Universidade Federal do Rio Grande – FURG

Instituto de Oceanografia

Programa de Pós-Graduação em Oceanologia

PIGMENTOS HIDROSSOLÚVEIS E LIPOSSOLÚVEIS EM FITOPLÂNCTON MARINHO PRODUTOR DE TOXINAS

Chariane Camila Werlang

Dissertação apresentada ao Programa de Pós-Graduação em Oceanologia, como parte dos requisitos para a obtenção do Título de Mestre.

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Rio Grande, RS, Brasil

Fevereiro 2020

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WERLANG, CHARIANE CAMILA
Pigmentos hidrossolúveis e lipossolúveis em fitoplâncton marinho produtor de toxinas/ Chariane Camila Werlang. – Rio Grande: FURG, 2020.
118 p.
Dissertação (Mestrado) – Universidade Federal do Rio Grande. Mestrado em Oceanologia. Área de Concentração: Ecossistemas Marinhos;
1.Diatomáceas. 2. Dinoflagelados. 3. Cianobactérias. 4. Pigmentos. .5. Toxinas. 6. Costa Atlântico Sudoeste.7. Talude Continental. I. Pigmentos hidrossolúveis e lipossolúveis em fitoplâncton marinho produtor de toxinas.

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DEFESA DE DISSERTAÇÃO DE MESTRADO – 02/2020

Às 09:00h do dia 20 de fevereiro do ano de dois mil e vinte, na Sala Estuários-Cidec-Sul - FURG/Carreiros, reuniu-se a Comissão Examinadora da Dissertação de **MESTRADO** intitulada **" Pigmentos Hidrossolúveis e Lipossolúveis em Fitoplâncton Marinho Produtor de Toxinas ",** da **Acad. Chariane Camila Werlang.** A Comissão Examinadora foi composta pelos seguintes membros: Prof. Dr. João Sarkis Yunes – (Orientador– IO - FURG), Dra. Luiza Dy Fonseca Costa (Co-orientadora – LCF/FURG), Dr. Marcio Silva de Souza (Co-orientador – Pós-Doc - IO-FURG), Profa. Dra. Eunice Machado (IO/FURG) e Prof. Dr. Luiz Laureno Mafra Junior (UFPR). Dando início à reunião, o Presidente da Sessão, Prof. Dr. João Sarkis Yunes, agradeceu a presença de todos, e fez a apresentação da Comissão Examinadora. Logo após, esclareceu que a Candidata teria de 45 a 60 min para explanação do tema, e cada membro da Comissão Examinadora, um tempo máximo de 30 min para perguntas. A seguir, passou à palavra a Candidata, que apresentou o tema e respondeu às perguntas formuladas. Após ampla explanação, a Comissão Examinadora reuniu-se em reservado para discussão do conceito a ser atribuído a Candidata. Foi estabelecido que as sugestões de todos os membros da Comissão Examinadora, que seguem em pareceres em anexo, foram aceitas pelo Orientador/Candidata para incorporação na versão final da Dissertação. Finalmente, a Comissão Examinadora considerou a candidata APROVADA por unanimidade. Nada mais havendo a tratar, foi lavrada a presente ATA, que após lida e aprovada, será assinada pela Comissão Examinadora, pelo Candidato e pelo Coordenador o Programa de Pós-Graduação em Oceanologia (PPGO).

Prof. Dr. João Sarkis Yunes
Presidente

Dra. Luiza Dy Fonseca Costa

Profa. Dra. Eunice Machado

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Agradecimentos

Agradecimentos à minha mãe Ivone Tarouco Goulart pelo suporte principalmente em momentos de dúvida.

Agradecimentos especiais ao meu orientador João Sarkis Yunes por todas as conversas proveitosas e suporte à algumas ideias durante a execução do projeto.

As minhas amigas chapecoenses Camila Zamprogna e Gabriela Galeti, pelo apoio diário e empatia.

Aos meus co - orientadores e amigos, Márcio Silva de Souza e Luiza Dy Fonseca Costa por me guiarem e orientarem diariamente na direção certa, e também pelos bolos risadas e conversas que com certeza fizeram minha jornada pela pós-graduação mais leve e divertida.

A todos os integrantes do Laboratório de Cianobactérias e Ficotoxinas da FURG, alunos de graduação, pós-graduação e pesquisadores de pós-doutorado, pela parceria e animação em dias difíceis, especialmente a aluna Larissa Andreola pela parceria durante as análises também.

Ao técnico e colega do Instituto Oceanográfico Marcelo Peres de Pinho pelo auxílio tanto na manipulação de equipamentos oceanográficos, quanto da conversão e análise de dos produtos resultantes dos mesmos.

A equipe do projeto “Bonito Listrado” pelo apoio logístico durante as coletas e transporte de amostras, sem os quais esse estudo não teria sido possível.

Aos professores Lauro Madureira e Stefan Cruz Weigert por ceder lugar ao laboratório de Cianobactérias e Ficotoxinas no desenho amostral do projeto “Bonito Listrado”.

A Amália De Toni por ceder parte das amostras coletadas durante seu projeto de doutorado, que integraram parte da presente dissertação, e com certeza fizeram toda a diferença na elaboração da mesma.

Aos professores integrantes do Programa de Pós-Graduação em Oceanografia Física, Química e Geológica/ Oceanologia por participarem de um momento tão importante na minha formação como pesquisadora.

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Capes pela bolsa de estudos que além de possibilitar minha estadia no curso, também me possibilitou estabilidade durante os dois anos de duração do mestrado.

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Capítulo V

Lista de acrônimos e Abreviações

A

ACAS- Água Central do Atlântico Sul (SACW).

AT- Água Tropical.

AP- Água de Plataforma.

ASP- Água Subtropical de Plataforma.

AD- Ácido Domóico.

B

BG- Baía da Guanabara.

C

CB- Corrente do Brasil.

C:CHL-*a:* razão entre biomassa em carbono e clorofila *a.*

CHL-*a:* Clorofila *a.*

***C*-**Carbono (*Carbon*).

D

DSP's - Diarrheic Shellfish Poisoning

dc-STX- Decarbamoil Saxitoxina.

DAD- Detector de arranjo de diodo (*Diode Array Detector*).

E

Elisa- Enzyme Linked Immunosorbent Assay (Imuno ensaio enzimático).

G

GTX- Gonyautoxinas.

H

HPLC- High Performance Liquid Cromathography.(Cromatografia líquida de Alta Precisão)

N

Neo-STX- Neo -saxitoxina.

N- Nitrogênio (Nitrogen).

O

OD- Oxigênio Dissolvido.

P

pSTX- Fração particulada de saxitoxina (*particulate saxitoxin*).

PC- Plataforma central

S

STX- Saxitoxina.

SST- Sea Surface Temperature (Temperatura superficial do oceano).

SSS- Sea Surface Salinity (Salinidade superficial do oceano).

U

UV- Ultra violeta.

