian Coast (Villareal and Carpenter 2003, Cropp et al. 2013). These events also occur in oligotrophic regions such as the Atlantic and Pacific gyres. In addition, blooms are often associated with large-scale, mid-scale and small-scale oceanic features, including edges and Langmuir circulation gyres (Capone et al. 1997, Tyrrell 2003). Recently, there has been an increase in the number of reports of *Trichodesmium* blooms, with new locations constantly mapped. This increase is partially attributed to reports from several oceanic regions that previously had been poorly investigated but are now receiving greater attention. This increase in marine research revealed the global occurrence of *Trichodesmium* spp. These new reports include, for example, regions such as the Andaman Sea (Arun Kumar et al. 2012), India (Srinivas and Sarin 2012) and Southeast Brazil (Rorig et al. 1998; Silva et al. 2008; Carvalho et al. 2008). Blooming events are commonly associated with ambient conditions such as hot weather, calm waters and a shallow mixing layer (Carvalho et al. 2008). Additionally, chemical analyses of the corresponding seawater have revealed low concentrations of essential nutrients, demonstrating that *Trichodesmium* spp. tend to live in oligotrophic waters (Capone et al. 1997).

As a result of the enormous number of blooming events over the world, the high biomass of *Trichodesmium* is a key component of the total primary production of the oceans, especially in areas where there is a deficiency of nutrients for other organisms. Currently, a considerable amount of effort is devoted to estimating CO_2 assimilation by autotrophic aquatic organisms and the impact these autotrophs have on biogeochemical cycles (Metz et al. 2005). This complex chemical system involves a down flux processing of carbon and its storage in deep oceanic water masses, helping to remove carbon dioxide from the atmosphere. This process, which mainly occurs in the photic zone of the ocean, influences the temperature of our planet by preventing major oscillations in and balancing CO_2 concentrations. As *Trichodesmium* globally contribute a large amount of biomass, the species of this genus represent a significant portion of the carbon cycle. Additionally, because of their capacity for assimilating atmospheric nitrogen (LaRoche andBreitbarth, 2005), *Trichodesmium* spp. also exert a key influence on N inputs to oceans. The ability to fix atmospheric N, in fact, adds a special upgrade to the removal of CO_2 from the atmosphere while balancing the nitrogen concentrations between the surface and depth (Peterson 1979).

The high biomass and capacity for adaptation in regions where other autotrophic plankton cannot adapt render *Trichodesmium* the most important diazotrophic marine organism, although it is not the only important diazotroph to occupy the oceans. The order Chroococcales, unicellular cyanobacteria with a coccoid shape, also contains a number of important diazotrophic representatives in the genera *Prochlorococcus* and *Synechococcus*. Several species can also have diatom-diazotroph associations, such as those of *Richelia intracellularis* with diatoms, forming a symbiotic relationship (Zehr and Ward 2002). These other organisms are picoplankton and nanoplankton, the most diminutive phytoplankton, and also occupy large oligotrophic areas, similar to *Trichodesmium*, but are typical of tropical regions and oligotrophic gyres (Zubkov et al. 2002, Follows et al. 2007).

When the main regions inhabited by diazotrophs are considered, there is a pattern that can be partially explained by physical and chemical factors that act to limit their existence (Ward et al. 2013). These variables are under study, and several are well documented, but many other influences are not yet fully explained. This complex biogeography pattern, especially for

Trichodesmium, appears to involve the successful physiological adaptation of species to numerous environmental variables, which is discussed in this work (Fig. 1).

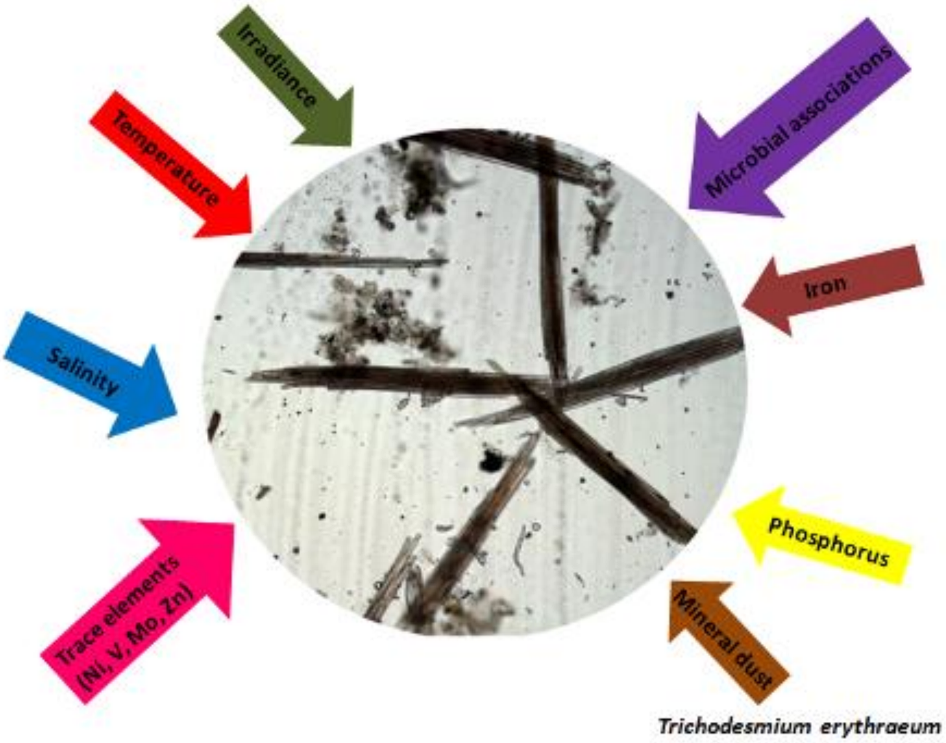

Fig. 1: Interactions among variables that limit the growth of *Trichodesmium* in oceans.

Ecophysiological contribution to success

Nitrogen fixation coupled with photosynthesis

It is possible that the most important physiological adaptation that *Trichodesmium* and several other prokaryotes developed is the capacity to acquire nitrogen from the atmosphere. This biochemical process inputs a new amount of bioavailable nitrogen into an ecosystem. Diazotrophs can be found in both aquatic and terrestrial environments, and *Trichodesmium* contribute the largest biomass in the marine environment (Capone et al. 2005). The amount of the global oceanic nitrogen added by diazotrophic fixation was calculated to be 110 Tg N.Y^{-1}, which is equivalent to almost half of the total nitrogen sources, foremost river contributions and atmospheric deposition (Gruber and Sarmiento 1997). In addition, the *Trichodesmium* N_2

fixation rates alone contributes to 42 Tg N yr^{-1} during blooms, and 20 Tg N yr^{-1} during non-blooms (Westberry and Siegel 2006).

The contribution of new nitrogen input as a result of *Trichodesmium* metabolism has intrigued scientists for decades. Many studies have attempted to measure this rate of fixation and relate it to the roles of *Trichodesmium* in biogeochemical processes in the ocean. In the Caribbean Sea, for example, the annual input from N_2 fixation was calculated to be 1.6 x 10^{12} mol, the major contribution among all diazotrophs (Capone et al. 2005). There have also been efforts to link these measurements with the global biomass using complex computer models. The model input must take into account the known variables that generally limit the growth of diazotrophs, such as the availability of P and Fe in the water (Ward et al. 2013). Nevertheless, this map of the total biomass and annual amount of new nitrogen fixation (Gruber and Sarmiento 1997) is still due for an update and is likely out of date because new reports of *Trichodesmium* biomass are released every year.

The process of acquiring atmospheric N_2 itself is well understood. The final result of nitrogen fixation transforms N_2 into $NH_{3.}$ This process has a high ATP (adenosine triphosphate) demand and requires the enzyme nitrogenase to catalyze the reaction to break the triple bond of the N_2 (Seefeldt et al. 2004). This process also involves the iron-rich protein ferredoxin to obtain electrons for the reaction, which is the reason these organisms require a relatively large amount of Fe:

$$N_2 + 8H^+ + 8e^- + 16ATP \xrightarrow{\text{nitrogenase}} 2NH_3 + H_2 + 16ADP + 16Pi$$

The general equation is an exergonic reaction that requires hydrolyzed ATP, transformed by the dinitrogenase enzyme to produce ADP (adenosine diphosphate) and Pi (inorganic phosphate) as a result (Seefeldt et al. 2004). The enzymatic complex nitrogenase is commonly composed by two units, dinitrogen reductase (Fe-protein) and dinitrogen (MoFe-protein), that interact cooperatively, where the Fe-protein transfers electrons to reduce $N_{2,}$ while the MoFe-protein is the active site itself (Howard and Rees 1996). Under Mo limiting conditions, the enzyme can be composed by a Fe-Fe only complex. Additionally, a number of studies of terrestrial diazotrophs suggest that the protein can also substitute Mo and Fe by V, producing a less efficient nitrogenase complex (Bishop and Eady 1985).

The most widely studied biochemical process involves the Mo-Fe protein. The reaction is described as having two possible pathways for NH_3 formation, distal and alternate (Fig. 2). In the first pathway, the N_2 is bound to Mo followed by a reduction and proton addition (by dinitrogen reductase). One atom of N is successively hydrogenated to ammonia and released, and the other N atom is only then hydrogenated to ammonia (Shrock 2008). Another alternative process for NH_3 formation is the alternate reduction of the N-N atoms, with cleavage occurring later in the reaction. Both pathways produce key intermediates, proposed to be diazene (HN=NH) and hydrazine (H_2N-NH_2) (Seefeldt et al. 2004).

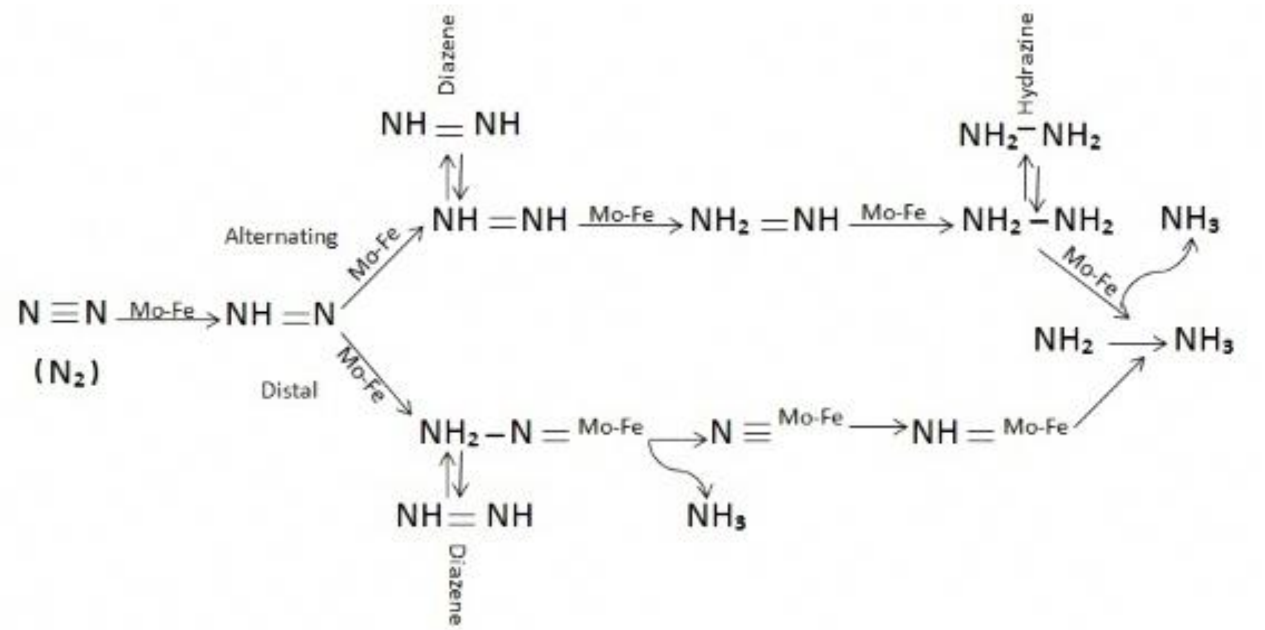

Fig. 2: Mo-Fe nitrogenase reaction by two possible mechanisms, alternating and distal. Adapted from Seefeldt et al. 2009.

This reaction is directly inhibited by O_2, which causes genetic or protein damage when present (Lin et al. 1998). To protect the nitrogenase enzyme from both reversible and irreversible damage, nitrogen-fixing organisms typically develop special cells adapted to fix nitrogen. These cells have lower concentrations of pigments and are called heterocysts. This type of cell also possesses a thick cell wall to protect enzymes against oxidation by O_2 (Fogg 1973). The genus *Trichodesmium* does not create heterocysts and is able to perform photosynthesis and nitrogen fixation simultaneously (Carpenter and Price 1977). It is already known, however, that the trichome cells have a degree of specialization. The most internal cells appear to have fewer pigments, such as chlorophyll-*a*, and mainly fix nitrogen instead of CO_2. Thus, several studies have suggested that in a colony, the inner portions of the trichomes are responsible for nitrogen fixation while the outermost cells perform the photosynthesis (Capone et al. 1997).

Because of their influence on photosynthesis and cell physiology, temperature and light appear to be important for limiting diazotrophic growth. A study conducted by Breitbarth and collaborators (2007), for example, evaluated the N_2 fixation and growth rates of *Trichodesmium* exposed to various temperature levels. Their results demonstrated that the culture could not grow at temperatures below 20° C or above 34° C. The maximum growth rate occurred at approximately 27° C. Additionally, nitrogen fixation followed the same temperature trend, with the optimum fixation rate occurring between 24° C and 34° C and the maximum rate at 27° C.

Gas vesicles

Gas vesicles are physiological structures present in numerous organisms from the phylum Cyanobacteria. This organelle, which evolved from a vacuole structure, is present in prokaryotic and eukaryotic cells and is a gas-filled space that provides cell buoyancy. This mechanism involves a change in the density of the vesicle by using substances such as carbohydrates or by simple disruption of the structure, providing a change in the general cell density (Walsby 1978).

For *Trichodesmium,* and for other cyanobacteria, the capacity of the gas vesicles to resist pressure is inversely proportional to their width (Gantt et al. 1984, Hayes and Walsby 1986). As the genus has many tiny vesicles, the organism is able to migrate to the photic zone boundary. In this case, the main biochemical process that controls vertical migration is the change in the carbohydrate concentration in the vesicles, which increases cell density and acts as ballast. Laboratory experiments suggest that this migration to photic zones is physiologically possible and that *Trichodesmium* spp. can survive for 3 to 6 days and are able to recover from light deprivation (White et al. 2006). An acceptable hypothesis to explain this migration is the movement for P acquisition by targeting the phosphocline, however, additional experiments must be conducted to fully explain the vertical movement (Villareal and Carpenter 2003).

An interesting ecological behavior that is observed in *Trichodesmium* is the aggregation of the trichomes into colonies. This aggregation results in two distinctive structures, parallel (tuft-shaped) or radial (puff-shaped) colonies. These conformations help the colony to sink into deeper waters (Villareal and Carpenter 2003) and, in the case of puffs, increase the capacity for acquiring Fe, an essential but trace nutrient in open waters (Rubin et al. 2011).

Nutrient storage

To grow in oligotrophic oceans, *Trichodesmium* have developed a strategy for storing nutrients inside cells that is common to other phytoplankton. The organism stores phosphate as polyphosphate granules and nitrogen products. Additionally, this storage has been demonstrated to be important in the regulation of buoyancy by carbohydrate ballasting (Romans et al. 1994). Additionally, the use of genetic tools enables the identification of the genes and isolation of the corresponding proteins that are produced to bind and store specifically Fe and P in the cell (Castruita et al. 2006).

Pigments

Cyanobacteria, cryptophytes and rhodophytes have phycobiliproteins as accessory pigments (Kirk 1994). These pigment-protein complexes are important components of the light harvesting complexes in these taxa and allow cyanobacteria, for example, to harvest wavelengths that are not absorbed by chlorophylls and carotenoids (Lawrenz et al. 2011). These pigments can be found in *Trichodesmium* photosynthetic apparatus, especially in the PSII antennae, and are divided into phycoerythrins, phycocyanins and allophycocyanins. Another specific optical characteristic of *Trichodesmium* is the high absorption of UV associated with the mycosporin-like amino acids, which represents an important adaptive advantage against chloroplast degradation and allows *Trichodesmium* to survive floating in surface waters (Dupouy et al. 2008).

Oceanographic processes that influence *Trichodesmium* growth

The environmental physical-chemical conditions appear to strongly delimit the biogeography of *Trichodesmium* and, as far as currently known, restrict this genus to tropical and subtropical waters (LaRoche and Breitbarth 2005). When considering these parameters, common characteristics among the studies suggest that the occurrence of the genus is associated with stratified waters, a high light intensity and low concentrations of nutrients, especially inorganic nitrogen (Hood et al. 2001). One of the first studies to link the distribution of *Trichodesmium* with environmental patterns was published by Carpenter and Price (1977). During their three research cruises to the Sargasso and Caribbean seas, these authors observed that the greatest surface concentrations of the genus occurred when the wind speed was less than 5 knots. During cruises along Hawaii between 1989 and 1992 (Letelier and Karl 1996), the biomass and distribution of *Trichodesmium* were characterized in an oligotrophic area. The results indicated a positive relationship between biomass and hot periods of calm seas, low winds and a shallow mixed layer. Additionally, the capacity of *Trichodesmium* to fix N_2 from the atmosphere appeared to enhance the growth of the local phytoplankton biomass by the release of the nitrogenated compounds (Letelier and Karl 1996).

The active growth of *Trichodesmium* is also affected by irradiance and salinity. These responses were determined by analyzing both environmental data and laboratory experiments. The optimal growth for the organism, when submitted to varying light, was demonstrated to be 7 $W.m^{-2}$ (Ohki et al. 1986). When varying the salinity, *Trichodesmium* were able to develop in a range of 22–37, with optimum growth between 30 and 37 (Hegde et al. 2008). Field studies of the coastal waters of Bengal Bay and the Arabian Sea have also demonstrated that the local species of *Trichodesmium* can form massive blooms within the salinity range from 29–31 (Jyothibabu et al. 2003).

There are nutrients, however, that appear to directly enhance the growth of *Trichodesmium* when present. Several studies have reported a relationship between high concentrations of atmospheric Fe and the occurrence of *Trichodesmium.* A bloom in the Arabic Sea during the intermonsoon period, in 1995, was related to the low wind speed patterns and high Fe concentrations in the atmosphere (Capone et al. 1998). The AMT (Atlantic Meridional Transect) cruise also reported a relationship between a high biomass of *Trichodesmium* and a shallow mixed layer in combination with a high concentration of Fe at the surface (Tyrrell et al. 2003).