UV-VIS- Ultra violeta visível

Resumo

Os fatores que influenciam a dinâmica do fitoplâncton na região Sul-Sudeste do talude continental e plataforma interna do sudoeste do Atlântico Sul são relativamente conhecidos, mas a maneira como estes afetam a produção de pigmentos lipossolúveis e hidrossolúveis no fitoplâncton ainda é pouco discutida. Também há poucos trabalhos que abordem a quantificação de organismos tóxicos de variados grupos (dinoflagelados, diatomáceas e cianobactérias), juntamente com a quantificação de toxinas como o AD e a STX nessa região oceânica. Por isso, o objetivo deste trabalho foi descrever a comunidade fitoplanctônica (abundância e em biomassa em carbono), a composição dos principais pigmentos (por espectrofotometria) e a concentração de ficotoxinas (por cromatografia de alta eficiência) em certos setores do Oceano Atlântico Sudoeste, para analisar a influência de variáveis oceanográficas (temperatura, salinidade, camada de mistura) sobre essas variáveis biológicas. Foram analisadas amostras advindas de dois cruzeiros: um em junho (inverno) e outro em setembro (primavera) de 2018 no âmbito do projeto "Bonito Listrado" na região costeira do Rio de Janeiro (sudeste); e amostras coletadas ao longo do talude sul-sudeste (projeto TALUDE-V, novembro de 2012) de maneira independente. Baseando-se nos dados de 2018, foi possível observar uma significativa diferença ($p<0,05$) tanto entre a biomassa (clorofila-*a*) quanto entre a abundância fitoplanctônica no inverno e na primavera. Essas diferenças foram relacionadas com a profundidade da camada de mistura ($r^2= -0,626$, $p<0,05$) e temperatura ($r^2= 0,641$, $p<0,05$). O inverno apresentou maior concentração de CHL-*a,* caracterizado por abundância de *Pseudo-nitzschia* spp.*,* enquanto a primavera se caracterizou por predominância de diatomáceas do gênero *Rhizosolenia* e dinoflagelados, associando-se com uma maior concentração de carotenoides e ficobiliproteínas. Em relação a toxinas (AD e STX), houve diferença significativa ($p<0,05$) apenas para STX, provavelmente relacionada à presença de dinoflagelados dos gêneros *Alexandrium* e *Gymnodinium* e a cianobactéria *Trichodesmium* spp*.* Quanto a novembro de 2012 (primavera),

foram revisitados os dados de abundância ao longo do talude sul-sudeste, sendo possível adicionar nesta dissertação que a abundância de *Trichodesmium* não foi relacionada (r^2= 0,0125) com a concentração de ficobiliproteínas. Por outro lado, *Trichodesmium* foi apontado como um dos potenciais produtores de STX (de 0,087 a 2,61µg L^{-1}) ao longo do talude sul-sudeste do Brasil, embora outros organismos, particularmente dinoflagelados, devam ser mais importantes para a produção de STX.

Palavras-chave: Diatomáceas, Dinoflagelados, Cianobactérias, Pigmentos, Toxinas, Costa Atlântico Sudoeste, Talude Continental.

Abstract

Within the Southwestern Atlantic Ocean, the relationship between environmental parameters and plankton dynamics is relatively well known. However, their effects over the production of lipo and hydrosoluble pigments are still poorly studied. There are also a few studies that estimate the numbers of many kinds of potentially toxin-producing phytoplankton (such as dinoflagellates, diatoms and cyanobacteria), along with the quantification of toxins [Domoic Acid (DA) and Saxitoxins (STX)] in that ocean region. There were analyzed biological samples collected under the umbrella of two projects: two surveys (Bonito Listrado project) corresponding to June (winter) and September (spring) of 2018, and one survey (TALUDE-V project) from November, 2012 (springtime). Therefore, the main goal was to describe the phytoplankton community (abundance and biomass), a broad pigment composition (by spectrophotometry) and major toxin analysis (by High Performance Liquid Chromatography) in certain sectors of southwestern Atlantic Ocean. At the same time, there was assessed the influence of oceanographic variables (temperature, salinity, mixed layer depth) over these biological variables. Regarding the 2018 surveys, there was a significant difference ($p<0.05$), both in terms of abundance of cells and (carbon) biomass, between the winter and spring periods. This difference was associated with the mixed layer depth ($r^2= -0.626$, $p<0.05$) and temperature ($r^2= 0.641$, $p<0.05$). Winter high abundance was related mostly to the numbers of pennate diatom *Pseudo-nitzschia* spp., while springtime showed a predominance of diatoms from the genus *Rhizosolenia* and dinoflagellates.There was only a significant difference ($p<0.05$) in STX concentration between these sampling periods, probably related to the relatively high numbers of dinoflagellates *Alexandrium* and *Gymnodinium*, and the cyanobacteria *Trichodesmium*, in spring. Considering the dataset revisited (2012), the abundance of *Trichodesmium* spp. trichomes showed no co-dependency with concentration of phycobiliproteins ($r^2= 0.0125$), and *Trichodesmium* was considered as potential STX-producer (low amounts) as well as dinoflagellates in higher amounts.

Key-words: Diatoms, Dinoflagellates, Cyanobacterias, Pigments, Southwest Atlantic Coast, Continental Slope

CAPÍTULO I: INTRODUÇÃO

1.1 Características físicas gerais da região costeira Sul-Sudeste.

A região da plataforma continental brasileira estende-se da latitude 34°S à 22°S, e pode ser dividida em quatro setores, e constitui em exemplo de plataforma dominada por eventos hidrodinâmicos [Mahiques et al., 2004], e corresponde a parte do Brasil com ambiente subtropical e temperado. Os quatros setores compreendem as zonas do cone do Rio Grande, o setor de Mostardas-Florianópolis, o embaíamento de São Paulo, e setor de São Tomé a Cabo Frio [Mahiques et al.,2010]. A largura da plataforma continental apresenta grande variação geomorfológica, hidrodinâmica e geoquímica.

Os processos geoquímicos nessa última área incluem constante aporte de nutrientes durante o inverno, de origem continental ou por ressurgências ligadas a Água Central do Atlântico Sul (ACAS). Nesse caso, águas que apresentam como características baixas concentrações de nutrientes durante o verão, passam a ser ricas em nitrogênio (N) e fósforo (P) com a chegada do inverno, sendo que as concentrações de silicato (Si) tendem a permanecer em altas independente de ciclos sazonais[Moser & Gianesella-Galvão,1997].

A dinâmica dos processos físicos na plataforma continental do Atlântico Sudoeste foi amplamente revisada por Piola et al. [2018]. É basicamente controlada por diferenças em características morfológicas, climáticas e, oceanográficas, sendo que toda a região que compreende o gradiente latitudinal de 55ºS a 23ºS pode ser dividida em 3 zonas. A primeira zona, mais ao sul representa a região da plataforma da Patagônia que é mais ampla e se estende de 38º a 55º S. A segunda, a plataforma central, um pouco mais estreita de 28 º a 38º S, formando um platô. E por último, a terceira, que corresponde à região do embaíamento sul brasileiro que se estende de 23º a 28ºS.

Essa região oceânica, e a costa do Brasil são caracterizadas por hidrodinâmica em constante mudança e redistribuição de água devido principalmente a vórtices da Corrente do Brasil (CB), conforme esta flui em direção ao Sul do país [Silveira et al., 2000]. Também se incluem nessa dinâmica, a intrusão da Água Central do Atlântico Sul (ACAS) ao longo da plataforma continental, além de outros fenômenos de ressurgência costeiras na região sudeste próxima a Cabo Frio [Castro et al., 2006]. Esses processos tornam a área oceanograficamente complexa, tanto do ponto de vista espacial quanto temporal, especialmente considerando também, a influência das plumas da Baía da Guanabara (BG)

e Rio Sepetiba (RS) sobre a dinâmica na região interna (< 50 m) da plataforma continental [Coelho, 2010].

Quanto aos processos de circulação, estes são controlados principalmente por efeitos baroclinicos (plataforma externa) e ventos locais (plataforma interna) [Palma & Matano, 2009]. Esses fatores somados ao espalhamento das plumas advindas de BG e RS por aproximadamente 120 km [Moser et al., 2016], favorecem a queda da salinidade na camada superficial da coluna de água, especialmente na região sudeste. Como a influência sazonal é importante nesse local, esse processo é mais acentuado durante o inverno [Stevensen, 1998; Souza & Robinson, 2004]. No entanto, com a chegada da primavera as águas superficiais dessa zona vão se tornando gradualmente mais salinas, quentes e estratificadas, devido a intrusão da CB ao longo da porção externa da plataforma continental [Campos et al., 1994].

Ainda na região da PC pertencente ao Brasil, mais especificamente de Cabo de Santa Marta até Cabo Frio há forte influência de frentes de salinas, principalmente por conta de descargas continentais vindas do rio La Plata e do sistema Lagoa dos Patos/Mirim, além da BG. Já no gradiente costa-oceano (a 70-80 km de distância da costa) também há uma limitação pela intrusão de água salina da CB, quente e geralmente pobre em nutrientes. Nesse caso, a CB proporciona trocas de energia entre as regiões costeira e oceânica [Piola et al., 2018].

De forma que essas trocas também são auxiliadas pelo estreitamento considerável da plataforma continental, favorecendo o transporte de Água Tropical (AT) (T > 20 °C; S > 36) para a camada de mistura, e da ACAS (T < 20 °C; 35 < S < 36) para a picnoclina [Castro & Miranda, 1998]. No que se refere a salinidade, é a Água de Plataforma (AP) que se espalha como uma frente de baixa salinidade (<35), pela porção mais próxima a costa. Nesse caso, a plataforma pode ser dividida também em 3 zonas distintas (interna, média e externa) de acordo com as características termohalinas apresentadas no transecto costa-oceano.

A região interna apresenta uma frente térmica de fundo representada pelo limite da isoterma de 18ºC, com a intrusão da ACAS próxima ao fundo. No verão esse limite pode se estender de 10 a 40 km da costa, mas no inverno pode chegar até 80 km [Castro, 2014]. Em geral essas águas são pouco estratificadas se comparadas com a porção média, e dominadas pela AP. Já nas porções média e externa da plataforma continental, há um acentuado gradiente de salinidade, principalmente próximo a superfície, formando uma

barreira halina [Piola et al., 2018], marcada pela transição entre a região interna (misturada) e a média (mais estratificada) e caracterizada pela presença da AT. Por fim, a porção externa é caracterizada pela presença da AT e intrusão da ACAS, mas de maneira desigual, dependendo da sazonalidade. No outono a proporção se mantém praticamente igual, mas durante a primavera e verão a ACAS supera em duas vezes a proporção da AT, desempenhando um papel principal na fertilização dessa região já que se trata de águas mais frias e ricas em nutrientes.

Considerando como esses eventos são importantes para a estruturação do fitoplâncton, é essencial levar em consideração a variação desses fatores em estudos que visam discutir as comunidades dessas áreas [Mena et al., 2019] de uma perspectiva que englobe fatores físicos, químicos e ecológicos, já que a variação na temperatura, salinidade e consequentemente na estratificação da coluna d'água, são as principais forçantes responsáveis pela estruturação das cadeias tróficas nos oceanos [Smith Jr. et al., 1997].

1.2. Dinâmica do fitoplâncton na região de estudo

Por se tratarem de áreas consideradas de baixa produtividade e biomassa, mas com alta diversidade planctônica [Longhurst & Pauly, 2007; Jales et al., 2015], os oceanos tropicais e sub-tropicais próximos a costa brasileira formam regiões em que a produção primária (PP) é controlada tanto por variáveis físicas (atmosféricas, oceânicas, geomorfológicas) quanto por fatores biológicos como interações ecológicas [Coelho-Souza et al., 2012]. Essas interações entre as comunidades em um ambiente tão dinâmico, são reguladas por meio de competição por recursos, principalmente por nutrientes [Moser et al., 2014],os quais apresentam-se em concentrações muito baixas, favorecendo assim, membros do pico e nanoplâncton, e, por consequência, afetando a diversidade [Hanson et al., 2007; Ciotti et al., 2007].

O balanço entre diversidade e biomassa tipicamente apresentado por essas comunidades pode mudar devido a modificações na estrutura da coluna d'água (Figura A.), tanto por marcadas mudanças sazonais, como por outros processos como intrusão de águas mais frias e ricas em nutrientes, ou pela chegada de plumas de rios [Tenembaum et al., 2006]. Esses eventos aumentam a representatividade e a contribuição de espécies com maior biovolume pertencentes ao microplâncton, como diatomáceas e dinoflagelados [Gonçalves-Araujo et al., 2018].

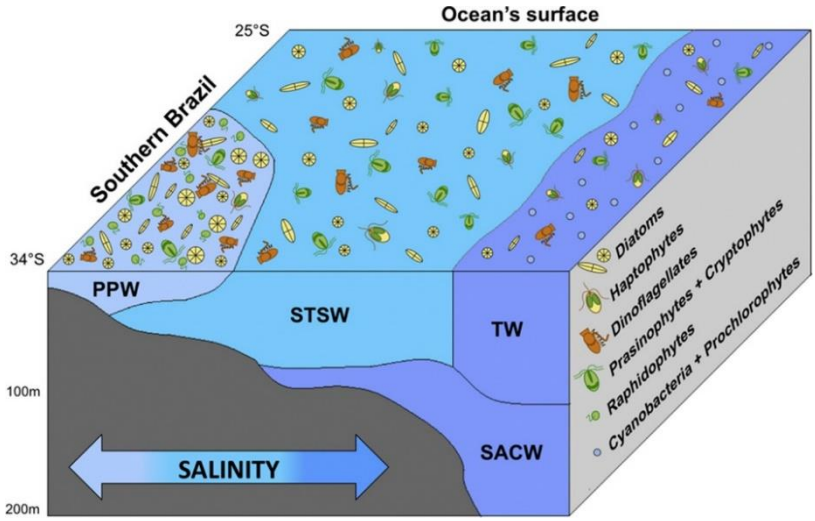


Figura A. Exemplo de distribuição de comunidades fitoplanctônicas ao longo do gradiente 34º a 25ºS conforme a estruturação da coluna d'água e presença de massas de águas (*PPW:La Plata River Plume Water*, *STSW: Subtropical Shelf Water,TW: Tropical Water, SACW: South Atlantic Central Water*) na região entre 100 e 200 m da plataforma continental Sul brasileira apresentada por Gonçalves-Araujo et al. [2018].