A previous study conducted in the eastern subtropical North Atlantic, during 2007 and 2008, reported the occurrence of *Trichodesmium* in a region with a high input of atmospheric iron and also suggested another limiting nutrient for these cyanobacteria. According to the study, the waters have sufficient available Fe, and the main limiting element to diazotrophic activity should be phosphorus (Fernandez et al. 2013). The necessity of P acquisition, with a minimum concentration required, was also observed for a South Pacific cruise in 2003 (Moutin et al. 2005).

Evidently, *Trichodesmium* blooms are related to low concentrations of nutrients, with the exceptions of Fe and P, and this relationship was reinforced by a study conducted off the coast of India (Martin et al. 2013). That study reported that the genus *Trichodesmium* was common during the pre-monsoon period, associated with the typical physical-chemical parameters for the organism (high temperature and salinity, water stratification and low levels of nutrients). Since the 1970s, the constant eutrophication caused by anthropogenic sources has led to the disappearance of the entire genus off the coast of India.

Nitrogen requirements

Species of this genus are thought to increase the rate of nitrogen fixation when inhabiting oligotrophic regions. Two cruises investigated the N_2 fixation rates of *Trichodesmium* along the Canary current region, influenced by eddies (Agawin et al. 2013). These authors reported that in stations with higher NH_4 concentrations, the rate of fixation was lower, whereas there was a higher rate in eddy regions of low NH_4. This discrepancy occurs because of the high energetic cost to acquire the nutrient from the atmosphere; hence, when there are dissolved nitrogen sources, these diazotrophs are able to store energy by absorbing nitrogen directly from the water. Earlier studies have already suggested that the genus, although able to grow without dissolved nitrogen compounds in the water, are affected by its presence, which suppresses N_2 fixation (Holl and Montoya 2005).

In contrast, the combination of high available nitrogen with limiting concentrations of other nutrients, such as P and Fe, interrupts this sensibility to slow down N_2 uptake. *Trichodesmium* cultures exposed to various concentrations of PO_4^{2-} and NO_3^- demonstrated that the fixation rates were not dependent on the dissolved nitrogen concentrations but were related to phosphate availability (Knapp et al. 2012). However, Spungin and collaborators (2014) reported that in laboratory experiments, *Trichodesmium* could maintain inputs of new nitrogen to the cells in the upper mixed layer of oceanic waters even in phosphorus-limited areas.

These results indicate that although nitrogen fixation is well understood, a number of variables determine its rate, and further investigation is required to completely understand the physiological regulation of diazotrophs. Thus, similar to other elements, nitrogen does not limit the occurrence of *Trichodesmium*, but appear to regulate their growth rates because N_2 fixation requires a high energetic cost compared to dissolved N. The need to acquire atmospheric nitrogen causes this genus to grow at low rates compared to other phytoplankton, and the max specific growth rate (μ) has been estimated to be 0.14 d^{-1} (LaRoche and Breitbarth 2005).

Phosphorus

An important compound that controls the growth of *Trichodesmium*, as demonstrated in numerous studies, is phosphorus in its various forms. Although this genus is known to store P, the measurements of this nutrient *in situ* do not always explain a high biomass (Moutin et al. 2005). *Trichodesmium* colonies can satisfy their P needs by supplementing dissolved inorganic phosphorus (DIP) uptake with phosphorus (P) cleaved from dissolved organic phosphorus (DOP) via alkaline phosphatase (Sohm and Capone 2006). A number of studies conducted by manipulating conditions in the laboratory have demonstrated that *Trichodesmium* can use both organic and inorganic dissolved phosphorus (DOP and DIP, respectively). The uptake of P from the DOP pool appears to be very important for *Trichodesmium* growth, and species of this genus can obtain considerable amounts of P from the DOP pool (Sohm and Capone 2006). In the Eastern Atlantic, an addition of 200 µM of DIP combined with 2 nM of Fe stimulated nitrogen fixation in the native *Trichodesmium* populations, reinforcing the importance of this macronutrient to their metabolism (Mills et al. 2004). Additionally, Stihl and collaborators (2001) measured the alkaline phosphatase activity in cultures with added DIP and determined that the enzyme activity was an indicator of P limitation. However, P is more commonly found in an organic form in the oceans, and more experiments, especially those to explain the real significance of the organic phosphorus, should be performed.

Furthermore, the combination of Fe and P limits the growth of *Trichodesmium*. Studies have demonstrated that the growth behavior differs among oceans. In the Atlantic, for example, *Trichodesmium* is more likely to be P-limited, according to the Redfield ratio. A study conducted in the Central Atlantic observed a limitation by P in combination with light under environmental conditions (Sañudo-Wilhemy et al. 2001). The most visually important input of iron in the Southeast North Atlantic, for example, is provided by the mineral dust from the Sahara desert, which increases the level of primary production. Satellite data indicate that this atmospheric event constantly provides a supply of Fe to these surface waters (Sañudo-Wilhemy et al. 2001). In this case, the seasonal availability of P, especially during spring, driven by then vertical diffusive flux, controls the level of diazotrophic activity (Fernández et al. 2013). In contrast, the Fe input is much lower in the North Pacific and appears to limit the growth there (Landing et al. 1987).

As an adaptation to P-limited resources, several studies have noted that this genus can acquire P even when the surface water is depleted. A unique capability for vertical migration, in this case, plays an essential role because the colonies can migrate to the phosphocline (Villareal and Carpenter 2003). As this nutrient varies seasonally, depending on the ascendance of water masses, for example, P variation can at least partially explain the seasonal cycle of *Trichodesmium* biomass, when coupled with other variables (physical and chemical dynamics) (Fernández et al. 2013). However, *Trichodesmium* does not need to compete for P with other phytoplankton and can coexist with picoplanktonic organisms in P-limited areas because of the presence of the phosphatase enzyme (Sohm and Capone 2006).

Iron

The element iron (Fe) is an important trace metal to all phytoplankton because it is contained in several important photosynthetic proteins. Diazotrophic phytoplankton also require an additional demand for this element because there is Fe in the nitrogenase enzyme and several other important proteins (Raven 1988, Sañudo-Wilhemy 2001). Because of this extra demand, the value of Fe in the Redfield ratio for C:N:P:Fe is higher compared to other phytoplankton and causes this element to be a limiting factor for N fixation and, as a consequence, for the growth of diazotrophic phytoplankton (Moore et al. 2009). While using atmospheric nitrogen, it is estimated that *Trichodesmium* devotes more than half of its Fe content for the enzyme nitrogenase, but while using ammonium for growth, the Fe in the cells is more strongly associated with photosynthetic compounds. This difference is also reflected in the optimal Fe requirement, based on the Fe:C ratio, which is lower for NH_4^+ use (Kutska et al. 2003b).

The main source of Fe to the open ocean is basically limited to inputs from atmospheric dust. However, this input appears sufficient to maintain the permanent *Trichodesmium* populations in specific regions such as the Central Atlantic Ocean (Sañudo-Wilhemy et al. 2001), Australia (Cropp et al. 2013) and the Northern Indian Ocean (Srinivas and Sarin 2012). For these three regions, undoubtedly, the most intensively studied and important contribution of atmospheric deposition comes from the Sahara desert, which fertilizes the equatorial region of the Atlantic Ocean (Tyrrell 2003, Agawin et al. 2013).

The availability of Fe to phytoplankton is associated with both dissolved and organic forms, and notably, *Trichodesmium* is unable to absorb Fe directly from dust. However, members of this genus appear to have individualized strategies to transform inorganic Fe to organic forms. One of these curious processes has been well described in the laboratory (Rubin et al. 2011) and involves the shape of the entire colony. In this study, the scientists discovered that the trichomes were able to transport dust and Fe oxides to the interior of the puff-shaped colonies, where there is an anoxic microenvironment. This process seems to enhance iron acquisition by transforming inorganic Fe into dissolved Fe. Additionally, single trichomes also appear to have the ability to dissolve iron oxides.

Another mechanism for maximizing the availability of total Fe in water is to form associations with other bacteria (Roe et al. 2012). Heterotrophic bacteria have important iron transport systems that transform unavailable forms of Fe to dissolved forms. This aggregation accounts for the iron requirement for *Trichodesmium* growth.

The waters where *Trichodesmium* blooms are typically found are generally far away from continents and, consequently, from continental iron input. This location is crucial to subjecting *Trichodesmium* to varying degrees of seasonal and biogeographical limitations. Recently, novel methods have been developed to evaluate Fe-limitation in the oceans indirectly. An efficient molecular method, for example, is able to measure the expression of a gene (*isiB*) that is induced by Fe-limitation. The method is based on clade-specific, quantitative reverse transcriptase-PCR (Chappel et al. 2012). The method has been applied to areas of the Pacific and Atlantic Oceans, where the organisms were P-limited, Fe-limited or limited by both, with positive results.

Another molecular method involves the measurement of photosynthesis and nitrogen fixation enzymes that utilizes iron in their molecules. This quantification demonstrated an internal regulation mechanism whereby, in the case of Fe-limitation, the photosynthetic apparatus is preferentially maintained. Additionally, the study was able to demonstrate iron-stress-induced protein accumulations and the ability of the organism to acclimate to iron-depleted conditions, even in the presence of iron, by expressing an iron-starvation protein (Richier et al. 2012).

These studies and techniques contributed important information concerning the physiological regulation of *Trichodesmium*. Iron seems to be the most important limiting element controlling the rate of N_2 fixation and limiting the occurrence of diazotrophs in the oceans.

Nickel and other elements

Although phosphorus and iron are well known to limit the occurrence of *Trichodesmium*, recent studies have suggested that nickel is an important element for N_2 fixation. An insufficient supply of this metal, coupled with low light intensity, can limit this process. When exposing cultures to a trace metal-defined culture and natural seawater, in which there were sufficient Fe and P, an increase in the Ni concentration was directly related to the rate of nitrogen fixation. This element seems to be involved in the protection of nitrogenase from superoxides that are generated during photosynthesis (Ho 2013).

Additionally, the genus has other metal requirements, such as vanadium, molybdenum and zinc. These elements are also related to nitrogen fixation by composing the enzyme nitrogenase and, indirectly, by the expression of antioxidants. The high intracellular concentrations of trace metals confer a unique biogeochemical signature to *Trichodesmium* (Nuester et al. 2012).

Global Warming

The consequences of global warming to phytoplankton communities are difficult to establish but have garnered significant interest. An increase in temperature strengthens the stratification (weak upwelling), modifies the intensity and the direction of oceanic currents and also acidifies the ocean by increasing the levels of CO_2 uptake by the oceans. This unbalanced system strongly rearranges the phytoplankton assemblages, affecting the equilibrium. Complex interactions among the new physical-chemical parameters can also alter organisms' physiological processes; consequently, they can adapt to the new conditions. The changes in the environmental conditions benefit certain species and cause extinctions of other species. Although the overall impact cannot be fully predicted, scientists have tried convincingly (Hallegraeff 2000).

Regarding *Trichodesmium* specifically, an experiment was conducted in the subtropical North Atlantic Ocean to measure the impact of a hypothetical acidification on marine diazotrophic and unicellular marine cyanobacteria, including *Synechococcus* and *Prochlorococcus* (Lomas et al. 2012). The results indicated that the changes in pH and CO_2 levels increased N_2 fixation rates in *Trichodesmium* and promoted a rapid cellular acclimation in all the groups, suggesting that these organisms will not suffer a negative impact, at least directly, from the impacts of global warming. In addition, laboratory experiments with *Trichodesmium* cultures have demonstrated that under high CO_2 levels, *Trichodesmium* spp. compensate for P limitation by utilizing a flexible resource reallocation mechanism, enabling higher N_2 fixation and growth rates of *Trichodesmium* in oceanic waters (Spungin et al. 2014). In this study, the authors demonstrated that both pCO_2 and P significantly influenced growth rates, which ranged from 0.22 (+/- 0.1) day $^{-1}$ under low pCO_2 and P-limited conditions to 0.58 (+/- 0.18) day^{-1} under high pCO_2 and P-rich conditions. These authors have stated that elevated CO_2

concentrations can compensate for low phosphorus availability and enable higher rates of carbon and nitrogen fixation for *Trichodesmium* in a warmer future ocean.

Mapping the occurrence of Trichodesmium: studies using remote sensing

An important observation regarding studies of *Trichodesmium* is that, because they are oceanic organisms, the opportunity to locate large concentrations *in situ* depends on the availability of an equipped ship. This ship would also need to be able to cover large areas and have a significant cruise length to achieve a high probability of finding aggregations of *Trichodesmium*, without a synoptic analysis. This method has a high cost, including ship maintenance and personal labor. An alternative method for mapping these bloom events without being onboard a ship is to use remote sensing, a relatively recent technique that is capable of detecting important aggregations of *Trichodesmium* events in open oceans and allows for performing synoptic and large-scale studies.

The use of satellites to detect and quantify phytoplankton blooms is possible because different groups of organisms have different pigments and proportions of pigments from each other. When considering the genus *Trichodesmium* in terms of pigment composition, it has a strong light absorption by a specific pigment called phycoerythrin, which produces a characteristic spectral feature at a wavelength of approximately 500 nm. Phycoerythrin represents a potential indicator by reflectance for remote sensing (Fig. 3). Hence, the unique characteristic of *Trichodesmium* pigments, coupled with their morphology, enable the color detection of bloom events in oceans (Westberry et al. 2005, Nair et al. 2008).

Fig. 3: Satellite image showing a *Trichodesmium spp.* bloom in the Capricorn Channel off the Queensland coast of Australia taken on December 3rd, 2002 (Source, NASA's Earth Sciences and Image Analysis).

Remote sensing has provided an important technique for mapping *Trichodesmium* biomass in surface waters (Davis and McGilicuddy, 2006). For this purpose, specific algorithms have been developed for detecting the *Trichodesmium* exclusive pigments compared to other phytoplankton groups (Subramanian et al. 1999ab, Hu et al. 2010). The detection of *Trichodesmium* blooms, or spots, from remote sensing has received great scientific interest since the 1980s (Dupouy et al. 1988, Borstad et al. 1992). A number of relevant empirical algorithms were developed for the Coastal Zone Color Scanner (CZCS) optical sensor images (Dupouy et al. 1988, Subramanian and Carpenter 1994), as the first described works in this research field. Currently, recent efforts have focused on inherent and apparent optic properties (absorption spectrum, reflectance and backscattering) of *Trichodesmium* (Subramanian et al. 1999ab) and the development of empirical (Subramanian et al. 2002) and semi-analytical algorithms (Westberry et al. 2005) to be applied to multispectral data from the optical sensor Sea-viewing Wide Field-of View Sensor (SeaWiFS). However, these algorithms only result in qualitative data, i.e., the presence or absence of dense concentrations of *Trichodesmium*. Additionally, the low spatial resolution of the sensor can limit the detection of the organism when the trichome aggregations are not sufficiently extensive.

The bio-optical model developed by Westberry et al. (2005) was adapted from Subramanian et al. (1999) using SeaWiFS images. The model was optimized to detect the presence of *Trichodesmium* aggregations in surface waters at a minimum concentration of 3,200 trichomes/L and 75% accuracy, although this technique did not produce good feedback for chlorophyll-*a*. Several physiological characteristics of *Trichodesmium* can cause variations in the emitted light-spectrum. A number of other factors can also influence pigment variations in healthy populations, including the presence of another group of phytoplankton; trichomes can also shadow each other and exhibit non-homogeneous distributions in ocean surface waters (Westberry et al. 2005).

Additionally, the optical characteristics that enable the identification of the genus are primarily the high light absorption in the blue wavelength region because of the colored dissolved organic matter (CDOM) that is aggregated on the trichomes and colonies; the flat absorption spectrum, defined by the “package-effect” of the colonies; auto backscattering, especially in the red and infrared region, because of the presence of gas vesicles, which can occupy approximately 80% of the intracellular space; and the high fluorescence in the green region, which is attributed to the presence of the phycoerythrin pigment (Westberry et al. 2005).

The utilization of NASA’s Moderate Resolution Imaging Spectroradiometer (MODIS) sensor is suitable because it provides a sufficient spectral and spatial resolution for remote sensing data of the ocean color. Hence, empirical methods are being developed based on hyper-spectral radiometric data information, collected immediately above the water surface when there is a dense aggregation of *Trichodesmium* (McKinna et al. 2011). An optical algorithm of binary classification is also applied to detect the presence or absence and the size of *Trichodesmium* aggregations. These methods performed well when applied at the Great Barrier Reef-Coral Sea – Northwestern Australia, an area where blooms are frequently observed (McKinna et al. 2011).

There is a need for further investigations and better optimization of bio-optical models, especially for obtaining quantitative data on *Trichodesmium* abundance, in addition to the presence and absence of data for the members of this genus. This development will lead to better comprehension of the aggregation dynamics and the importance of *Trichodesmium* to oligotrophic ocean environments.

Concluding remarks

Studies involving *Trichodesmium* were initiated when this genus was separated from the genus *Oscillatoria* and a first description of nitrogen fixation without heterocysts was published (Carpenter and Price 1977). Since then, great effort has been devoted to their study. Hence, its worldwide distribution and physiological requirements are now better understood.

Trichodesmium spp. are very important because of their biomass and roles in the biogeochemical process. At present, studies are mainly focused on mapping their occurrence as a prerequisite to estimating the total biomass. Remote sensing techniques will help scientists to create more precise maps of the occurrence of *Trichodesmium*. However, *in situ* measurements of *Trichodesmium* blooms must be coupled with the images to validate the data and to implement a better approach for developing bio-optical algorithms because the genus migrates vertically. In sum, we need to better understand the ecology and physiology of *Trichodesmium* spp. in the marine ecosystem.

Given the current state of investigations, there is a good probability that it will soon be possible to build a high-precision map that couples *Trichodesmium* biomass and nitrogen levels in various regions of the world with more refined data available each year. This map will be possible because several important areas that were only recently discovered, such as the southwest Atlantic Ocean and the Indian Ocean, are now receiving greater attention. In additional, the already existing studies on *Trichodesmium* physiology should be refined as more results are published and will reveal the importance of *Trichodesmium* to oceans globally.

Trichodesmium spp. are well-adapted organisms found in all tropical and subtropical oceans. Members of this genus contribute significantly to the phytoplankton biomass and influence the biogeochemistry of carbon and nitrogen in oceans. The biogeography of *Trichodesmium* is limited by multiple co-existing factors, including water temperature, salinity, sunlight and nutrients. Despite the fact that phosphorus and iron are already known to be limiting nutrients for members of this genus, their exact importance and mechanisms of acquisition still need to be clarified. Additionally, numerous studies have suggested that there are other important limiting compounds to *Trichodesmium*, such as Ni, V, Mo and Zn, that deserve special attention and should also be investigated.