Além das condições oceanográficas a disponibilidade de luz influencia a morfologia, fisiologia e por consequência a ecologia dos organismos do microplâncton [Reynolds, 1997]. Dessa mesma forma, esta interação afeta a abundância relativa dessas espécies, que desenvolvem estratégias tanto para maximizar a capacidade fotossintética, quanto para administrar ferramentas metabólicas. Desta maneira, algumas espécies são capazes de ser melhores competidoras frente ao ecossistema e sua dinâmica espaço-temporal, que seleciona organismos com diferentes adaptações frente a diferentes disponibilidades de recursos [Margalef, 1977; De Castro & Moser, 2012].

Essa dinâmica no plâncton muda de acordo com essas variações que seguem padrões de escalas espaciais e temporais, acabando por determinar a composição taxonômica das comunidades do fitoplâncton, sendo essas intrínsecas à formação e ao estruturamento dos ecossistemas marinhos [Brandini, 2018]. Por exemplo, em momentos em que a zona eufótica é mais rica em nutrientes há presença expressiva de grupos com

razão superfície: volume menor como as diatomáceas microplanctônicas ou formadoras de cadeias. Por apresentarem frústulas, têm maior tendência a sedimentar. Conforme os nutrientes vão se tornando menos disponíveis, os nanoeucariontes (flagelados) e procariontes (cianobactérias) com maior razão superfície-volume se tornam os principais produtores primários na zona eufótica [Flombaum, 2013]. Portanto, mesmo em condições tão contrastantes, a bomba biológica de carbono se mantém estável nas regiões tropicais e sub- tropicais [Brandini, 2018].

Para tanto, os organismos fitoplantônicos muniram-se de variados artifícios de rápida resposta a variações da coluna d´água [Reynolds, 1997]. Como estratégia para lidar com esse ecossistema em constante mudança, variados grupos desenvolveram diferentes recursos de controle fisiológico, assim facilitando a competição. Por consequência também acabaram incrementando a capacidade de expansão do seu nicho ecológico [Polechová & Strock, 2018]. Foram bem-sucedidos nessa tarefa, por exemplo, devido a presença de flagelos que permitem a migração, formação de agregados que dificultam a predação, além do aumento da concentração de certos pigmentos que facilita a fotossíntese, e da biossíntese de toxinas. Portanto, conhecer essas características das comunidades fitoplanctônicas torna-se essencial para discutir sua dinâmica, condições de estruturação e estado fisiológico.

1.3. Pigmentos lipossolúveis

Diferentemente de membros do Reino Plantae, o grupo polifilético de microalgas, cianobactérias e dinoflagelados apresenta um amplo espectro de pigmentos que auxiliam ou desempenham papel principal durante o processo de fotossíntese [Proença, 2006]. Historicamente, esses pigmentos foram amplamente utilizados para estimativas de biomassa do fitoplâncton tanto pela limnologia quanto pela oceanografia, por meio da utilização e aprimoramento de diversas técnicas como a espectrofotometria, e nos últimos anos a cromatografia [Streit et al., 2005].

Para a determinação de biomassa fitoplanctônica a clorofila-*a* (CHL-*a*) é amplamente utilizada é o principal pigmento lipossolúvel responsável pela fotossíntese. As clorofilas somam aproximadamente 10 tipos conhecidos [Proença, 2006]. Apesar de todos esses grupos lipossolúveis (incluindo carotenos e xantofilas) apresentarem arranjos

de carbono muito similares, há diferenças estruturais nítidas que conferem a essas moléculas características apolares [Roy et al., 2011].

A CHL-*a*, por exemplo, apresenta um complexo coordenado de magnésio [Scheer, 2006] (Figura B.) contendo anéis tetrapirrólicos, acoplados ainda a cinco anéis isocíclicos e, podendo apresentar-se também ligados a Zn. Mesmo apresentando relativa variedade que acompanha a diversidade das espécies planctônicas fotossintéticas, todas as clorofilas contêm as estruturas comuns já mencionas das [Porra et al., 2011], mas conforme o estado de oxidação (por foto inibição) também podem inferir mudanças de estrutura.

Dependendo do nível de oxidação podem se apresentar em três formas. No caso da CHL-*a*, o anel de porfirina contém um grupo metil (-CH_3) que juntamente com o seu formato assimétrico confere a estrutura uma característica insaturada [Xu et al., 2001]. Por isso, variados métodos têm sido desenvolvidos ao longo do tempo para extrair CHL-*a* tanto de amostras naturais quanto de cultivo de fitoplâncton [Jeffrey & Humpfrey, 1975], comumente por meio de utilização de solventes orgânicos e quantificação por meio de espectrofotometria, fluorimetria ou cromatografia líquida.

Por também apresentarem característica insaturada os carotenos e xantofilas (Figura B.) podem ser extraídos e quantificados da mesma forma que a CHL- *a*, mesmo diferindo em estrutura e espectro de absorção da luz [Roy et al., 2011]. Enquanto a CHL-*a* absorve luz visível próxima do espectro vermelho ~ 665 nm e é vulnerável a fotooxidação [Taiz & Ziegler, 2004], os carotenos e xantofilas são em geral fotoprotetores, e absorvem luz em comprimentos de onda mais energéticos ~480nm

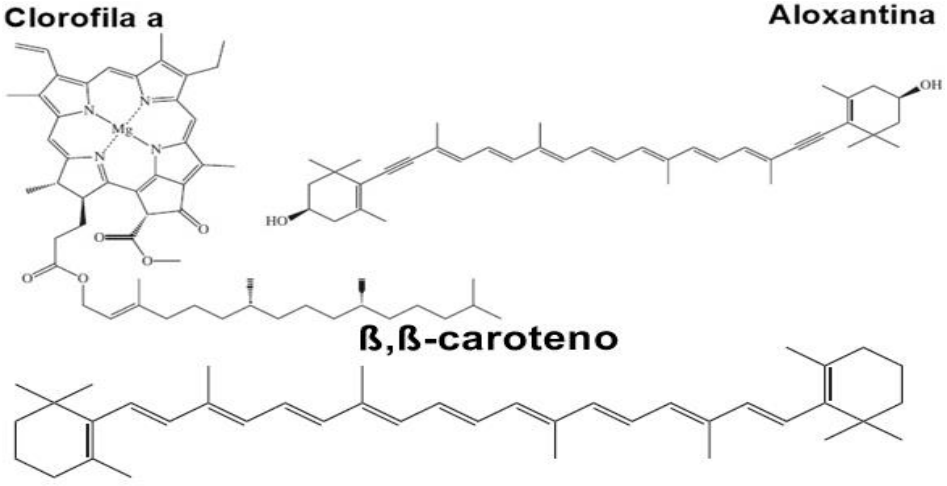


Figura B. Desenho esquemático representando as estruturas químicas da CHL-a, um exemplo de composto do grupo dos carotenos (β,β-caroteno) e das xantofilas (Aloxantina) adaptado de Roy et al. [2011].

[Porra et al., 2011]. Para tanto contam com estrutura do tipo isoprenóide em que o esqueleto de C40 é a estrutura molecular básica [Britton et al., 2004]. Apesar de carotenos e xantofilas apresentarem estruturas similares de hidrocarbonetos, os carotenos por padrão apresentam essa estrutura em sua forma pura, já as xantofilas são derivadas de carotenos oxidados. Nesse caso, pelo menos um radical contendo oxigênio foi adicionado ao esqueleto longo de carbono [Lorh, 2011].

Juntamente com a CHL- *a* e as ficobiliproteínas (pigmento hidrossolúveis restritos a alguns grupos), os carotenóides formam o principal centro de reação (fotossistema II) responsável pela fase clara da fotossíntese [Engel & Poggiani, 1991]. Nesse caso, mesmo podendo ter papel ativo durante o processo de fotossíntese, os carotenóides não são utilizados como ferramenta para índices de biomassa, pelo menos não por espectrofotometria, mas são excelentes indicadores de estresse luminoso, fotoaclimatação ou em alguns casos estresse nutricional [Jeffrey & Montoura, 1997; Roy et al., 2011].

Por isso, a quantificação de CHL-*a* é extensamente utilizada na oceanografia, tanto para estimativas de biomassa e a produção primária, quanto para determinação de estoque de carbono refratário [Weise et al., 2010]. Quanto aos carotenóides são mais utilizados em estimativas de estresse por diversos fatores, entre eles a foto-proteção ou foto-estresse [Chase et al., 2017]. Juntamente com as estimativas de outros pigmentos como as ficobiliproteínas, os pigmentos são importantes para inferências sobre o estado fisiológico das comunidades fitoplantônicas. Também indicam a ocorrência de grupos específicos e as influências de fatores como a temperatura, a salinidade e a disponibilidade de luz que podem variar conforme a sazonalidade [Rai & Rajashekhar, 2014].

1.4. Pigmentos hidrossolúveis

Alguns grupos do fitoplâncton apresentam pigmentos distintos da CHL-*a* e carotenóides associados a seus fotossistemas. Esse grupo é chamado de ficobiliproteínas e possui características muito distintas dos outros dois grupos [Spolaore et al., 2006], e ao contrário da CHL- *a* não é comum a todos os organismos fotossintéticos. Na verdade, a ocorrência desse grupo de pigmentos está restrita a poucos filos de micro- e macroalgas como as criptófitas e rodófitas e às cianobactérias [Roy et al., 2011]. Portanto, sua quantificação não está vinculada a estimativas de biomassa, mas sim à ocorrência

daqueles grupos específicos, bem como a indicação de estresse luminoso ou foto aclimatação [Christaki et al., 2015].

Esse grupo de pigmentos é dividido em três tipos de molécula, mas que possuem a mesma base proteica com ligação covalente entre os cromóforos de cisteína-aminoácidos chamados ficobilinas, pertencentes aos tetrapirroles de cadeia aberta [Eriksen, 2008] (Figura C). Essas estruturas diferem apenas em propriedades espectrais e no arranjo dos radicais em função dos aminoácidos centrais [Tandeau de Marsac, 2003]. Também absorvem energia luminosa no assim chamado "*green gap*", onde a CHL- *a* absorve apenas parcialmente [Sidler, 1994].

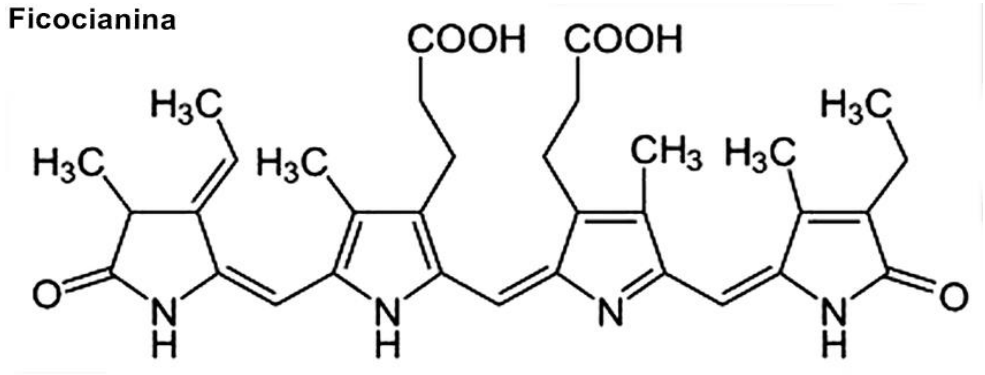


Figura C. Exemplo da estrutura química da ficocianina desmonstrando a base proteíca do pigmento e arranjos dos radicais do H_3C modificado de Hosseini et al. [2013].

Os três pigmentos desse grupo são responsáveis pela absorção nos comprimentos de onda próximos aos espectros verde e azul da luz visível [Hong Zhao et al., 2011]. Juntos formam o chamado complexo antena, localizado na membrana do tilacóide e constituído pela ficoeritrina, responsável pela absorção de luz a 565 nm, pela ficocianina (a 620nm), e aloficocianina (a 650nm). Em rodófitas e cianobactérias esse complexo antena é organizado em estruturas específicas chamadas ficobilissomos, já em criptófitas esses pigmentos parecem estar dispersos na membrana, mesmo que a causa dessa diferença ainda não seja clara [Roy et al.,2011].

Diferentemente dos cromóforos da CHL-*a* que ocorrem de maneira isolada e são eficientes receptores de luz, as ficobilinas não são receptores fotossintéticos muito eficientes, e em geral se desnaturam facilmente [Hong Zhao et al., 2011]. Porém o arranjo no qual são apresentadas (ficobilissomos) favorecem o balanço quantitativo entre cada pigmento. Logo em situações de mudanças drásticas de luz são uma poderosa ferramenta adaptativa. Assim mesmo em situações de limitação luminosa, ou mesmo superexposição

a luz, a fotossíntese pode ser mantida em níveis ótimos por meio de maior sintetização desses pigmentos por parte do organismo [Roy et al., 2011].

Os resultados da quantificação desses pigmentos são bons indicadores tanto da ocorrência dos grupos que produzem esses compostos, quanto do estado fisiológico em que essas comunidades se encontram no ambiente [Wang et al., 2007]. Portanto a quantificação desses pigmentos em amostras naturais pode auxiliar a entender a dinâmica das comunidades fitoplanctônicas ao longo do tempo, bem como interações com o meio ambiente tais como flutuações de temperatura, salinidade, nutrientes e luz [Grossman et al., 2003].