Furthermore, another main challenge relies on understanding the physiological behavior of *Trichodesmium* and how it is affected by environmental variables, such as ocean acidification, and its adaptive advantages against competition with and coexistence with other phytoplankton, especially regarding resource availability. A better knowledge of the actual biomass and distribution of the dense aggregations of *Trichodesmium* acquired from ocean color images can certainly offer answers regarding their climate regulation status, denoting them as the most important nitrogen fixing organisms in the oceans.

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CAPÍTULO II

Trichodesmium in south-southeastern Brazilian shelf slope: species abundance and coexistence with other microplanktonic organisms

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ABSTRACT

Marine cyanobacteria *Trichodesmium* occur in tropical and subtropical oligotrophic oceans and contribute to the nitrogen and carbon cycle. Although there are plenty of reports about their occurrence, just a few are located in southern Brazilian waters. This work aimed to collect and identify these diazotrophic species near the south-southeastern Brazilian shelf slope, as well as the microplanktonic organisms that co-occur within some aggregations. Physical (temperature and salinity) properties were measured during two consecutive autumn periods (04–11 June of 2013 and 10–20 May of 2014). Mixed layer depth (MLD) was derived from density profiles and wind information (direction and intensity) was obtained from an anemometer mounted on the foremast ~18m above the sea surface. Biological samples were collected through vertical hauls with a plankton net, integrating the first 30 m of the water column. Overall, cyanobacteria trichomes were more abundant in the northern stations, under lower wind speed (< 8 knots) and shallow MLD (< 40 m). Besides the previous reports of *T. erythraeum* in the coastal and shelf parts of this region, other four species (*T. clevei*, *T. hildebrandtii*, *T. radians* and *T. thiebautii*) were also observed during these two consecutive autumns.

KEYWORDS: Brazilian shelf slope; coexistence; diatoms; diazotrophs; dinoflagellates

1. INTRODUCTION

Trichodesmium (Ehrenberg, 1830) is an important marine member of cyanobacteria. They are able to form colonies of different shapes and often form aggregations that can be found along tropical and subtropical oligotrophic oceans through the world (Karl *et al.*, 2002). The *Trichodesmium* biogeography is associated with commonly external factors as hot and calm weathers and the presence of a shallow mixed layer (Agawin *et al*., 2013).

In addition to the capacity of acquiring atmospheric nitrogen, *Trichodesmium* occurs in high biomass, playing an important role in the biogeochemical cycle of nitrogen and carbon. The global estimative of N_2 fixation rate of 42 Tg N yr^{-1} during blooms, and 20 Tg N yr^{-1} during non-bloom conditions, proof the genus domination among the diazotrophs (Westberry and Siegel, 2006). More recently, studies on *Trichodesmium* are complemented by remote sensing. This tool gives a better resolution for the biomass estimate and total N_2 uptake (McKinna *et al*., 2011). By all this information, new reports on the genus occurrence, especially in regions that it was not observed before, are of great importance.

The majority of studies and, hence, more reported occurrences of *Trichodesmium*, are located in North Atlantic, North Pacific and Australian Coast. As the genus has a preference for low-nutrient regions, it is also well studied in the Atlantic and Pacific gyres (Villareal and Carpenter, 2003; LaRoche and Breitbarth, 2005). On the other hand, there is a relative great amount of studies and reports in other areas not focused before, like the Andaman Sea (Arun Kumar *et al*., 2012) and the offshore region of India's coast (Srinivas and Sarin, 2013). These new records of *Trichodesmium* occurrence suggest that they may be more significant than previously thought in terms of primary productivity, especially where nutrients are depleted. Other poorly known locations are the huge coast and shelf sites of Brazil, with only a few studies published until now (Carvalho *et al.*, 2008; Monteiro *et al*., 2010, 2012) and, unfortunately, with a relatively great amount of white papers and/or unpublished data.

Other members of phytoplankton and zooplankton species usually co-occur with *Trichodesmium*. One important association observed is between *Macrosetella gracilis* and the colonies, which provide food and substrate for this copepod's eggs and larvae (O'Neil *et al.,* 1996). Also, the diatom *Rhizosolenia hebetata* in symbiosis with the diazotroph cyanobacterium *Richelia intracellularis* were also reported during high biomass of *Trichodesmium* (Madhu *et al*., 2013). Understanding the structure of phytoplankton community is therefore an important key for the knowledge of *Trichodesmium* ecology and its potential biotic interactions with other species along the southern Brazilian shelf slope.

2. MATERIAL AND METHODS

2.1. Sampling and physical parameters

This study was carried out in a large area encompassing the Brazilian shelf slope between 24º and 35º S (Fig. 01), in the autumn (04–11 June of 2013 and 10–20 May of 2014), on board the R/V *Atlântico Sul* (Federal University of Rio Grande – Brazil), as part of the named "TALUDE" project (Table I).

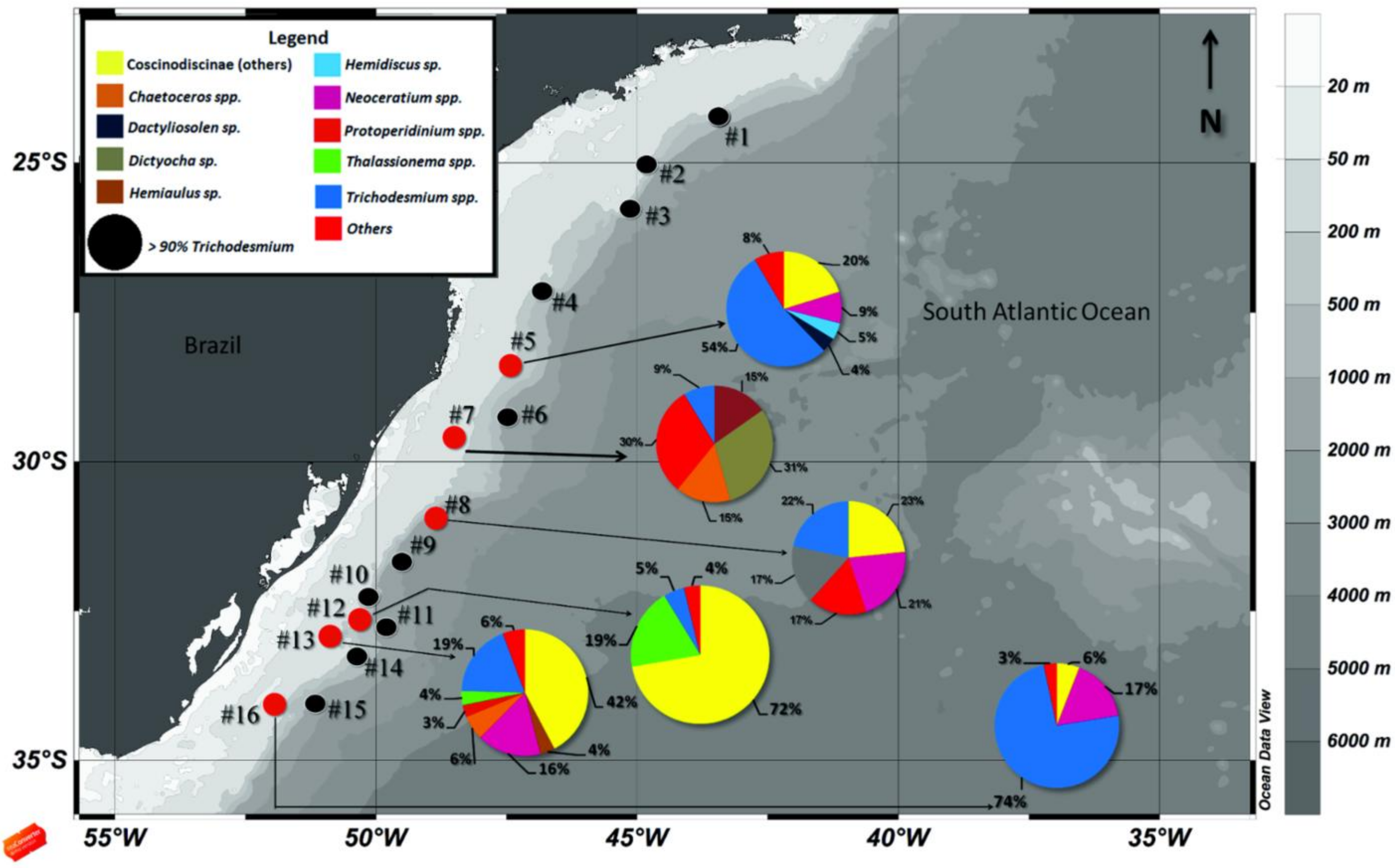

Figure 01: Map of the study area showing the location of sampling stations along the south-southeast Brazilian shelf slope. Inset box brings a legend with colors meaning the main microplankton species as sectors of pie charts and a black circle denoting sites where *Trichodesmium* spp. contributed >90% to total amount of microplankton assemblages. Small red circles represent sites where *Trichodesmium* spp. contributed <90% to total amount of microplankton assemblages.

Vertical profiles of temperature and salinity were obtained with a SeaBird® 911+ CTD (conductivity+ temperature+ depth) sensor. Classification of surface water masses was based on the thermohaline intervals found in Möller *et al.* (2008). The mixed layer depth (MLD) was determined from vertical density profiles ($\partial\rho/\partial z$), excluding the first 5 meters of depth due to noise data. Wind speed and direction were obtained from anemometer mounted on the foremast ~18m above the sea surface. Besides showing both sea surface temperature (SST) and salinity (SSS), these parameters were averaged across a 30 m of water column in order to relate to biological samples and other environmental features.

Table I. Major parameters of water and weather conditions at the stations. Temperature (°C) and salinity values are averaged on a 30 m of the water column, followed by corresponding standard-deviation values (in parentheses). *Mixed Layer Depth, **Sea Surface Temperature, ***Sea Surface Salinity.

Stations	**Lat (S)**	**Long (W)**	**Sampling dates**	**Local depth (m)**	**SST****	**T (°C)**	**SSS*****	**Salinity**	**MLD* (m)**	**Surface water mass**	**Wind speed (knots)**	**Wind direction**
#1	24°16.6'	43°22.2'	06/11/13	692	24.0	23.7 (0.109)	36.8	36.9 (0.390)	45	TW	6	SE
#2	25°07.6'	44°51.4'	06/08/13	190	23.6	24.6 (0.502)	36.2	37.3 (0.420)	17	TW	4.5	NO
#3	25°48.8'	45°04.2'	06/07/13	1287	23.2	22.9 (0.104)	35.8	35.8 (0.380)	40	TW	8	SE
#4	27°6.4'	46°24.7'	06/04/13	830	23.2	23.1 (0.157)	36.8	36.8 (0.112)	80	TW	4.6	SE
#5	28°6.6'	48°00.0'	05/20/14	138	21.8	22.9 (0.548)	36.0	36.7 (0.350)	14	TW	5	NW
#6	29°22.9'	47°08.8'	05/19/14	1876	22.8	22.8 (0.009)	36.8	36.8 (0.007)	35	TW	11.6	SW
#7	29°26.0'	48°24.8'	05/18/14	140	22.7	22.8 (0.009)	36.7	36.8 (0.007)	35	TW	10.4	SW
#8	30°45.6'	48°24'	05/17/14	1505	20.6	20.7 (0.672)	34.3	34.5 (0.584)	80	STSW	12.2	W
#9	31°39.3'	49°29.8'	05/15/14	1816	21.8	22.9 (0.548)	36.0	36.7 (0.350)	43	TW	15.7	NW
#10	31°56.5'	49°57.2'	05/14/14	680	20.6	20.7 (0.672)	34.3	34.5 (0.584)	18	STSW	4	SE
#11	32°12.9'	49°41.1'	05/14/14	1870	21.8	22.9 (0.547)	36.0	36.7 (0.349)	40	TW	5.8	SW
#12	32°18.2'	50°06.6'	05/14/14	806	21.8	22.9 (0.557)	36.0	36.7 (0.339)	40	TW	11	SW
#13	32°33.6'	50°16.9'	05/13/14	575	20.6	20.9 (0.723)	31.1	31.4 (0.554)	47	PPW	18	W
#14	32°55.3'	50°17.6'	05/11/14	676	20.8	22.3 (0.783)	35.0	36.1 (0.612)	15	TW	6	NW
#15	33°04.0'	50°05.0'	05/12/14	1166	23.5	23.5 (0.004)	36.9	36.9 (0.004)	20	TW	6.7	W
#16	33°40.4'	51°23.5'	05/10/14	127	20.6	20.8 (0.894)	31.1	31.3 (0.664)	18	PPW	24	SE

2.2. Biological samples

Two kinds of sampling points were performed: one was occupied on dense aggregations of *Trichodesmium* that were visible by naked eyes and, another sampling point was chosen when there were no clearly visible *Trichodesmium* aggregations. These sampling efforts were usually made under suitable weather conditions such as clear sky and calm sea. During the sampling period of 2013, only sampling in the northernmost section (stations #1–#4, see Fig. 01) was carried out, as the main goal was to work exclusively with high densities of *Trichodesmium*. Since aggregations of trichomes were not commonly found during the autumn of 2014, the sampling was performed equally across the study area albeit without visiting of northernmost section.

Biological samples were collected in vertical hauls with ring plankton net (30 cm mouth diameter, 50 µm mesh size) down to 30 m depth of water column. These samples were, then, preserved in 4% buffered formaldehyde. *Trichodesmium* species were identified following Komárek and Anagnostidis (2005) and quantified as number of trichomes. As far as possible, co-occurring microplankton (>20 µm) were also identified and counted (at least 100 cells) using an inverted microscope Zeiss Axiovert A1 coupled with AxioCam MRc and sedimentation chambers (Utermöhl, 1958), at ×200, ×400 and ×1000 magnification, according to specific literature (Hasle and Syvertsen, 1996; Steidinger and Tangen, 1996). Since a huge number of trichomes of *Trichodesmium* spp. were difficult to be identified during the counting, they were grouped together as presented in the Fig. 01. The total abundance of the genus was calculated by the sum of the single trichomes and their mean values in the colonies (Carpenter, 1983). Both *Trichodesmium* and other taxa were, then, divided by the 30-m water column × 0.03-m mouth diameter of the plankton net. Finally, the abundance was shown in trichomes (*Trichodesmium* spp.) and cells (remaining microplankton organisms) per liter.

2.3 Statistics

Multivariate analyses were conducted in order to verify any spatial pattern in species composition and to describe the relationship between some abiotic factors and that biological feature.

A statistical test nMDS (non-parametric multidimensional scaling) was chosen to generate an ordination diagram and spatially group the planktonic taxa identified and counted (Wickelmaier, 2003), using a dissimilarity matrix based on the non-metric *Bray-Curtis* index (Bray and Curtis 1957). Then, an analysis of similarities (ANOSIM) was applied (Clarke, 1993) to verify possible differences among the groups of sites. These analyses were carried out using the free software Past (v. 1.81) (Hammer *et al.*, 2008).

Canonical correspondence analysis (CCA) was performed in order to identify the main patterns of microplankton species variability, with respect to environmental variables (Ter Braak and Prentice, 1988). Biotic variables were represented by total abundances of *Trichodesmium* and other microplankton taxa. Environmental variables included: sea surface temperature and salinity, MDL, wind speed, latitude and type of water mass. That latter categorical variable was ascribed as follows: Tropical Water (TW) = 1, Subtropical Shelf Water (STSW) = 2 and Plata Plume Water (PPW) = 3, and latitude was used as a covariate. All variables were log-transformed before analysis to reduce the influence of the different scales in the sets of analyzed variables. To test the significance of the CCA, Monte-Carlo tests were run based on 499 permutations under a reduced model ($P < 0.05$). Three sampling stations (#1, #15 and #16) were excluded from the final CCA analysis because they represented very extreme conditions in previous analyses.

3. RESULTS

In considering the environmental conditions for both two sampling periods (Table I), the main surface water mass in the region was the Tropical Water (TW, $S \geq 36$), with average temperatures (for the first 30 m of depth) >22.3°C. The Sub-tropical Shelf Water (STSW, $33.5 < S < 35.5$) influenced the stations #8 and #10, both with temperature of 20.7 °C. In the stations #13 and #16, the Plata Plume Water (PPW, $S < 33.5$) was noticeable, with temperatures ≤ 20.9. The wind direction varied greatly, with a relative predominance of southerly or westerly directions. Interestingly, winds produced a weak speed of 4 knots at #10, with 90% of trichomes of *Trichodesmium* spp. or more intense speeds up to 24 knots at #16, with 74% of *Trichodesmium* spp. MLD was also variable, with a shallowness of 14 m up to a more deeper depth down to 80 m (Table I).

A map of the study area enumerating the microplanktonic taxa shows that *Trichodesmium* spp. were the greatest contributors to total amount of microplankton in both autumns. In some southernmost sites, the diatoms Coscinodiscineae and *Thalassionema* spp. and the dinoflagellates *Neoceratium* spp. and *Protoperidinium* spp. co-occurred with the cyanobacteria (Fig. 01). Stations #2 and #3 presented a much more elevated concentration of trichomes: 2.1×10^6 and 4.4×10^6 trichomes L^{-1}, respectively. These two stations and other four ones (#1, #4, #10 and #11) were sites wherein there was good eyesight of surface patches of *Trichodesmium* spp., whose abundance attained more than 8×10^3 trichomes L^{-1}. On the other hand, there were no visible patches of *Trichodesmium* in the rest of stations, where abundances ranged from only 4 to 2.5×10^3 trichomes L^{-1} (Table II).

In general, the other microplankton organisms presented low relative abundances across the sampling points, with *Trichodesmium* spp. encompassing almost 100% of the microplankton assemblage at station #2 and the other organisms contributing to around 10% of the total microplankton assemblages in the others stations with *Trichodesmium* spp. reaching at least 90%. When *Trichodesmium* spp. contributed <90%, there was some variation in species abundance of microplankton (Fig. 01, Table II). The stations #14 (18 taxa), #10 (17 taxa) #1 and #5 (13 taxa each) showed other organisms in reasonable numbers while #6, #8, #12 and #15 showed lesser amount of microplankton, in general, <500 organisms L^{-1} (Table II).

Some microplanktonic organisms were very important in the study area, even considering the station #10 which had a considerable number of *Trichodesmium* spp. (Table II). The centric diatoms from the sub-order Coscinodiscineae, dinoflagellates *Neoceratium* spp. and silicoflagellates (Class Dictyochophyceae) were fairly common across the study area. Moreover, the calanoid copepod *Macrosetella gracilis*, with their eggs and larvae commonly attached to the *Trichodesmium* trichomes, were present in all sampling points. In the southernmost part of study area, an unidentified pteropod mollusk was not uncommon. However, both *M. gracilis* and this latter organism were not quantified.