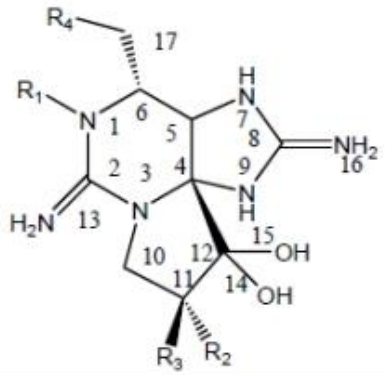
1.5 Organismos produtores de saxitoxinas e seu análogos

A capacidade de síntese de compostos tóxicos por organismos fitoplânctônicos autotróficos e heterotróficos é em geral abordada quando da ocorrência de florações, e suas implicações econômicas e ecológicas. No entanto, a produção de ficotoxinas não se deve apenas a eventos de densidade massiva desses organismos [Hallegraeff, 2010]. Além disso, mudanças em fatores ambientais, como as interações ecológicas parecem ser gatilhos para a produção de toxinas, principalmente em diatomáceas e dinoflagelados [Visciano et al., 2016].

Pelo menos 100 diferentes espécies de fitoplâncton marinho são registradas como produtoras de toxinas [Moestrup et al., 2009]. Dentre elas se destacam gêneros de dinoflagelados produtores de saxitoxinas e toxinas diarréicas algumas diatomáceas produtoras de ácido domóico. No caso de cianobactérias apenas um gênero é ligado à produção de saxitoxinas, mas como pelo menos quatro espécies de ocorrência no Brasil, não sendo claro qual delas pode ser responsável por esses registros [Proença et al., 2009].

As toxinas listadas como saxitoxinas fazem parte de um grupo de mais de 58 compostos estreitamente correlacionados, que historicamente foram agrupados e nomeados dessa maneira pelos sintomas demonstrados por humanos e mamíferos e portanto, listados como toxinas paralíticas [Farabegoli et al., 2018]. Porém, recentes avanços nos estudos sobre as estruturas químicas desses compostos demonstraram algumas diferenciações ou ramificações entre essas estruturas. Todos os compostos presentes nesse grupo possuem um esqueleto de tetrahidropurinas, que são diferenciadas de acordo com a posição dos radicais, mesmo assim, todos são lipossolúveis. Por

exemplo, a saxitoxina (STX), (Figura D) que é um alacalóide não terpeno, faz parte desse grupo juntamente com seus outros análogos como as goniautoxinas (GTX), neo-saxitoxina (Neo-STX), decarbamoil-saxitoxina (dc-STX), decarbamoil-neosaxitoxina (dc-NeoSTX), decarbamoil- goniautoxinas (dcGTXs) e outros [Wiese et al., 2010].



Division	Name [a]	R1	R2	R3	R4
Carbamate	STX	H	H	H	$OCONH_2$
	NeoSTX	OH	H	H	$OCONH_2$
	GTX1	OH	OSO_3^-	H	$OCONH_2$
	GTX2	H	OSO_3^-	H	$OCONH_2$
	GTX3	H	H	OSO_3^-	$OCONH_2$
	GTX4	OH	H	OSO_3^-	$OCONH_2$
	GTX5 (B1)	H	H	H	$OCONHSO_3^-$
Decarbamoyl	dcSTX	H	H	H	OH

Figura D. Estrutura química básica da STX e parte de seus análogos adaptado de Cusick &Sayler [2013].

A produção de STX em ambiente marinho é restrita a algumas espécies de dinoflagelados, e recentemente também associadas à ocorrência de cianobactérias do gênero *Trichodesmium* [Proença et al., 2009; Detoni et al., 2016a; Bif et al., 2019]. Entre os dinoflagelados é documentada em pelo menos três gêneros (*Alexandrium*, *Gymnodinium* e *Pyrodinium)* que são conhecidos desde a década de 70 por produzirem STX, compondo inúmeras espécies [Fuentealba et al., 1981]. Sua ocorrência é cosmopolita e foi registrada na Costa do Oceano Pacífico Norte e Sul, Mediterrâneo e Atlântico [James et al., 2010]. No Atlântico Sul, as ocorrências de *Gymnodinium catenatum* e *Alexandrium* spp. foram registradas na costa Sul-Sudeste do Brasil, no Uruguai e toda a costa da Argentina. Já *Pyrodinium bahamense* tem ocorrência registrada apenas na costa norte do Uruguai [Band-Schmidt et al., 2019].

No Brasil, assim como no restante do contorno da América Latina banhada pelo Atlântico Sul, o registo dessas espécies é limitado a poucos estudos, em geral, estudos taxonômicos e ecológicos, ou estudos da quantificação de toxinas ligadas a florações comuns em cultivos de bivalves [Pereira Alves et al., 2018]. Desde 1997, pelo menos uma espécie de *Gymnodinium* sp. foi registrada como responsável pela produção de STX na costa brasileira [Proença et al., 2001], embora o registro de espécies como *Gymnodinium catenatum* date da metade do século XX [Odebrecht et al., 2002]. Desde o primeiro registro em 1966, a distribuição de *G. catenatum* tem se expandido do Sudeste para o sul da Costa brasileira [Band-Schmidt et al., 2019].

Outros dinoflagelados ligados à produção de STX pertencentes ao gênero *Alexandrium* também têm expandido sua distribuição, registrados primeiramente no Uruguai, passando a ser encontrados também na costa brasileira, em direção ao Rio de Janeiro e Bahia [Band-Schmidt et al., 2019]. Das sete espécies do gênero que ocorrem em águas brasileiras, pelo menos duas são produtoras de STX: *Alexandrium tamarense/catenella* e *Alexandium minimum,* que têm sido ligadas a eventos tóxicos desde 1996 na costa do Rio Grande do Sul [Perish et al., 2006], mas atualmente têm sua distribuição registrada também na costa de Santa Catarina, assim como Rio de Janeiro e chegando até a Bahia [Menezes et al., 2018].

Mesmo com o aparente crescimento e expansão na distribuição desses gêneros ao longo da costa brasileira, estudos sobre produção/concentração de toxinas continuam limitados [Nabout et al., 2015]. Parte dessa restrição encontra-se nos próprios métodos de quantificação dessas toxinas que têm limitações de custos e equipamento como a cromatografia líquida de alta performance (HPLC), ou de limite de detecção e determinação de análogos com o ensaio enzimático (ELISA). Mesmo com essas limitações, a quantificação de toxinas por HPLC oferece dados confiáveis e a possibilidade de estabelecimento de padrões de composição de toxinas [Band-Schmidt et al., 2019].

Os estudos sobre a composição de toxinas em amostras naturais têm demonstrado a ocorrência de GTX1-4, Neo-STX, dc-STX e STX, ligadas a espécies de cianobactérias e dinoflagelados [Proença et al., 2009; Band-Schmidt et al., 2019]. No entanto, a proporção em que os análogos se apresentam pode variar, mas em geral está ligada à origem e à distribuição geográfica dos organismos produtores. Esse aspecto também está associado à composição do fitoplâncton local, além da proporção entre as próprias

espécies produtoras de STX [Band-Schmidt et al., 2010]. Alguns fatores bióticos, como a co-ocorrência de espécies e o decaimento por reação enzimática também podem afetar a proporção entre a concentração de STX e a diferenciação dos análogos [Farabegoli et al., 2018] e devem ser levadas em consideração. Juntamente com interações com outros membros da biota local, as mudanças de temperatura, salinidade e radiação solar podem causar mudanças entre a proporção de análogos tornando o estudo dessas variáveis ambientais ainda mais importante do ponto de vista ecológico e oceanográfico.