Table II. Abundance of microplankton groups (trichomes L^{-1} for *Trichodesmium* spp. and cells L^{-1} for the remaining organisms) at the stations. **N. concilians, N. contortum var. karstenii, N. extensum, N. fusus, N. horridum, N. pentagonum, N. praelongum, N. symmetricum, N. teres, N. trichoceros, N. vultur var. sumatranum.*

Taxa	**Stations**															
	#01	**#02**	**#03**	**#04**	**#05**	**#06**	**#07**	**#08**	**#09**	**#10**	**#11**	**#12**	**#13**	**#14**	**#15**	**#16**
Cyanobacteria																
Trichodesmium spp.	918351	4405541	2139531	157416	1180	263	4	13	936	8185	26567	26	1138	2500	461	1504
Other oscillatoriales	268	108	90	30						17						9
Diatoms																
Chaetoceros spp.	670			36			7			22				834		
Climacodium frauenfeldianum					35			10		65		5		194		
Coscinodiscineae	804				444	29	14	14	21	117	10	381	26	5592	9	120
Dactyliosolen sp.					91					4				139	5	
Hemiaulus sp.	1116		45		18		7		11	104			13	500	5	
Hemidiscus sp.					104									46		
Planktoniella sol	45				5					4						
Pleurosigma sp.	42		23	6						9				19		
Rhizosolenia spp.	357		67	30	36					26		10		56		9
Thalassionema spp.										9		100	13	472		86
Dinoflagellates																
Amphisolenia bidentata	90			24												
Ceratocorys hórrida														9		
Dinophysis spp.														9		
Neoceratium spp.*	2411	54	402	269	194			13	11	294		30	10	2167	13	334
Ornithocercus spp.	268		67	6	9				16	65		5		65		9
Protoperidinium spp.	581	54	45	12										408		9
Pyrophacus sp.					22			5	11	43						
Schuetiella mitra																9
Others																
Dictyochophyceae	45		23		9		14	5		22	5			83		
Ciliate										4				9		
Radiolarian					5					30	5			65		26
Foraminiferan	328		23	96	9					39				56		
Total abundance	925376	4405757	2140316	157925	2161	292	46	60	1006	9059	26587	557	1200	13223	493	2115

Based mainly on biometric parameters (length and width of a cell) as well as some phenotypic features (presence/absence of calyptra and shape of apical cell), five species of *Trichodesmium* (Komárek and Anagnostidis, 2005) were identified and generally co-occurred across the sampling stations (Figs. 02 and 03).

T. clevei (J. Schmidt) Anagnostidis et Komárek 1988 was found as free-swimming or in fascicles containing 20-30 trichomes, cylindrical and slightly coiled. Most of the trichomes were blue-green, but found also in dark reddish hue. Cell diameter ranged from 5.9 to 7 µm, longer than wide, with apical cell rounded and without calyptra. Their occurrence was already registered in Gulf of Siam and Australia, but it is the first time that species is described for Brazilian waters.

T. erythraeum (Ehrenberg ex Gomont, 1892) was found as free-floating or in colonies, purple-red trichomes, constricted at the cross-walls and gradually attenuated at the ends. Cells were 6-11 µm long, with the apical cell generally containing a calyptra. It is well-known as forming blooms in tropical and subtropical oceans, including the Atlantic, and was already described in the south-southeastern Brazilian shelf slope (Siqueira *et al*., 2006; Carvalho *et al.*, 2008).

T. hildebrandtii (Gomont, 1892) was easily distinguished among the other species because of its diameter ranging from 13 to 22 µm, with shorter cells. It was found as single trichomes or in colonies with a dark-reddish color, with attenuated cells at the ends and apical cells containing or not a calyptra. It can be found in warm seas all over the world, but has not been described in Brazilian waters before.

T. radians (Wille) Golubié 1977 was clearly characterized as wavy trichomes, occurring alone or in star-shaped colonies. Their cells were always blue-green with large gas vesicles and diameter with a range of 6 to 9 µm, longer than wide. Apical cells were rounded and without calyptra. It is largely distributed around the world’s oceans, but this is the first report of its occurrence in Brazilian waters.

T. thiebautii (Gomot ex Gomont 1892) was found as free-swimming and forming colonies partly rope-like contorted or radially-arranged. Cells were nearly isodiametric, not constricted at the cell walls, with apical cells rounded. This species has a wide range of distribution. This is the first occurrence reported for the south-southeastern Brazilian shelf slope.

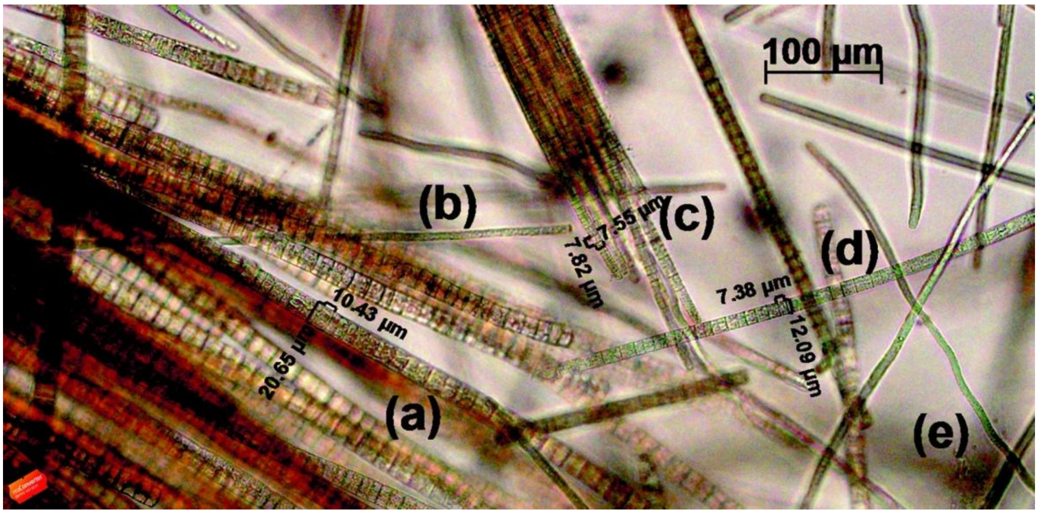

Figure 02: Five species of the genus *Trichodesmium* co-occurring in a sample (×200 of magnification): *T. hildebrandtii* (a), *T. clevei* (b), *T. thiebautii* (c), *T. erythraeum* (d) and *T. radians* (e). There are annotated the length and width of cells only on the trichomes of *T. hildebrandtii*, *T. thiebautii* and *T. erythraeum.*

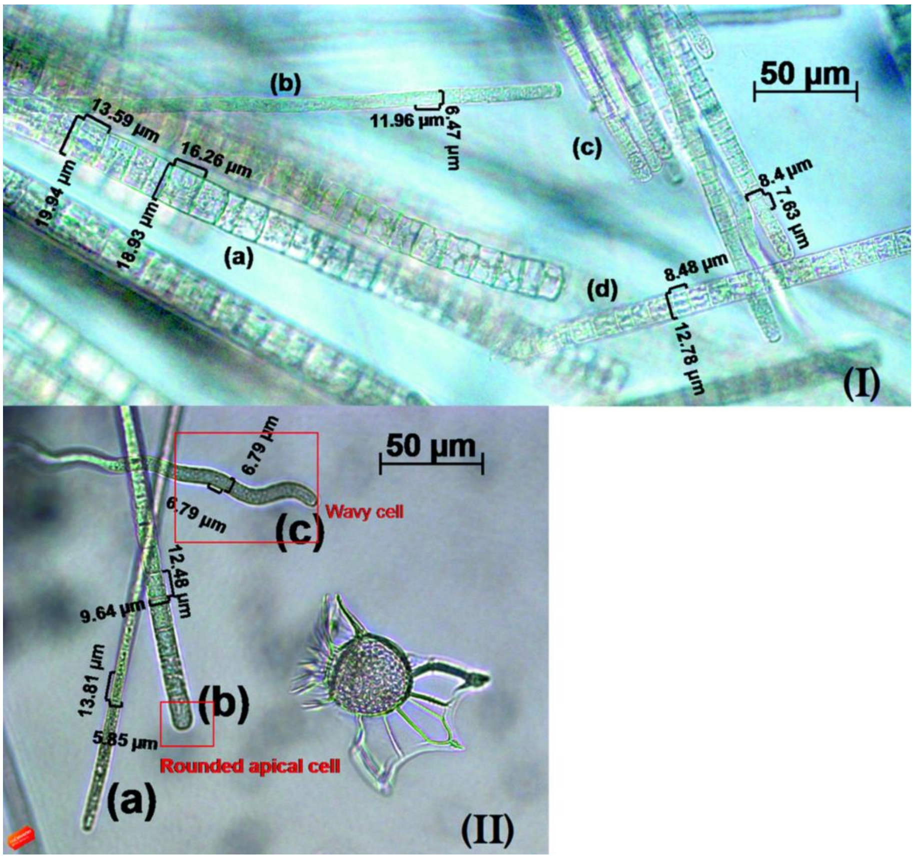

Figure 03: Details of the *Trichodesmium* trichomes co-occuring in other samples (×400 of magnification). In the first image (I) *T. hildebrandtii* (a), *T. clevei* (b), *T. thiebautii* (c) and *T. erythraeum* (d) with their cell measurements shown. The second image (II) shows cell measurements of *T. clevei* (a), and a rounded apical cell of *T. thiebautii* (b) and the wavy trichome of *T. radians* (c).

The ordination diagram derived from the nMDS displayed the sampling stations due to the number of trichomes of *Trichodesmium*, i.e., a group A composed of the stations with >8,000 trichomes L^{-1} and a group B with <2,500 trichomes L^{-1} each sampling station (Fig. 4). These groups were significantly different (ANOSIM, $r^2 = 0.48$, $p < 0.0001$). Results of the CCA analysis (Fig. 5) were used to investigate the association of microplankton species or higher taxonomic categories to environmental variables. A Monte-Carlo test of the F-ratio, applied during the CCA analysis, showed that the five environmental variables (temperature, salinity, MDL, wind speed and water mass types) plus the covariate latitude contributed significantly to the observed spatial distribution of microplankton groups ($p<0.01$). In fact, environmental variability explained 44% of the spatial variability in microphytoplankton community composition and structure and the first two significant canonical roots cumulatively explained 71.5% of the observed variance.

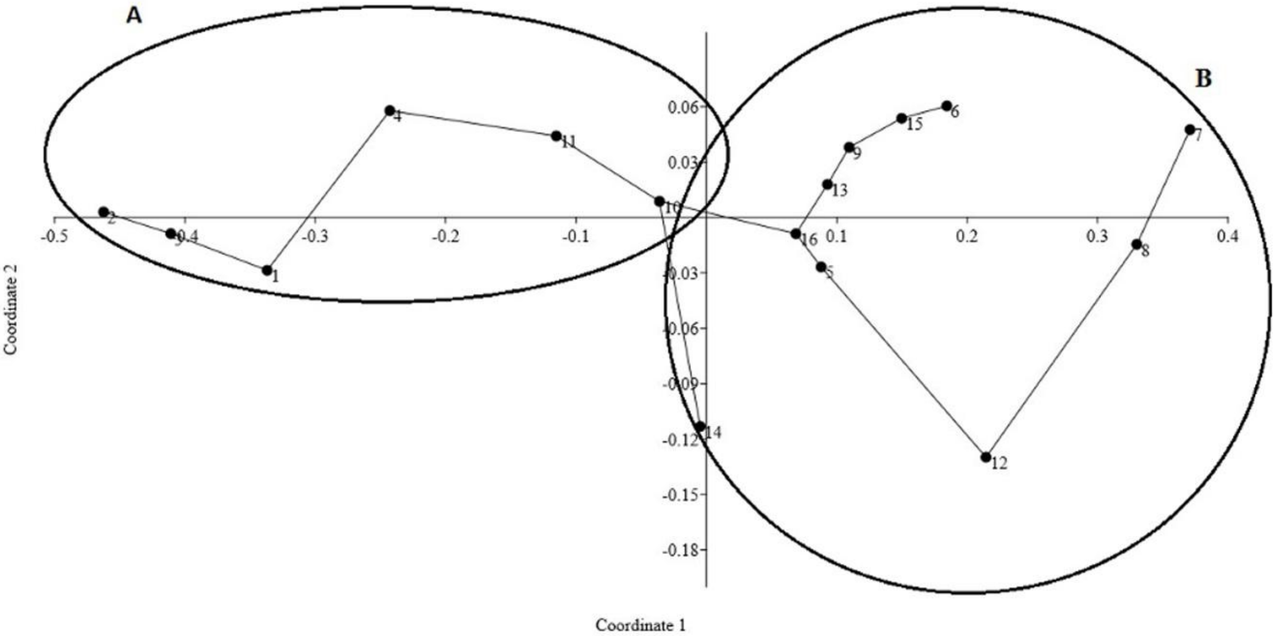

Figure 04: nMDS ordination diagram, dividing the stations into two distinctive groups according to *Trichodesmium* abundance. Group A, where the stations presented > 8,000 trichomes L^{-1} and Group B, containing the stations with < 2,500 trichomes L^{-1}. The ANOSIM test revealed statistical differences between the two groups: *p*-value < 0.01, $R^2 = 0.58$ and Stress = 0.04 (less than 0.5).

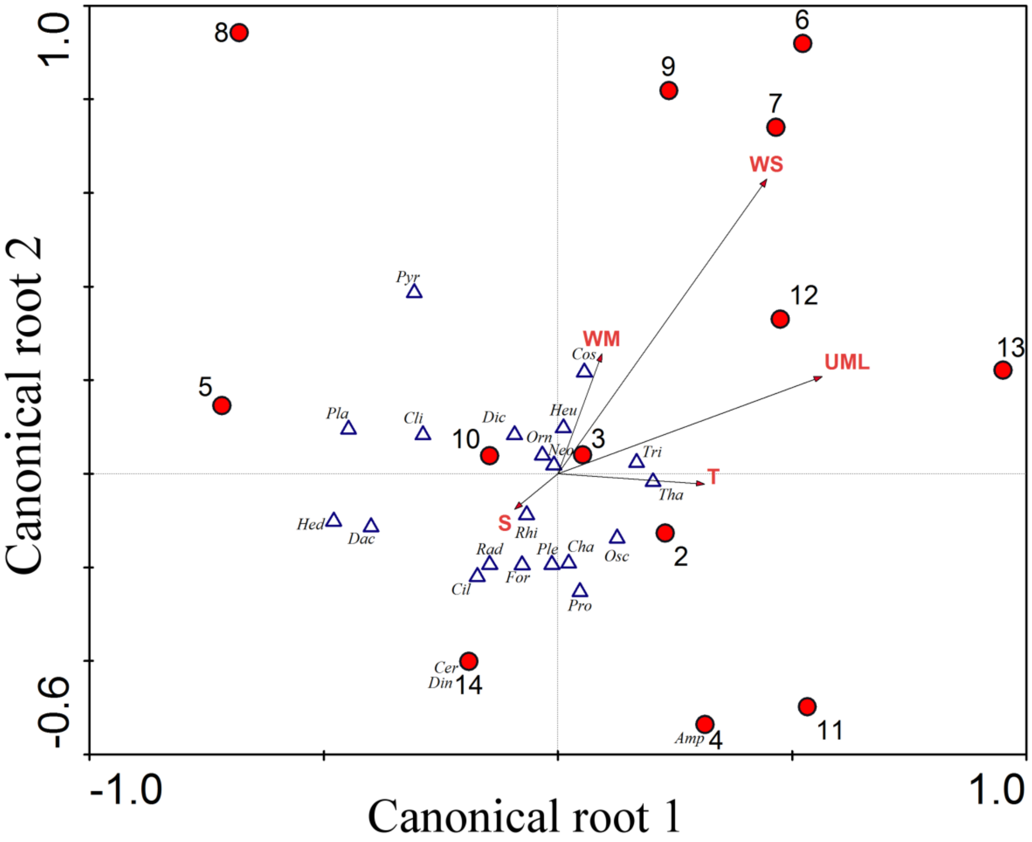

Figure 05: Canonical Correspondence Analysis showing the relationship between the abiotic variables and surface microplankton abundance. The first two canonical roots represent 71.5% of microplankton groups versus environment relationships (1st root explains 40.3% and 2nd root explains 31.2%).The red circles represent the stations, numbered from 1 to 16. For the abiotic variables, represented by arrows: MDL = Upper Mixed Layer, WS = Wind Speed, WM = Water Mass, S = Salinity, T= Temperature. For the taxa, represented by triangles: Tri = *Trichodesmium spp*., Osc = Other Oscillatoriales, Dia = *Diatoms*, Cha = *Chaetoceros spp*, Cli = *Climacodium frauenfeldianum*, Cos = *Coscinodiscineae*, Dac = *Dactyliosolen sp*., Heu = *Hemiaulus sp*., Hed = *Hemidiscus sp*., Pla = *Planktoniella sol*, Ple = *Pleurosigma sp*., Rhi = *Rhizosolenia spp*., Tha = *Thalassionema spp*., Amp = *Amphisolenia bidentata*, Cer = *Ceratocorys horrida*, Din = *Dinophysis spp*., Neo = *Neoceratium spp*., Orn = *Ornithocercus spp*., Pro = *Protoperidinium spp*., Pyr = *Pyrophacus sp*., Sch = *Schuetiella mitra*, Dic = *Dictyochophyceae*, Cil = *Ciliate*, Rad = *Radiolarian*, For = *Foraminiferan*.

4. DISCUSSION

All of the samples were collected during good meteorological and sea conditions, with low to moderate wind speed (Villareal and Carpenter, 2003; Agawin *et al*., 2013). The four southernmost stations were under influence of two distinctive water masses: the shelf #16

(127 m of local depth) and offshore #13 were both affected by PPW; and stations #14 and #15, far offshore, were affected by TW (see Table I) (Möller *et al*., 2008). As the expeditions were carried out on autumn, the colder and less saline PPW, from *Río de La Plata* waters, formed a tongue and reached these two former stations (Möller e*t al*., 2008). For the other stations, TW was the most typical water mass, but STSW also appeared, denoting mixing of waters in that region.

The most noticeable environmental feature was the *Trichodesmium* aggregations were clearly observed at station #2, accumulating at the first centimeter of water surface. Accompanying a virtually complete predominance of trichomes, there was a distinct accumulation of objects as feathers, wood and plastic. These findings suggest that the region was under a Langmuir cell condition, with *Trichodesmium* spp. being accumulated in the convergence part, as previously reported in other studies (Evans and Taylor, 1980). The ability of keeping at these first centimeters of water surface, even when forced to sink, could be explained by the presence of gas vesicles inside the cells (Villareal and Carpenter, 2003).

The highest densities of *Trichodesmium*, when their aggregations were macroscopically visible, could be associated with winds producing <8 knots of speed. This condition has been frequently reported in other studies and became a classical oceanic feature for the observations of *Trichodesmium* (Villareal and Carpenter, 2003; LaRoche and Breitbarth, 2005). This kind of association between *Trichodesmium* and some environmental driving forces, including the wind speed, was denoted in our CCA's ordination diagram (Fig. 5). Furthermore, two groups of stations related to the concentration of *Trichodesmium* were confirmed through the nMDS and ANOSIM analyses (Fig. 4). When the wind speed started to slow and blew steadily over the sea surface, the trichomes and colonies could easily form aggregations in the windrows, as observed in our field sampling. Thus, in conjunction with a shallow MLD in the station #02 (Table I), there might have been facilitated a kind of agglutination of colonies and isolated trichomes, as well as other organic and inorganic particles.

Since *Trichodesmium* is known to present a low growth rate, related to the high costs of acquiring N_2 (LaRoche and Breitbarth, 2005), it is unlikely that dense aggregations observed in some stations can be characterized as bloom events. Thus, a *Trichodesmium*-dominated microplankton assemblage, or a great part of *Trichodesmium* spp. patches, could be simply associated to the convergence sites/windrows of Langmuir cells. Additionally, in spite of the capacity of doing vertical migration, which facilitates an organism mobility (Villareal and Carpenter, 2003), the presence of MLD generally <40 m in the majority of stations with higher numbers of *Trichodesmium* spp. (seen in group A, Fig. 4) might have contributed to this level of aggregations across our 30-m water column sampled.

At the same time, low concentrations of *Trichodesmium* spp. $<8 \times 10^3$ trichomes L^{-1} (related to group B; see Fig. 4) could be considered a transient condition, before some MLD stabilization and wind speed <8 knots were appropriated for *Trichodesmium* aggregations. On the other hand, under an 80-m MLD and >4.6-knot winds, the aggregations of *Trichodesmium* seemed to decrease drastically from $\sim 10^5$ trichomes L^{-1} at #4 to almost none of 13 trichomes L^{-1} at #8 (see Tables I and II). Probably, these conditions might have spread out the aggregations of *Trichodesmium* and, by chance, our vertical hauls of 30 m did not account for these organisms in a MLD of 80m. The importance of MDL on the composition and structure of microplankton organisms was also highlighted in the CCA's ordination diagram (Fig. 5). The levels of nutrients in our sampling area were low (data not shown), and they helped explain the low densities of microphytoplankton groups co-occurring with *Trichodesmium* spp. (Table II). There were some typical representatives of oligotrophic oceans such as the dinoflagellates *Neoceratium* spp. that often appeared through the sampling region, *Dinophysis* spp. and *Ornithocercus* spp., and the obligate heterotroph *Protoperidinium* spp. These kinds of organisms have been commonly classified as the major consumers of the phytoplankton biomass, as *Protoperidinium* spp., especially in oligotrophic oceans (Sherr and Sherr, 2007). Some of them can also associate with picocyanobacteria as the case of *Ornithocercus* spp. (Madhu *et al.*, 2013). Similarly, an association between diatoms (*Rhizosolenia* spp.) + the diazotrophic cyanobacterium *Richelia intracellularis* were observed in the samples, as previously seen in other oceans (Madhu *et al.*, 2013), highlighting the poor nitrogen condition in the study area.

5. CONCLUDING REMARKS

In short, the south-southeastern Brazilian shelf slope shows some environmental conditions as Langmuir cell formation, oligotrophic waters and calm weather, ideal to the growth and development of *Trichodesmium* spp. during autumn seasons, either in aggregations or in co-occurrence with other microplankton organisms. Despite the lack of studies on

Trichodesmium ecology and biogeography, the Brazilian coast is given a vast occurrence of the genus. Five species of *Trichodesmium* (*T. clevei*, *T. erythraeum*, *T. hildebrandtii*, *T. radians* and *T. thiebautii*) were identified, but have never been properly reported in the South Atlantic Ocean. Thus, this report suggests the importance of further studies on the biogeography and ecology of marine cyanobacteria, especially the relationships between the environmental variables and plankton composition. Some dinoflagellates and relatively large cells of diatoms were also important in non-*Trichodesmium*-dominated assemblages, showing a considerable species abundance for the study area. High abundances of *Trichodesmium* spp. are undoubtedly of great importance as CO_2 sinking of particulate matter and as substrate and food item to zooplanktonic organisms and other associated phytoplankton for this region.

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CAPÍTULO III

Distribution of *Trichodesmium* and association with iron-rich dust on the southwest Atlantic ocean

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ABSTRACT

Trichodesmium populations are largely distributed in oligotrophic oceans because of their capacity to fix atmospheric nitrogen. However, these organisms require additional amounts of elements, such as iron and phosphorus, to sustain their activity. This study collected biological, physical and chemical samples from a large area of the Brazilian shelf slope, and the horizontal and vertical distributions of *Trichodesmium* are presented in this paper. We show that the genus is spread throughout the study area, most concentrated at the surface. The highest concentrations of trichomes (> 157,000 trichomes L^{-1}) were located at the northernmost stations. The area had low concentrations of phosphate (< 1 µM) and total dissolved nitrogen (< 1.5 µM), which characterizes the region as oligotrophic. In contrast, we found alternative sources of mineralized elements as grains inside the colonies. These sources were analyzed by scanning electron microscopy coupled with EDS X-ray analysis, which revealed high concentrations of iron (10-20% of the total sediment composition) and other elements, including phosphorus and zinc. The stations also seemed to be influenced by the La Plata River input of sediments due to the presence of titanium. The trichomes were in association with other bacteria that could be involved in dissolution processes, increasing the availability of important nutrients.

Keywords: Brazilian shelf slope; Cyanobacteria; Electron Microscopy; Iron; Phytoplankton; *Trichodesmium*.

1. INTRODUCTION

The genus *Trichodesmium* (Ehrenberg, 1830) is member of the order Oscillatoriales, which includes marine species. The cyanobacterium is found in tropical and subtropical oceans around the world, from coastal zones to large-scale gyres (Capone *et al.*, 2005). Their cells are organized as trichomes that can form colonies with fusiform (tufts) or spherical (puffs) shapes. They are well-known to fix atmospheric nitrogen even without the presence of specialized cells, called heterocysts, and continue this activity while performing photosynthesis (Carpenter and Price, 1977).

The ability to fix N_2 as an evolutionary adaptation to live in oligotrophic areas made *Trichodesmium* a dominant group in these regions. The genus commonly forms large aggregations that contribute biomass and new nitrogen to the oceans (Karl *et al.*, 2002). However, the fixation process requires additional energy and nutrients, such as iron and phosphorus (Sañudo-Wilhemy *et al.*, 2001), so their growth rate is low compared to other phytoplankton organisms (LaRoche and Breitbarth, 2005).

Trichodesmium is especially important in oceanic regions that are poor in nitrogen but have large inputs of iron. These are suitable places to grow without competition for resources. One of those areas, the eastern North Atlantic, is constantly fertilized by iron-rich dust from the Sahara Desert. The genus has established permanent populations in this region and often forms large aggregations (Tyrrell *et al.*, 2003; Agawin *et al.*, 2013; Fernández e*t al.*, 2013). However, only the dissolved and organic forms of iron are available to phytoplankton, and the mineralized iron contained in the sediments cannot be directly absorbed. Thus, *Trichodesmium* developed a strategy to transform the iron from dust into its dissolved forms. This behavior was observed during an experiment using manipulated sediments under controlled conditions. The cells were able to trap the grains and transport them to the interior of the colony, which is more anoxic and reduced and is suitable for iron dissolution (Rubin *et al.*, 2011). An alternative way to maximize iron acquisition is by associating with heterotrophic bacteria that are also able to dissolve the element (Roe *et al.*, 2012).

Phosphorus is another important nutrient for *Trichodesmium* and also limits its growth. The genus can supplement the incorporation of dissolved inorganic phosphorus (DIP) with dissolved organic phosphorus (DOP) due to alkaline phosphatase (Sohm and Capone 2006).

In some regions, phosphorus is restricted to deeper waters. In this case, the gas vesicles in this genus evolved to numerous tiny and thick structures that help the entire colony to migrate vertically and reach different depths (Villareal and Carpenter, 2003).

Some trace elements, such as nickel, vanadium, molybdenum and zinc, are also important for *Trichodesmium* growth. These elements are related to nitrogen fixation, enzyme composition and cellular protection against oxidative stress (Nuester *et al.*, 2012; Ho, 2013). However, their biochemical role requires additional investigation.

The importance of *Trichodesmium* to the biogeochemical cycles has led to this organism being studied in detail around the world. However, only a few studies have focused on the South Atlantic ocean, so there is a lack of information about the genus' distribution, taxa and ecology in this region. Many unpublished data suggest that the organism occurs along the coast, especially in the northeast and southeast regions (Carvalho *et al.*, 2008; Monteiro *et al.*, 2012).

The objective of this study was to characterize *Trichodesmium* aggregations over a large area of the south-southeast Brazilian shelf slope. The study was initially focused on the vertical distribution of the genus in the water column, their occurrence as single trichomes or colonies and the availability of nutrients in the environment. However, observations under inverted microscopy noted the presence of dust inside the colonies, so a detailed analysis was performed to verify the dust content. Thus, this study coupled the vertical and surface analyses of *Trichodesmium* in the study area with analyses of the dissolved nutrients and geochemical analysis of the dust. Finally, the study identifies the main source of iron-rich dust to the southernmost *Trichodesmium* populations. The images in this study were taken by inverted and electron microscopy.

2. MATERIAL AND METHODS

2.1. Study area and physical parameters

The sampling area encompassed the Brazilian shelf slope between 24º and 35º S during two consecutives autumns. Stations 01 to 04 were sampled on 04–11 June 2013, and stations 05 to 16 were sampled on 10–20 May 2014 on board the R/V *Atlântico Sul* (Universidade Federal do Rio Grande, FURG – Brazil) as part of the “TALUDE” project. The temperature and salinity were obtained with a SeaBird® 911+ CTD (conductivity+ temperature+ depth) sensor. These data were used to classify the surface water masses (Möller *et al.*, 2008) and calculate the mixed layer depth (MLD), which was determined from vertical density profiles ($\partial\rho/\partial z$); the upper 5 meters were excluded due to noisy data (Table I). The water masses are represented by stars in the map of the study area (Figure 1). The image was constructed using the free software ODV (Ocean Data View V.4) and GIMP (2.8.10).

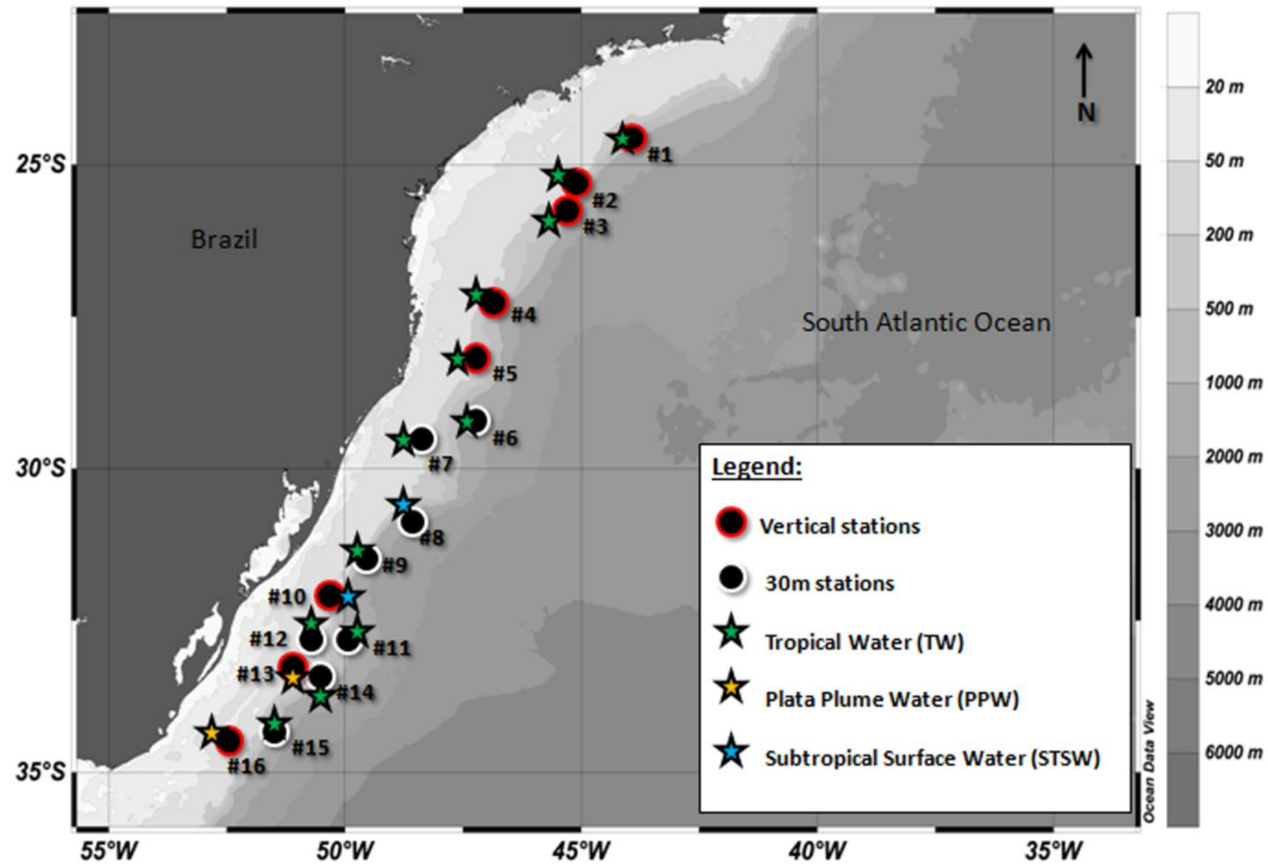

Figure 1: Map of the study area on the south-southeastern Brazilian shelf slope showing the sampling stations and surface water masses.

Table I: Sampling coordinates with the dates that the samples were collected, local depth and mixed layer depth (MLD).

Station	Lat (S)	Lon (W)	Date	Local depth (m)	MLD (m)
#1	24°16.6'	43°22.2'	06/11/2013	692	45
#2	25°07.6'	44°51.4'	06/08/2013	190	17
#3	25°48.8'	45°04.2'	06/07/2013	1287	40
#4	27°6.4'	46°24.7'	06/04/2013	830	80
#5	28°6.6'	48°00.0'	05/20/2014	138	14
#6	29°22.9'	47°08.8'	05/19/2014	1876	35
#7	29°26.0'	48°24.8'	05/18/2014	140	35
#8	30°45.6'	48°24'	05/17/2014	1505	80
#9	31°39.3'	49°29.8'	05/15/2014	1816	43
#10	31°56.5'	49°57.2'	05/14/2014	680	18
#11	32°12.9'	49°41.1'	05/14/2014	1870	40
#12	32°18.2'	50°06.6'	05/14/2014	806	40
#13	32°33.6'	50°16.9'	05/13/2014	575	47
#14	32°55.3'	50°17.6'	05/11/2014	676	15
#15	33°04.0'	50°05.0'	05/12/2014	1166	20
#16	33°40.4'	51°23.5'	05/10/2014	127	18

2.3. Nutrients

Water samples for nutrient analysis were collected using Niskin bottles that were attached to the ship's cable. They were coupled with messengers to collect punctual samples from a depth of 15 m to a maximum depth of 140 m depending on the local water depth. The stations that only had superficial biological samples were sampled at 15 m. The water samples were filtered with a cellulose filter (45 µM) and frozen in falcon tubes.

The nutrient analyses were performed using the following methods: total ammoniacal nitrogen (NH_4^+) (Koroleff, 1972); nitrate (NO_3^-) (Aminot and Chaussepied, 1983); nitrite (NO_2^-); phosphate (PO_4^{-2}) and silicate (Si) (Aminot and Chaussepied, 1983). All of the nutrients were analyzed by spectrophotometer using a manual for chemical analyses with the methods (Baumgarten e*t al.*, 2010). The nutrients NH_4^+, NO_3^- and NO_2^- were then summed and graphically represented as dissolved inorganic nitrogen (DIN).

2.2. Biological parameters

The biological samples were collected using a ring plankton net (30 cm mouth diameter, 50 µm mesh size) in previously established vertical extracts of the water column depending on the local depth. These samples were then preserved in 4% buffered formaldehyde.

During the first cruise, vertical samples of the water extracts at different depths were performed only when *Trichodesmium* aggregations were visible by the naked eye. On the second cruise, two different types of samples were collected: vertical extracts at different depths and surface extracts (1 vertical extract from 0-30 m depth), which were collected when the organisms were both visible by the naked eye and when there were no clearly visible aggregations. The vertical samples were collected to a maximum depth of 200 m. Sampling was usually conducted under suitable weather conditions, such as clear sky and calm seas.

The *Trichodesmium* abundance was quantified using an inverted microscope (Zeiss Axiovert A1) coupled with a camera for image acquisition (AxioCam MRc) and optional UV light (UV fs09 ph1-04). The images were manipulated using the free software AxioVs40x64 (V. 4.9 1.0) and GIMP. For the identification (Komárek and Anagnostidis, 2005) and abundance of *Trichodesmium*, there were used sedimentation chambers (Utermöhl, 1958). Because the organism was commonly found as colonies, these life forms were counted separately. The total abundance of trichomes was estimated by multiplying the average number of trichomes in one colony by the number of colonies and adding the number of single trichomes (Carpenter, 1983). The concetrations of *Trichodesmium* was shown in three different ways: single trichomes per liter, tufts and puffs (colonies) per liter and total trichomes per liter. Finally, the stations where the samples were collected in extracts were represented in abundance per depth.

2.4. Biogeochemical analysis

During the inverted microscopy analysis, many types of sediment were observed to be attached to the colonies, so scanning electron microscopy (SEM) (JEOL 6060 LV) coupled with energy-dispersive X-ray spectroscopy (EDS) was used at a fixed voltage of 15 kV. This technique provided qualitative information about the geochemical composition of the sediments by giving the percentage of each element of the total. For this analysis, representative stations were selected and prepared in replicate according to the number of colonies, location (south, central and north samples) and water mass influence. The sediments were randomly analyzed to create a database with *n* graphs. These results were used to calculate the mean contribution of each element to the station.