1.6 Ácido domóico e organismos produtores

Diferentemente de outras neurotoxinas como as STX, o ácido domóico (AD) e seus análogos são moléculas de aminoácidos cristalinos solúveis em água. Também contém cromóforos do grupo carboxila (-COOH), que agem como locais de absorção de luz na região do ultravioleta ~212 nm [Bates et al., 1998]. A estrutura do ácido domóico é formada por uma amina secundária com três grupos carboxílicos e uma ramificação da cadeia insaturada C8 ligada à posição C4 [Garcia-Altares, 2017] (Figura E). Assim como a STX, o AD também apresenta análogos, mas com diferenças na cadeia lateral do octadieno.

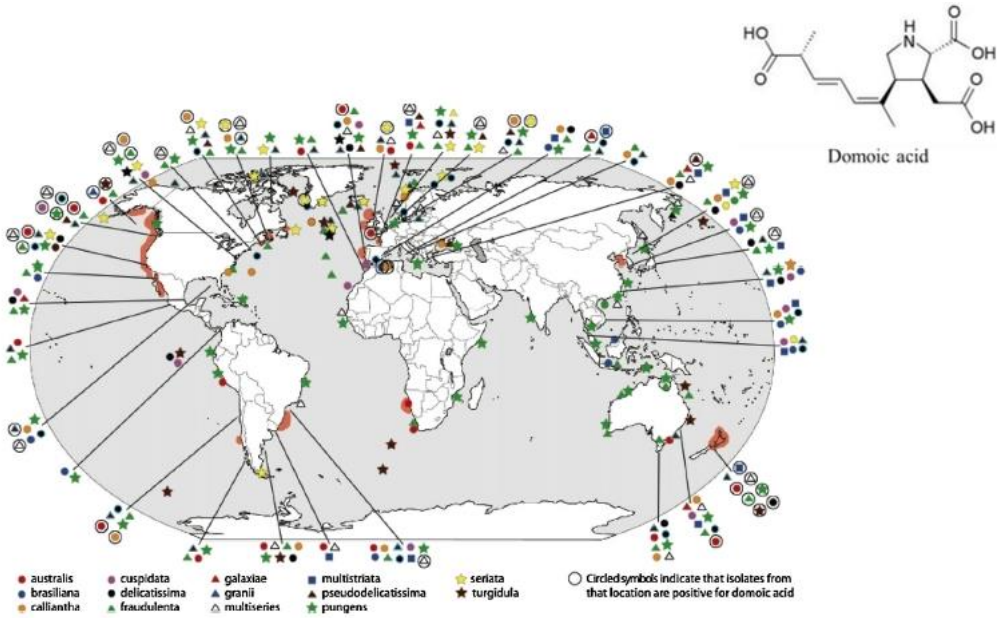


Figura E. Estrutura química do AD (canto superior direito) de Cusick &Sayler [2013] e distribuição de espécies toxigênicas de *Pseudo-nitzschia* registradas até o ano de 2012 [Trainer et al., 2012]. Áreas em vermelho no mapa representam interdição da produção de mariscos por níveis elevados de AD (>20µg g^{-1}).

Organismos do filo Bacillariophyta, especificamente da classe Bacillariophyceae, são responsáveis pela síntese dessa toxina de origem natural [Bates et al., 2018]. Dentro desse grupo, pelo menos dois gêneros (*Nitzschia* e *Pseudo-nitzschia*) são historicamente

ligados tanto a eventos de floração, quanto à quantificação de AD. A lista de organismos produtores vem aumentando desde o primeiro registro da existência do AD em 1987 [Bates et al., 1989]. A partir de então, inúmeros estudos tiveram por objetivo não só quantificar o AD, mas também evidenciar quais grupos de diatomáceas são responsáveis pela produção dessa toxina, bem como seu efeito no ambiente [Hanic, 2014].

Até 2012 pelo menos 37 espécies de *Pseudo-nitzschia* foram descritas, das quais 14 foram listadas como produtoras de AD [Lelong et al., 2012]. No entanto, nos últimos 8 anos, pelo menos 15 novas espécies foram adicionadas a esta lista, elevando o número de espécies descritas para 52, e dessas, pelo menos 26 são tidas como toxigênicas até o momento [Bates et al., 2018]. Já o gênero *Nitzschia* apresenta apenas duas espécies amplamente conhecidas como toxigênicas, *N. navis-varingica* e *N. bizertensis,* havendo variações na produção de AD dependentes de variáveis ambientais locais [Trainer et al., 2012].

Mesmo que produzam as mesmas toxinas, há diferenças diagnósticas características entre os dois gêneros. Os organismos membros de *Pseudo-nitzschia* são diatomáceas penadas que possuem simetria bilateral e ao contrário de *Nitzchia*, formam longas cadeias de células [Hasle, 1994]. Essas variam em comprimento, dependentes da turbulência local, bem como da disponibilidade de nutrientes. A identificação a nível de espécie por microscopia óptica é dificultosa. Em específico, apenas ultraestruturas presentes nas frústulas são diagnósticas, e sua visualização é permitida apenas por técnicas de microscopia de transmissão ou varredura [Lelong et al., 2012]. Para facilitar a morfometria em microscopia óptica e permitir uma identificação posterior mais precisa, o gênero *Pseudo-nizstchia* é separado em dois complexos com base no eixo transapical (largura) da frústula: *seriata* (>3 µm) e *delicatissima* (<3 µm) [Hasle & Syvertsen 1997].

Dentro do complexo *seriata,* pelo menos 5 espécies de *Pseudo-nitzschia* foram registradas na costa brasileira até 2012, sendo elas *P. australis, P. calliantha, P. fraudulenta, P. pungens* e *P. multiseries.* Já a variedade de espécies do complexo *delicatissima* em águas brasileiras é relativamente menor, contando com *P. brasiliana, P. delicatissima* e *P. cuspidata* [Lundholm et al., 2002]. No entanto, ainda há lacunas sobre as espécies de *Nitzschia* produtoras de AD que ocorrem tanto na costa brasileira quanto no oceano adjacente. Até o momento, o registro da espécie *Nitzschia navis-varingica* está restrito a parte do Pacífico e em ambiente lagunar do Mediterrâneo, embora haja

evidências de que sua distribuição é cosmopolita e ocorra também em oceanos tropicais do mundo todo [Bates et al., 2018].