The same biological samples that were used for inverted microscopy were used for the SEM analysis, but they followed a special preparation process so they could be properly visualized and qualified. Initially, several milliliters of every replicate were filtered using a nucleopore 5 µM membrane until an ideal concentration was reached. The filter was then covered by another filter, washed three times with Milli-Q water to remove the marine salts and successively washed with increasing concentrations of an ethanol solution (5%, 25%, 50%, 75%, 95% and 100%) for dehydration (Association of European Marine Biological Laboratories - ASSEMBLE).

After concentrating the *Trichodesmium* cells in the membranes, they were transported to the CEME-Sul (Centro de Microscopia Eletrônica da Zona Sul) at the same university that the experiments were performed (Universidade Federal do Rio Grande – FURG) for CO_2 critical point drying (CPD) preparation (Tousimis Autosamdri®, model 815). This method substitutes the sample's ethanol content with carbonic gas by exposing it to a controlled temperature and pressure. The process dehydrates and avoids any cell surface tension and provides a better and more realistic visualization. The last preparation step consisted of a metallic evolvement of the samples with gold (Denton Vacuum, Dorsk V). Finally, the membranes were positioned on stubs for posterior observation by SEM.

2.5. Statistics

Multivariate analyses were conducted using the surface samples (0-30 m extracts) to verify similarities in the trichome organizations (tufts, puffs or single trichomes) between the stations. The percentage contribution, in numbers of trichomes to the total number of trichomes in the sample, was calculated by Similarity Percentage Analysis (SIMPER) (Clarke, 1993). A statistical non-parametric multidimensional scaling (nMDS) analysis was then conducted, using a dissimilarity matrix, based on the non-metric *Bray-Curtis* index (Bray and Curtis 1957). This was used to verify any spatial pattern in the proportions of trichomes/tufts/puffs between the stations. An analysis of similarities (ANOSIM) was also applied (Clarke, 1993) to verify possible differences among the groups based on their Bonferroni–corrected *p*-values. Additionally, a cluster analysis based on Ward's method was used as another way to represent the similarities between the stations (Everitt *et al*., 2001). These analyses were performed using the free software Past (v. 1.81) (Hammer *et al.*, 2008).

The geochemical analysis of the sediments using SEM coupled with EDS resulted in percentages of the elements for each grain in the sample. These percentages were organized for each station and resulted in a mean of every element. The results were then presented as bar graphs in increasing order to show the total contribution of elements using the free software RStudio (V. 0.98.1079).

3. RESULTS

3.1. Area of study, *Trichodesmium* abundance and distribution of nutrients

The map of the study area shows the surface water masses at each station (Figure 1), which include tropical water (TW), Plata plume water (PPW) and subtropical surface water (STSW). More details about the water masses and mixed layer depths are given in Bif, Souza and Yunes (submitted).

The biological analysis showed a clear distinction between the tuft-shaped and puff-shaped colonies; however, the genus was mostly present as single trichomes at the stations. These trichomes had different contributions to the total number of trichomes per liter at each station. Table II provides information about the surface concentrations of *Trichodesmium*, which are categorized as colonies and single trichomes, and the nutrient concentrations collected at a depth of 15 m. The phosphate, dissolved inorganic nitrogen and silicate concentrations in the samples were low at all of the stations and are also shown in Table II. N:P ratios were lower than the Redfield ratio (16), except at station #4.

Table II: Abundance of trichomes/colonies per liter (0-30 m depth) and nutrient concentrations for each station.

	***Trichodesmium* abundance (trichomes/colonies L^{-1})**				**Nutrient concentration (μM)**			
Station	**Single Trichomes**	**Tufts**	**Puffs**	**Total**	**PO$_4^{-2}$**	**DIN**	**Si**	**N:P**
#01	47151	1758	2598	918351	0.1	0.668		6.68
#02	52325	15380	6385	4405325	0.12	1.16		9.67
#03	213731	1444	8185	2139531	0.13	1.05		8.08
#04	9816	84	654	157416	0.06	0.97		16.17
#05	1180	0	0	1180	0	0	12.15	0
#06	63	1	0	263	0	0.65	3.93	0
#07	4	0	0	4	0.26	1.45	18.69	5.58
#08	13	0	0	13	0	0.028	1.31	0
#09	336	2	1	936	0	0.59	0.75	0
#10	1385	24	10	8185	0	0.12	0	0
#11	967	125	3	26567	0	0	2.06	0
#12	26	0	0	26	0	0	3.45	0
#13	1500	2	3	2500	0.21	0.7	19.8	3.4
#14	338	1	3	1138	0.19	0.2	4.86	1.05
#15	61	2	0	461	0.32	0	3.55	0
#16	144	0	0	144	0.32	0	3.93	0

Statistical analyses were performed using the surface concentrations of *Trichodesmium* divided as colonies and single trichomes as criteria. The nMDS analysis organized the stations as three distinct groups, A, B and C (Figure 02), based on the contributions of puffs, tufts and single trichomes to the total abundance of *Trichodesmium*. The ANOSIM analysis found significant differences between the three groups based on the Bonferroni-corrected *p*-values, which were < 0.05 for all combinations. A Simper analysis was then carried out to calculate the average dissimilarities between the groups. The results showed 61.9% dissimilarity for puff *versus* tuft–dominated groups, 67.1% dissimilarity for puff *versus* single trichome–dominated and 70.3% dissimilarity for tuft *versus* single trichomes–dominated.

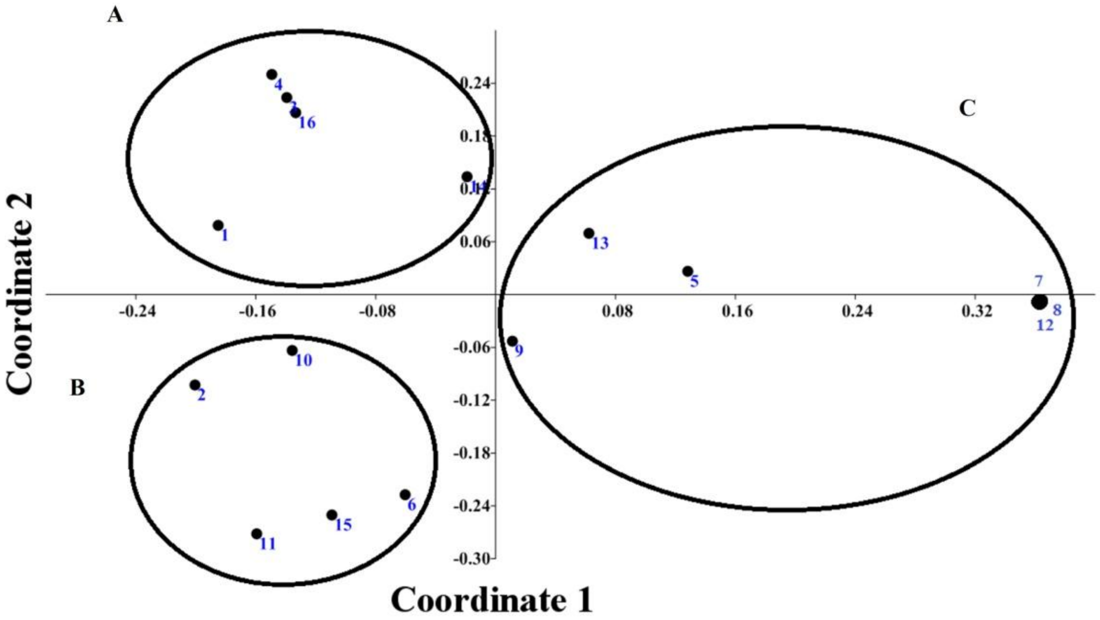

Figure 02: nMDS ordination diagram, which divides the stations into three distinct groups according to the *Trichodesmium* organization: Group A, where puffs contribute most of the total abundance, Group B, where tufts dominate, and group C, where single trichomes account for most of the total abundance. The ANOSIM test revealed significant differences between the groups: R^2 = 0.69 and stress = 0.045 (less than 0.5).

A cluster technique using Ward's method was also used as an alternative to show the group distribution. Instead of groups A, B and C, the groups are distinguished in the graph by different colors (red, green and pink). The analysis is based on the distance of similarity between the groups and between each station (Figure 03).

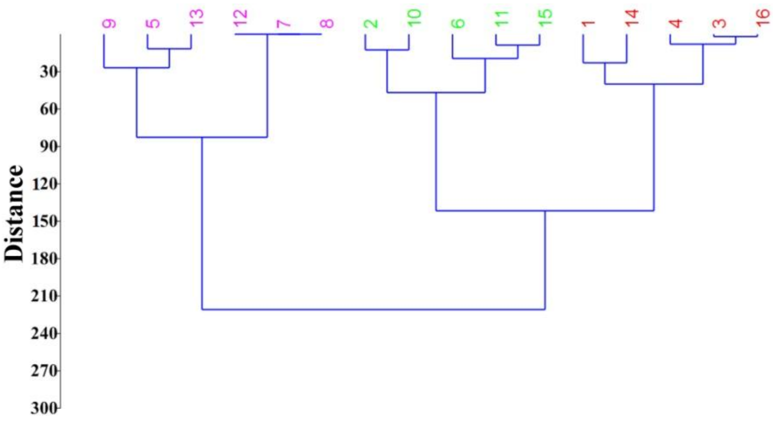

Figure 03: Dendrogram grouping the 16 stations into three groups: Group A in red, Group B in light green and Group C in pink. The numbers of the stations are the same as those shown in the index.

3.2. Vertical distributions of trichomes and nutrients

The distributions of total trichomes (tufts + puffs + single trichomes as trichomes L^{-1}) with depth from the biological samples that were collected in the water extracts are shown in Figure 04. The maximum MLD depths (Table I) are shown by the red lines. The distributions of tufts, puffs and single trichomes are also provided as separate graphs (see supporting materials). The highests abundances of *Trichodesmium* were found in the surface extracts, above 60-80 meters, as shown in the graphs.

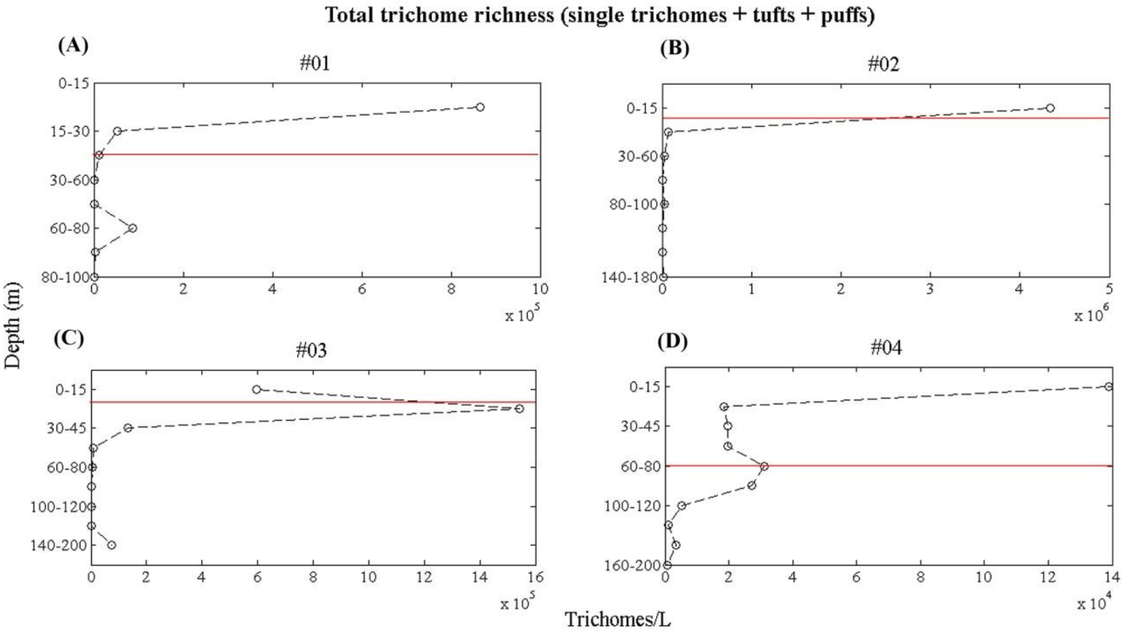
Total trichome richness (single trichomes + tufts + puffs)

Figure 04: Vertical distribution of *Trichodesmium* (total number of trichomes L^{-1}) in the stations (A to F). The red line indicates the maximum MLD depth.

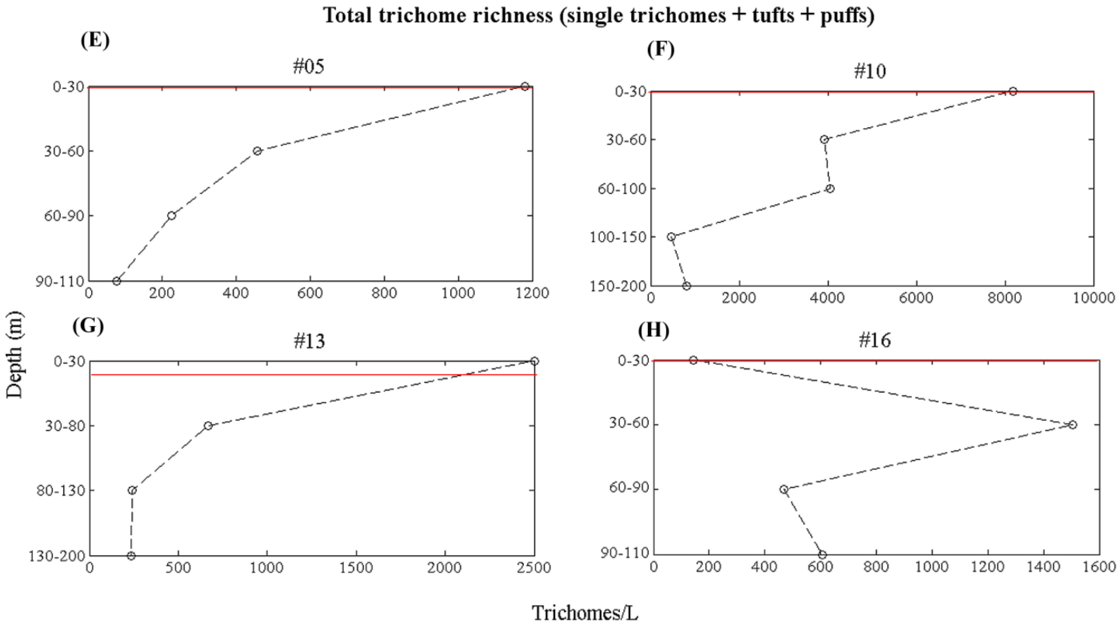

Figure 04 continued.

At the vertical stations (#05, #10, #13 and #16), punctual samples of water were collected for nutrient analysis (phosphate, DIN and silicate) to a maximum depth of 140 m (Figure 05). The concentrations of nutrients were higher at the minimum and maximum collected depth. Although, the low concentrations of inorganic nutrients characterize the environment as oligotrophic based on the typical concentrations of this type of water (Baumgarten e*t al.*, 2010).

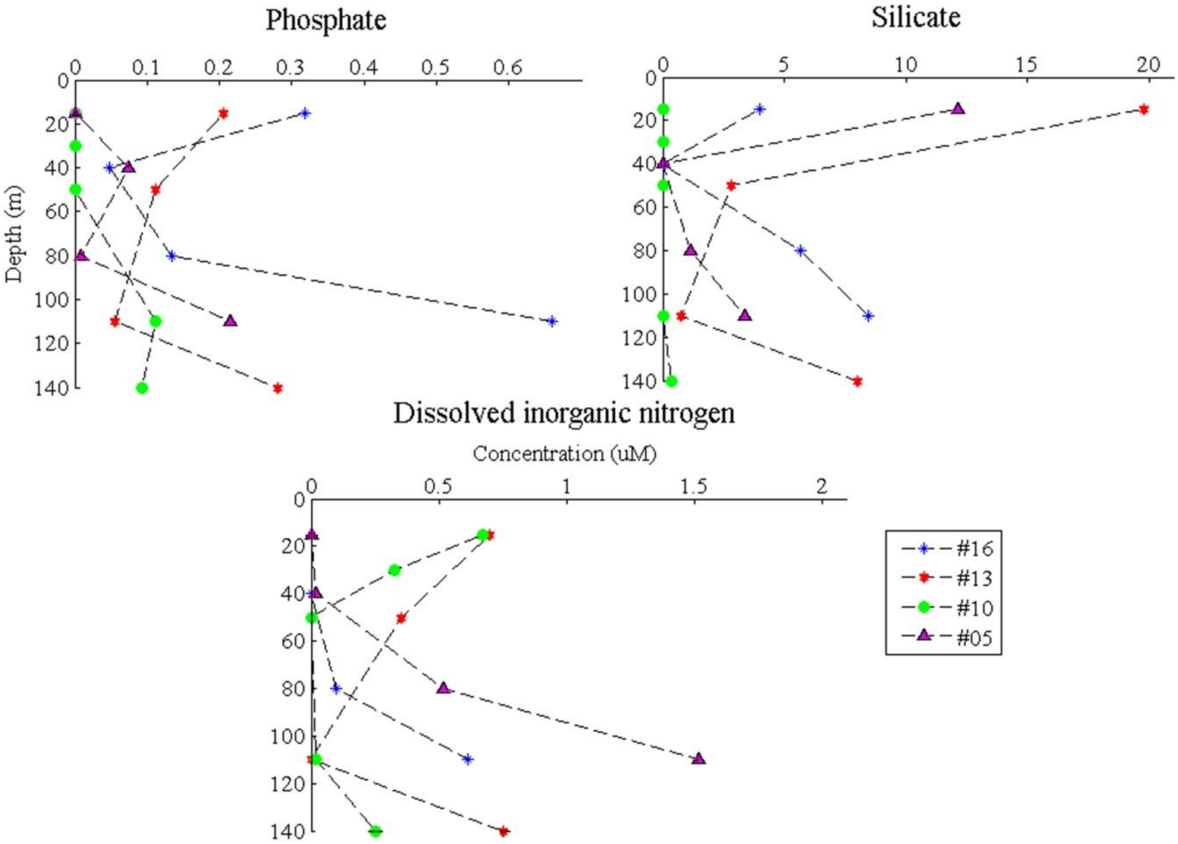

Figure 05: Vertical distributions of the phosphate (A), silicate (B) and dissolved inorganic nitrogen (DIN) (C) concentrations for stations #05, #10, #13 and #16.

3.3. Microscopy and sediment analysis

The inverted microscopy coupled with the Axio camera provided images of the *Trichodesmium* colonies that contain sediments. The puff-shaped colonies appeared to concentrate the dust inside the colony, while the tuft-shaped colonies contained sediments all over the trichomes (Figure 06). This content was observed in all of the stations in which the colonies were present and had different colors and shapes. Additionally, the dust appeared to evolve in the organisms’ mucilage that was preserved even after the cells were fixed in formaldehyde.

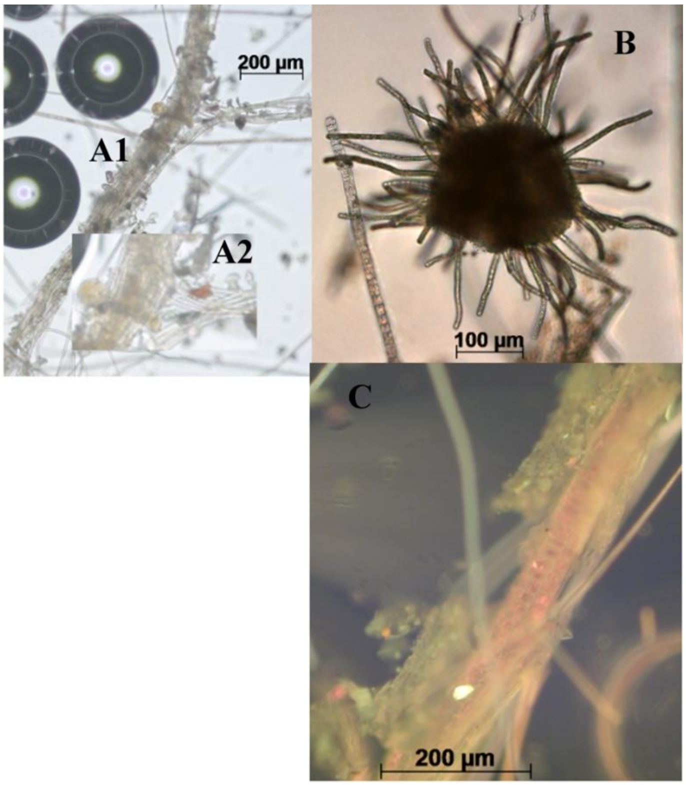

Figure 06: Images taken by inverted microscopy (Zeiss Axiovert A1) coupled with a camera (AxioCam MRc). Image A1 (10x magnification) shows a tuft-shaped colony with sediments of different colors (A2 is an enlarged view of A1). Image B (10x magnification) is a puff-shaped colony that contains sediments in its interior. Image C (20x magnification) was taken under UV light (UV fs09 ph1-04) and shows the different reflectance of a dust-rich trichome.

Samples that contained colonies were analyzed by electron microscopy (SEM) coupled with EDS analysis to verify the dust composition. This analysis identified the elements and their percentages in each selected area under the microscope. The data were then organized by the means of the elements for each station (Figure 07). The stations were chosen because of their distinguishing characteristics: #02 and #03 were colony-rich northern samples that were well-suited for dust observations, #09 and #10 were located in an intermediate area of study, and #16, the southernmost station, was influenced by the PPW water mass. The graphs show the different proportions of the elements at each station. Iron had the greatest concentrations. Other elements that were present in significant concentrations include rubidium, zinc and potassium. Curiously, the southern stations #09 and #16 contained significant concentrations of titanium.

In the northernmost station (#02), Fe represented > 20% of the elements in the sediment. Other trace nutrients, including P (≅ 4%) and Zn (≅2%) were also present in significant proportions. In station #03, Fe was ≅13% of the elements, followed by Zn (≅2%) and P (≅1%). In station #09, Fe contributed >20%, P contributed ≅3%, and Zn contributed ≅2%. In station #10, Fe contributed ≅10%, while Zn contributed ≅ 4%. Finally, at the southernmost station (#16), the proportion of Fe was >25%, Zn was ≅ 4%, and Mo was ≅ 2% (Figure 07).

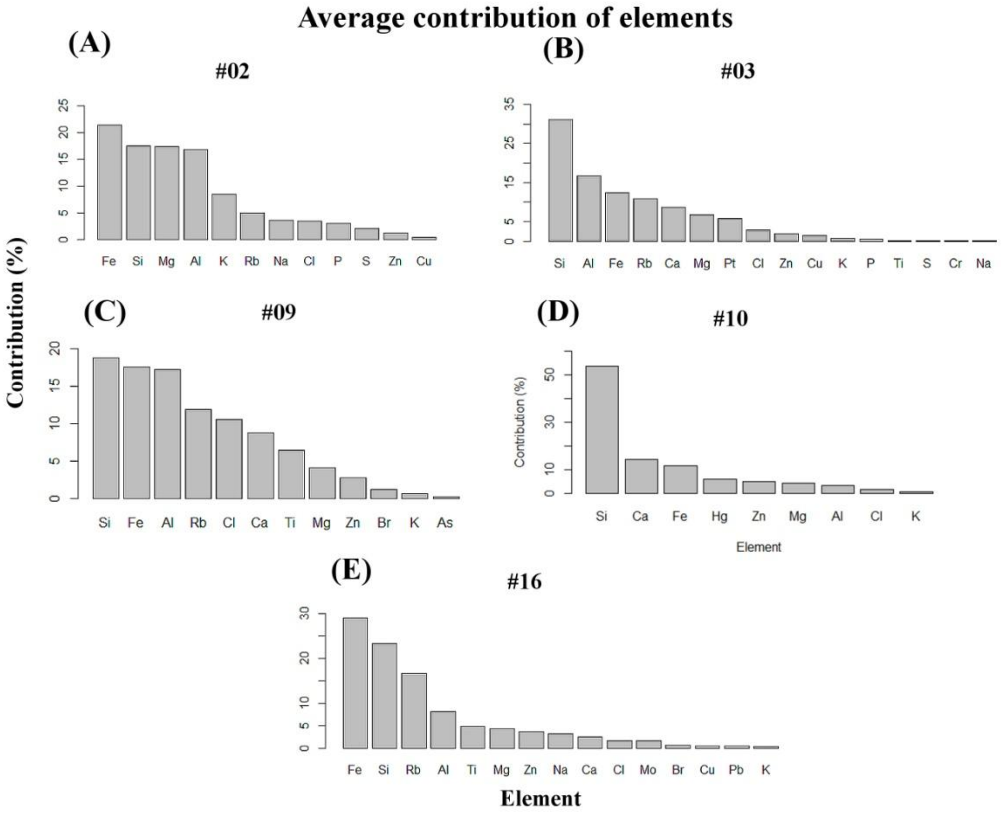

Figure 07: Average contributions of elements at stations #02, #03, #09, #10 and #16 (A to E, respectively).

The SEM observations provided detailed images of the sediments, which had different compositions, shapes and sizes (Figure 08). Some grains can be identified as having a biogenic origin, which was clearly represented by organism carapaces (Figure 08A). Additionally, the composition of the *Trichodesmium* mucilage (Figure 08c) was analyzed by EDS; it has the same composition as the grains that were attached to the nearby trichomes. Associations with heterotrophic bacteria, which are usually common in phytoplanktonic populations, are shown in image 08d.

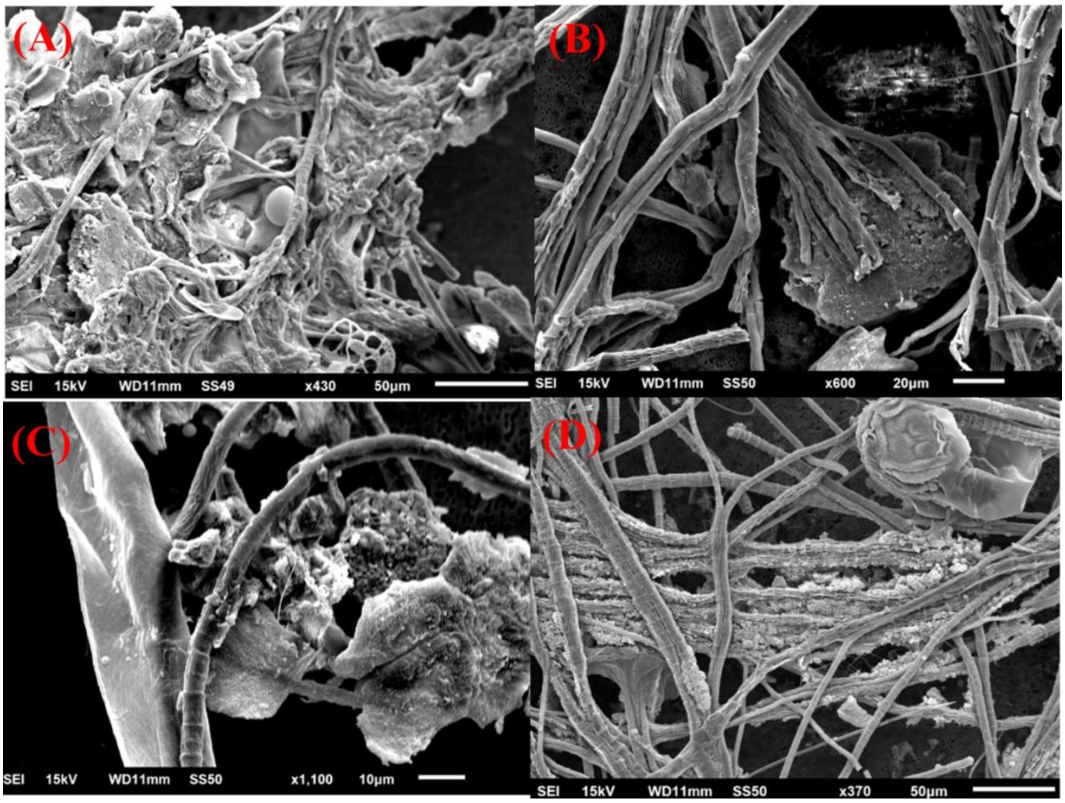

Figure 08: SEM images of *Trichodesmium* and sediments associated with the trichomes. Image A shows details of diverse sediments attached to the trichomes, including biogenic frustules. Image B shows trichomes attached to a sediment particle. Image C shows mucilage containing trichomes as well as sediment associations. Image D shows probable associations with heterotrophic bacteria.

4. DISCUSSION

The sampling stations were influenced by different surface water masses. Most of them (12 stations) were affected by tropical water (TW), stations #08 and #10 were influenced by subtropical surface water (STSW), and #13 and #16 were affected by Plata plume water (PPW) (Figure 01). The colder and less saline PPW affected 2 of the 4 southernmost stations that were shallow (Table I) and close to the continental shelf.

The surface *Trichodesmium* abundance was higher in the 4 northern samples, with a total concentration of > 157,000 trichomes L^{-1} (Table II). Stations #10 and #11 had concentrations of > 8,000 trichomes L^{-1}, and the trichomes could be seen by the naked eye. The concentrations of tufts and puffs were also highest in those stations; the maximum concentration of tufts was 15,380 colonies L^{-1} in #02, and the maximum concentration of puffs was 8,185 colonies L^{-1} in #03. In comparison, the *Trichodesmium* concentrations in the state of Pernambuco ranged from 1,000 to 1,700 filaments L^{-1} (Monteiro *et al.*, 2012). The statistical analysis grouped the stations by the contributions of the colonies and single trichomes to the total concentration of trichomes (Figures 02 and 03). The explanation for this aggregation is uncertain, but it may facilitate vertical migration (Villareal and Carpenter, 2003).

The availability of the dissolved phosphorus, DIN and silicon for *Trichodesmium* uptake in the collected stations was very low and well above the maximum concentrations for an oligotrophic environment (Table II) (Baumgarten *et al.*, 2010). The concentrations of PO_4^{-3} were less than 1 µM, while the total ammoniacal nitrogen, nitrate and nitrite were < 1 µM individually and ≤ 1.5 µM as DIN (Table II). However, the presence of dissolved nutrients at the surface and depth, even in those concentrations, are extremely significant in a low-nutrient environment. The relationship between high concentrations of nutrients and the presence of *Trichodesmium* was studied by Lenes and Heil (2010) in the Gulf of Mexico. The genus releases ammonium as a result of N_2 fixation, as well as particulate nitrogen and phosphorus, during cell death. The regeneration of compounds was proved to supply *Karenia brevis*, co-ocurring in the region, nutrient requirements and even support their blooms.

The N:P Redfield ratios differed between the stations. The four northernmost stations, which had higher abundances of total surface *Trichodesmium*, also had the highest ratios (> 6:1); the maximum ratio of 16:1 was found at station #04. However, all of the stations were equal to or below the mean Redfield ratio (16:1), which suggests an oligotrophic environment that favors N_2-fixing organisms (Arrigo, 2005) (Table II). These authors described the cellular Redfield ratio as the intracellular N:P ratio. The cellular ratio for survivalist-strategist organisms, which includes diazotrophs, is calculated to be > 30:1. These high values are explained by the need for additional P for N_2 fixation (Arrigo, 2005; LaRoche *et al.*, 2005). The cellular ratio for *Trichodesmium* varies between 66 along the northern Australian coast to 43.5-87 in the Gulf of Mexico, where the variation depended on the cellular concentrations of P (Villareal and Carpenter, 2003).

In a low-nutrient ratio environment, such as this study area, the addition of certain nutrients could increase the *Trichodesmium* growth rates. The fixation rates of cultures of *Trichodesmium* appear to increase when they were exposed to high phosphate concentrations (Knapp *et al.*, 2012). A constant supply of iron also maintains a high growth rate of *Trichodesmium* in the southeast North Atlantic (Sañudo-Wilhemy *et al.*, 2001) when combined with seasonal phosphate availability (Fernández *et al.*, 2013). Additionally, the vertical migration provided by the gas vesicles acting like ballast in *Trichodesmium* has been shown to increase phosphorus acquisition. This process could allow the trichomes and colonies to reach a phosphocline when present in the water (Villareal and Carpenter, 2003).

The vertical distribution of *Trichodesmium* was observed in all eight stations where they were collected (Figure 04). The highest concentrations were above the MLD, but a significant number of trichomes and colonies were also found in locations below the mixed water column. The low concentrations of phosphate, DIN and silicon that were observed in the surface stations extended to depth at the four stations in which samples were collected vertically (Figure 05). If the colonies were migrating for phosphorus acquisition (Villareal and Carpenter, 2003), they would find small quantities at the deepest waters.

The low concentrations of dissolved nutrients were, however, only one of the probable sources of elements. The genus was also concentrating sediments to use as a supply of nutrients. Microscope analysis showed high concentrations of dust in the colonies (Figure 06). These sediments were analyzed by electron microscopy (SEM) coupled with X-ray EDS analysis to identify elements that could be considered as nutrients for the trichomes. The results showed that the sediments were rich in iron and also contained other different trace nutrients that are important for *Trichodesmium* growth, such as phosphorus, zinc and molybdenum (Figure 07). Zn and Mo are known to be present in enzymes that are responsible for N_2 fixation. Thus, Zn is in enzymes that remove reactive oxygen species that are linked to oxidative stress. (Howard and Rees 1996; Nuester *et al.*, 2012

The process of transforming Fe-oxide dust to reduced forms by transporting it to the interior of the *Trichodesmium* colony under laboratory conditions was described by Rubin *et al.* (2011). Because P and Zn oxides follow the same process of dissolution, both elements may have also been acquired. The detailed images that were provided by SEM microscopy show the attachment of dust to the colonies (Figure 08AB). The *Trichodesmium* mucilage contained the same elements that were found in the sediments and probably acts as a mechanism for nutrient transportation along the entire trichome (Figure 08C). Additionally, a typical biofilm was observed on the surface of the trichomes (Figure 08D), which indicates associations with heterotrophic bacteria that may facilitate iron acquisition as they dissolve it for their own metabolism (Roe *et al.*, 2012).

Several other elements were found at high concentrations. Mg, K, Ca and Cl are major components of the water (Baumgarten *et al.*, 2010) and, coupled with Si and Al, are common in terrestrial and biogenic sediments (Deer *et al.*, 1966) (Figure 8A). Mg, K, Cl and Ca ions are the most abundant in the water (Baumgarten *et al.*, 2010), and Rb has long been known to be important for mitochondrial transport processes, including for gram-positive bacteria such as *Escherichia coli* and *Bacillus subtilis* (Zaristsky *et al.*, 1981; Bakker, 1982). However, the role of Rb in the cyanobacteria metabolism still needs to be studied.

The high concentrations of titanium and iron at stations #09 and #16 can be explained by the influence of the Plata plume water (Figure 01). Although station #09 was not directly influenced by the PPW at the time the samples were collected, the La Plata River plumes consistently reach the region, especially during the autumn and winter (Burrage e*t al.*, 2008). The La Plata basin system is one of the largest in the world, exceeding 25% of the total Mississippi River discharge. The system covers parts of Brazil, Bolivia, Argentina, Paraguay and Uruguay, and it’s most important tributary rivers are the Paraná and Uruguay Rivers (Berbery and Barros, 2002). A geochemical analysis of the sediments associated with the PPW shows significant concentrations of hematite, amphiboles (Fe-oxide) and kaolinite (Al-rich) in the region (Campos *et al.*, 2008). Ti is known to follow Fe in magmatic crystallization and enriches amphiboles (Salminen *et al.*, 2005). The Paraná and Uruguay Rivers carry heavy metals in their waters, including Ti, which is more closely related to the Uruguay River (Depetris and Pasquini, 2007).

5. CONCLUSIONS

Populations of *Trichodesmium* can be found along the entire south-southeast Brazilian slope at high to low concentrations. In spite of their capacity for vertical migration, they were concentrated at the most superficial extracts of water. The trichomes were found alone or in colonies as puffs and tufts. The nutrient analysis of the sampling stations showed that the environment had oligotrophic characteristics. However, the populations of *Trichodesmium* appear to acquire several elements, such as Fe, P and Zn, from sediments that attach to the colonies both by concentrating them in their interior and by making associations with other bacteria. In the southernmost region, the La Plata River appears to enrich the waters with sediments that contain iron, which is an important and limiting nutrient for the genus. Thus, the Brazilian coast needs to be investigated further to explain the interactions between *Trichodesmium* and the acquisition of elements from dust. This study provides an analysis of *Trichodesmium* behavior in the South Atlantic and demonstrates the importance of the genus to these waters, which are poor-nutrient regions where other phytoplanktonic organisms cannot establish permanent populations.

ACKNOWLEDGEMENTS

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SUPPORT MATERIALS

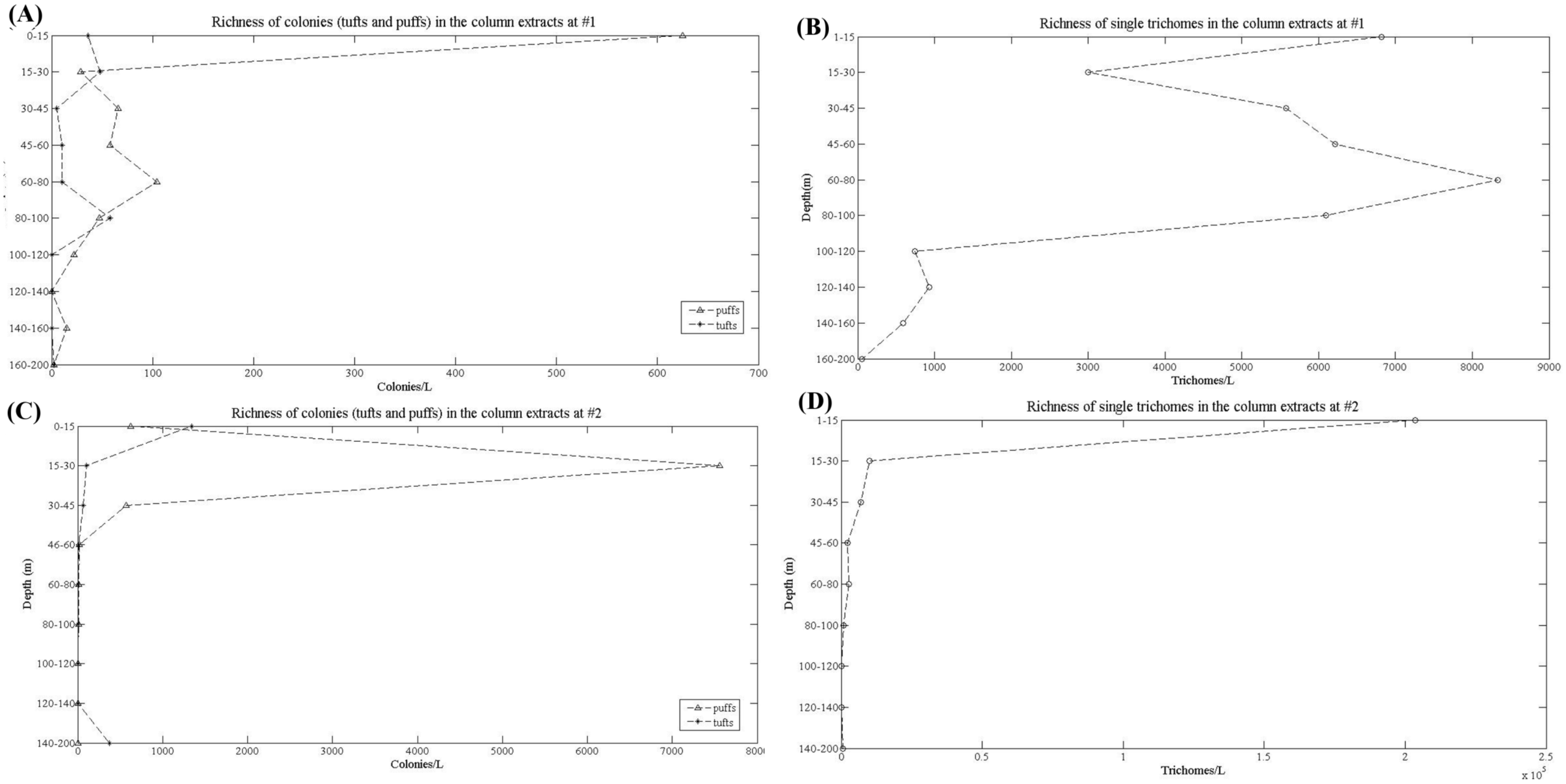
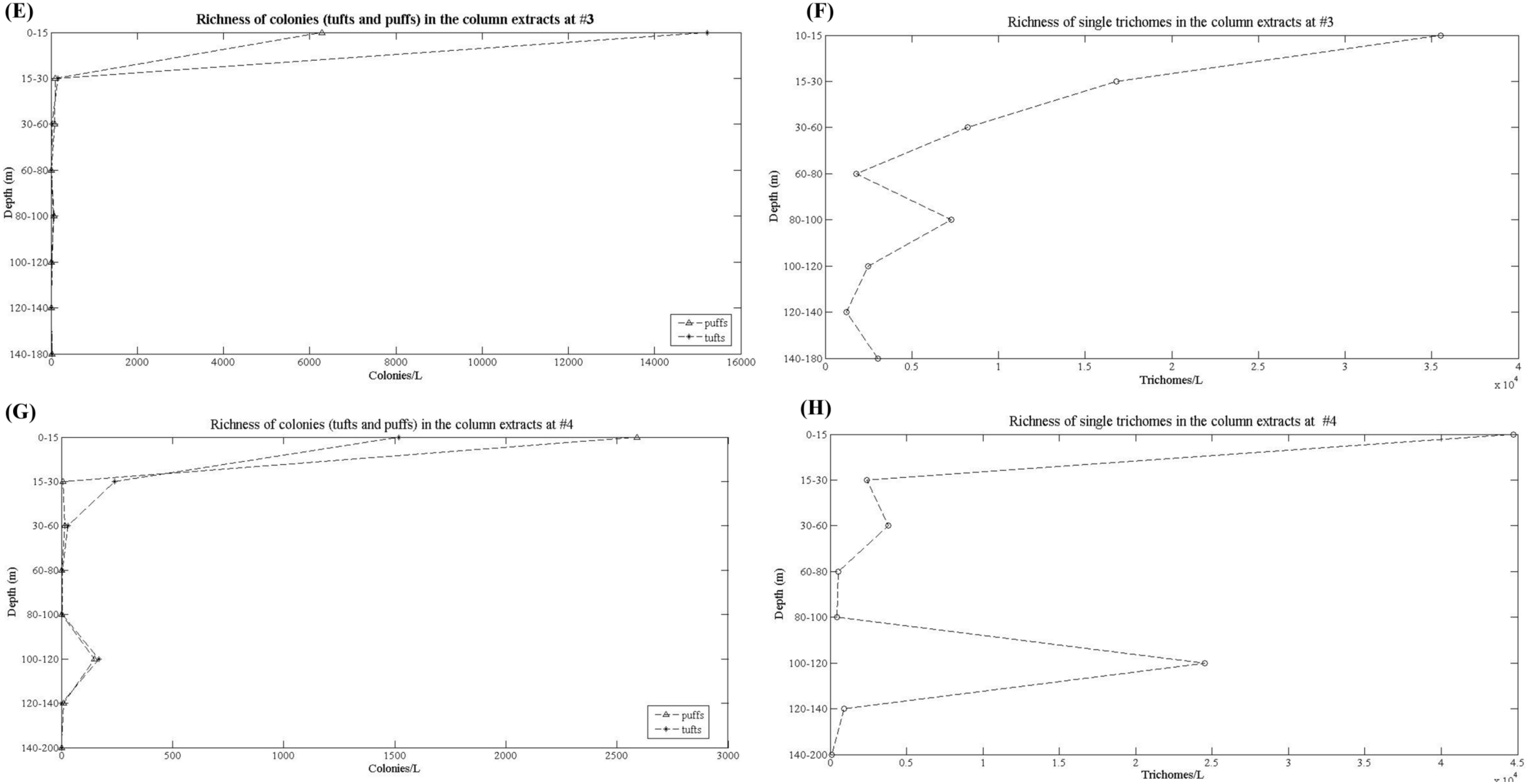


Figure 09: Vertical distribution of *Trichodesmium* (Colonies L^{-1} and single Trichomes L^{-1}) in the stations (A to P).



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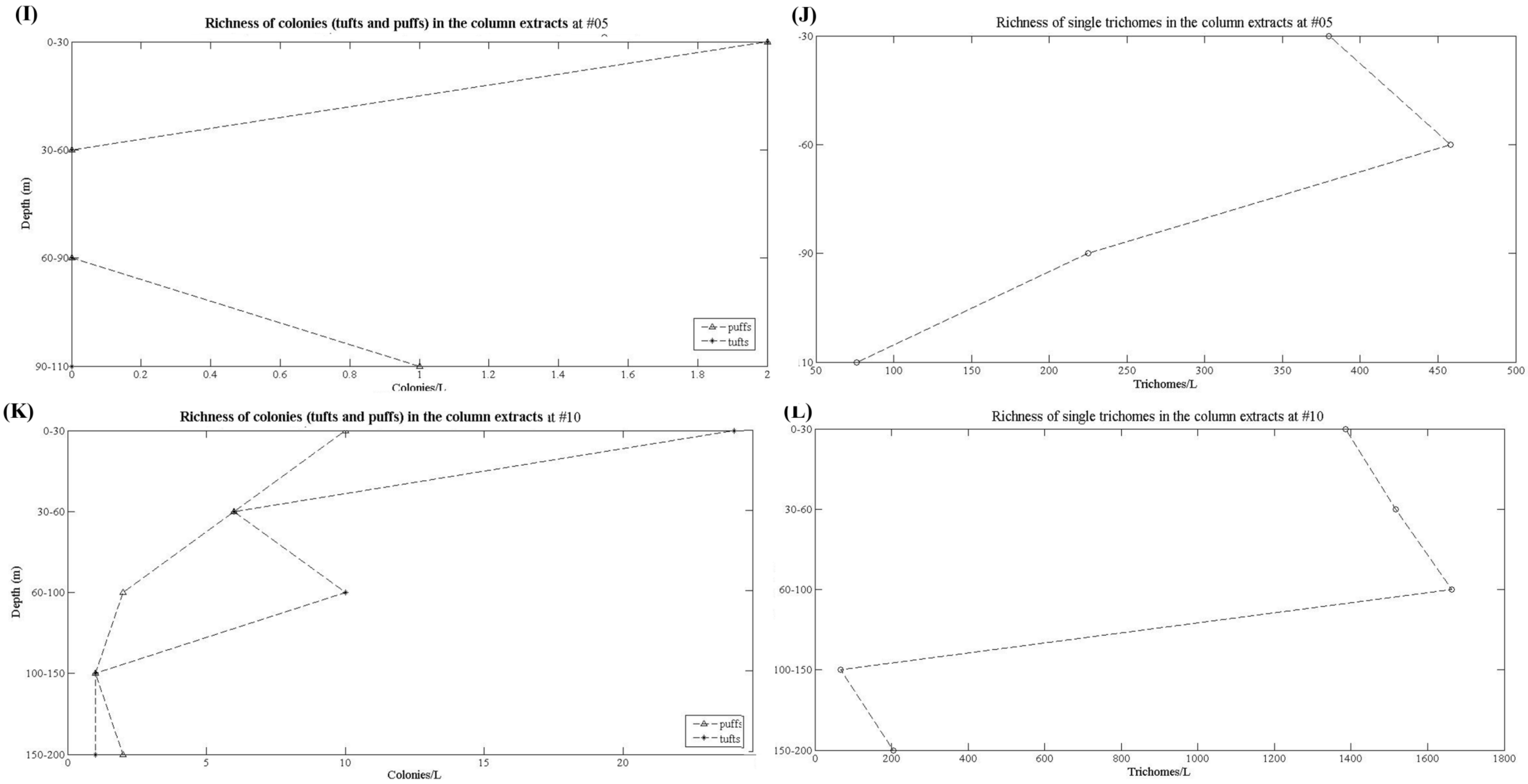

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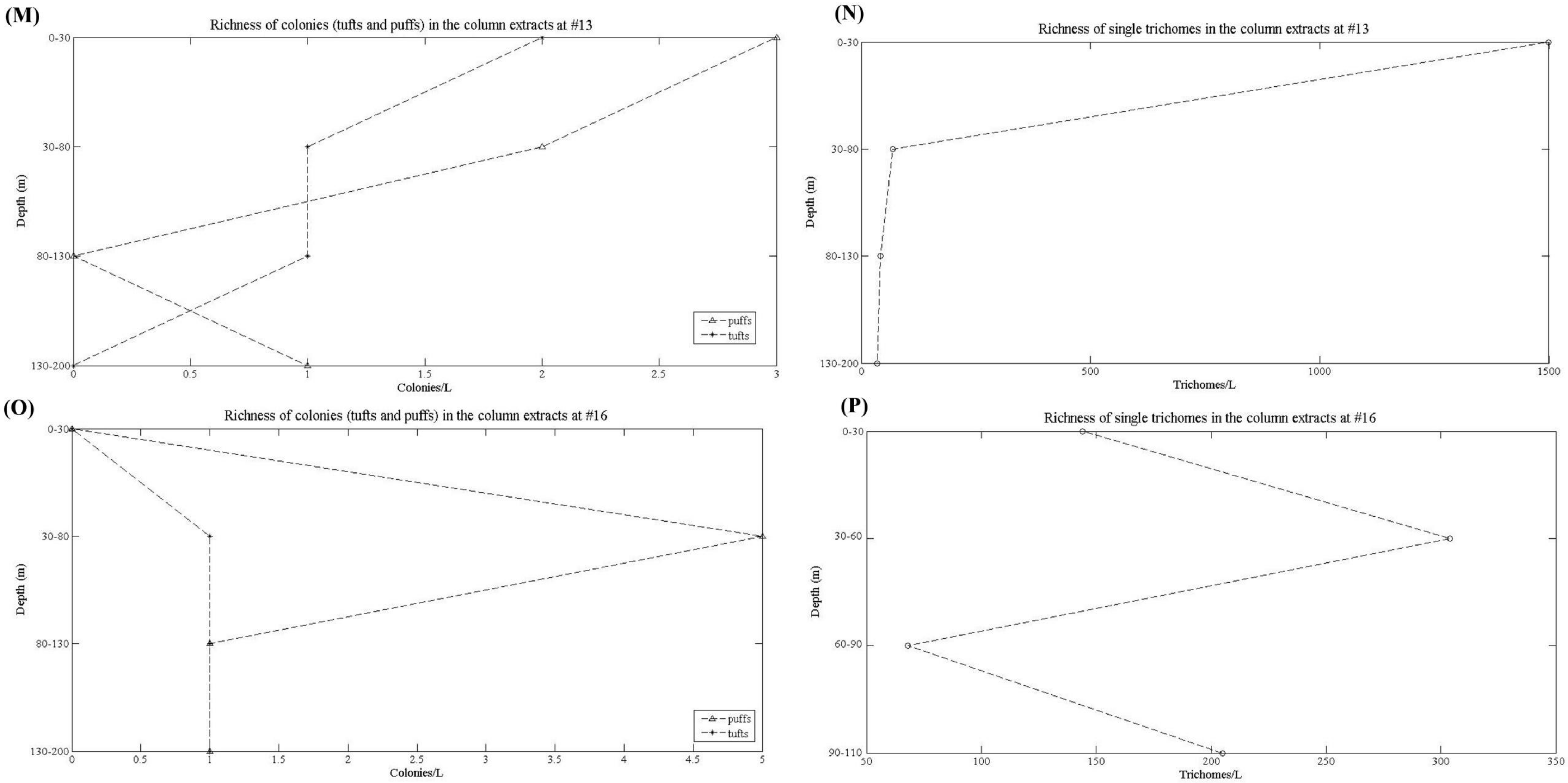

Figure 09: Continued

4. CONSIDERAÇÕES FINAIS

Na região do Talude Sul-Sudeste do Brasil, o gênero *Trichodesmium* mostrou-se presente em todas as estações em que ocorreram. Algumas condições ambientais já observadas e mencionadas em outros trabalhos puderam ser comprovadas, como a formação de células de Langmuir, baixas concentrações de nutrientes e mar calmo coincidindo com as maiores concentrações do gênero. Cinco espécies de *Trichodesmium* foram identificadas (*T. clevei*, *T. erythraeum*, *T. hildebrandtii*, *T. radians* e *T. thiebautii*) coocorrendo com outros organismos do fitoplâncton, principalmente representantes dos grupos Dinoflagelados e Diatomáceas. No entanto, estes outros organismos estavam presentes em baixa abundância.

Apesar da capacidade do *Trichodesmium* em realizar migração vertical, a distribuição do organismo na coluna d’água pareceu ser regida pela profundidade da camada de mistura. As análises de nutrientes mostraram que o ambiente possuía características oligotróficas. Mesmo assim, as populações de *Trichodesmium* utilizaram a estratégia de agregar sedimentos para o interior das colônias, o que facilita a sua dissolução. Estes por sua vez, ricos em elementos considerados nutrientes para a cianobactéria, como Fe, P e Zn. Ainda, foram observadas associações com bactérias no interior das colônias, o que facilitaria ainda mais a dissolução de elementos. Nas estações mais ao sul, o aporte do Rio da Prata parece ser a principal fonte dos sedimentos encontrados no interior das colônias, pois os grãos continham quantidades significativas de titânio. O elemento Fe foi encontrado em todos os sedimentos das estações analisadas por EDS, gerando uma fonte alternativa de nutriente para o *Trichodesmium*.

A costa Brasileira ainda precisa ser mais investigada para que as interações entre sedimento-colônias possam ser detalhadas. Este trabalho promoveu uma aproximação inicial do comportamento das populações de *Trichodesmium* no Atlântico Sul, e comprovou a importância do gênero nestas águas pobres em nutrientes, em que outros organismos do fitoplâncton não conseguem estabelecer populações.

5. SUGESTÕES PARA TRABALHOS FUTUROS

Este trabalho sugere fortemente a continuação de estudos de pesquisas sobre *Trichodesmium* na costa Brasileira, dada à importância do gênero nos ciclos biogeoquímicos. Como sugestões para trabalhos futuros incluem-se:

- Dedicar esforços de cruzeiros oceanográficos para o estudo do *Trichodesmium*, acompanhando o comportamento de uma mesma agregação ao longo do dia, assim como já foi feito em inúmeros estudos em outros oceanos (Villareal and Carpenter, 2003; Fernández e*t al*, 2013).
- A estimativa da fixação de Nitrogênio das populações, visto que estas contribuem de maneira distinta de acordo com a região oceânica, variando principalmente com a latitude, afim de estimar a contribuição do gênero para o ciclo de N_2 (LaRoche and Breitbarth, 2005);
- Cultivo e manutenção de cepas de *Trichodesmium* para estimativa de assimilação de nutrientes, taxas de fixação e liberação de compostos nitrogenados através de simulações ambientais em condições controladas (Knapp *et al*, 2012);
- Analisar mais profundamente os mecanismos de aquisição dos sedimentos pelas colônias e a assimilação destes elementos (Rubin *et al*, 2011);
- Avaliar a variação na biomassa de *Trichodesmium* com a sazonalidade;
- Identificar outras possíveis feições oceânicas controlando a distribuição espacial do gênero, principalmente de pequena escala como ressurgências localizadas, vorticidade, ondas internas e outras;
- Utilizar a biologia molecular como auxílio na identificação de espécies de *Trichodesmium*.

As parcerias com outras Universidades e laboratórios de pesquisa são de grande importância para o estudo do gênero, pois apenas mão de obra qualificada, saídas de campo e equipamentos de ponta permitir-se-ia coletar e analisar propriamente os dados ambientais.

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