Embora o gênero *Pseudo-nitzschia* seja um dos mais intensamente explorados cientificamente, estudos envolvendo AD na região costeira e oceânica ainda são limitados, tanto em nível global quanto nacionalmente. No Brasil, eventos tóxicos têm sido ligados à ocorrência de *Pseudo-nitzschia* desde 2009 [Fernandes & Brandini, 2010], mas a quantificação de AD está em geral ligada a complexos estuarinos [Mafra Jr. et al., 2006] ou regiões costeiras de cultivo de mariscos [Fernandes et al., 2013]. Os trabalhos ligados à região costeira ou oceânica no Brasil são escassos, sendo que o primeiro registro distante de sistemas de cultivo e em amostras de plataforma continental próximo ao RJ (sudeste brasileiro) é recente [Costa et al., 2019].

As limitações no registro e na análise de AD se devem em parte à própria natureza da toxina. Por se tratar de um composto hidrossolúvel, em geral sua concentração decai facilmente [Silver et al., 2010]. Esse processo é facilitado em águas onde a ação de mistura é ativa, ou onde a camada de mistura é mais profunda, e onde diatomáceas penadas como as *Pseudo-nitzschia* são abundantes e, até em alguns casos, parte dominante do fitoplâncton [Jager et al., 2008]. No entanto, a concentração de AD pode diminuir por meio de outros processos de remoção, como foto-degradação, ação bacteriana e absorção por ácidos húmicos em fase coloidal, tanto em águas costeiras quanto oceânicas [Lail et al., 2007].

Por geralmente não se acumular na coluna d'água, a concentração em amostras naturais é baixa [Lelong et al., 2012]. Portanto, as técnicas de detecção utilizadas na quantificação de AD devem ser sensíveis. Por esse motivo, o método mais utilizado para quantificação é o HPLC, que além de se tratar de um método sensível, conta com padrões certificados para a detecção e a quantificação de AD. Além disso a presença de cromóforos (-COOH) na estrutura da toxina facilita a leitura em detector UV [Trainer et al., 2012] que é amplamente utilizado em trabalhos internacionais.

Considerando o crescente interesse científico internacional sobre os grupos de fitoplâncton produtores de toxinas, e a importância de diatomáceas, dinoflagelados e cianobactérias nos ecossistemas marinhos brasileiros, o presente estudo buscou discutir e associar a comunidade de fitoplâncton a características físicas da coluna de água em partes da costa brasileira (sul e sudeste), além de quantificar (toxinas) e discutir a presença de espécies tóxicas associadas à produção de dois grupos de toxinas (STX e AD), que

podem ter importante impacto sobre a diversidade local nas áreas de estudos. O primeiro manuscrito apresentado no capítulo V se refere a ambas toxinas e organismos produtores na região costeira do Rio de Janeiro. Já o segundo manuscrito se refere apenas à presença de cianobactérias do gênero *Trichodesmium* e sua possível associação com as concentrações de STX quantificadas ao longo do talude continental Sul-Sudeste e possível contribuição de dinoflagelados.

CAPÍTULO II: OBJETIVOS

Geral

Discriminar pigmentos lipossolúveis e hidrossolúveis em organismos fitoplanctônicos marinhos produtores de toxinas.

Específicos

- Quantificar e qualificar os pigmentos produzidos pelo fitoplâncton;
- Relacionar a estrutura da comunidade à estruturação da coluna d'água;
- Caracterizar a comunidade planctônica na região de estudo;
- Determinar a ocorrência e abundância de organismos fitoplantônicos tóxicos;
- Realizar estimativas da concentração de toxinas.

CAPÍTULO III: ÁREA DE ESTUDO

A área de estudo compreendeu a região sul e sudeste da costa oeste do Atlântico Sul, acompanhando a feição costeira próxima ao Rio de Janeiro durante a execução do projeto "Bonito Listrado". Este ocorreu durante o inverno (24 e 25 de junho) e primavera (24 e 25 de setembro) de 2018, entre as latitudes 23º43'S e 22.6º41'S. Também foram incluídas amostragens realizadas durante o curso do projeto "TALUDE-V", referentes à primavera de 2012 entre os dias 11–24 de novembro, entre as latitudes 28°40'–23°33'S, compreendendo a região do talude ao largo do Cabo de Santa Marta e Cabo Frio. Uma

representação das duas áreas de estudo é apresentada na Figura F, e mapas detalhados são apresentados ao final de cada sessão do Capítulo V “Artigos científicos”.

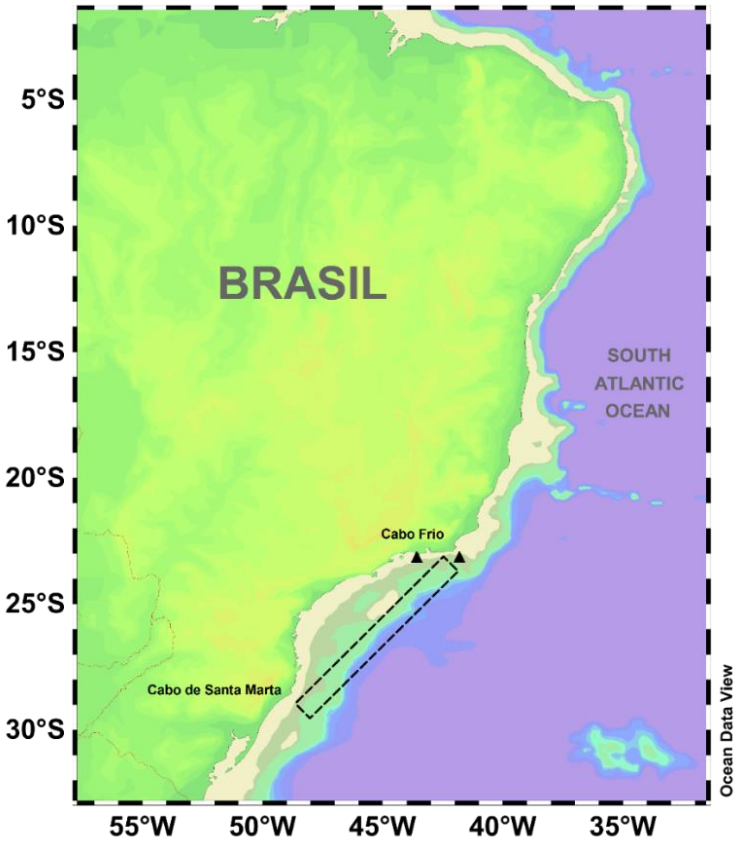


Figura F. Área de estudo no Oceano Atlântico Sul-Sudeste onde o retângulo pontilhado representa as amostras coletadas durante a primavera de 2012 pelo projeto "TALUDE-V”. Triângulos preenchidos representam os limites das estações amostradas na região costeira próxima ao Rio de Janeiro durante o inverno e primavera de 2018 pelo projeto “Bonito Listrado”.

CAPÍTULO IV: MATERIAL E MÉTODOS

4.1 Análise de parâmetros ambientais

Os dados de temperatura superficial (SST) e salinidade superficial (SSS) foram obtidos através do perfil vertical com um sistema CTD (Conductivity, Temperature and Depth) SEABIRD 925® acoplados com um sensor de fluorescência e oxigênio (OD) dissolvido (WetLab Eco-RTD®). A densidade potencial da água do mar foi obtida com base nos perfis de temperatura e salinidade. Através da derivada vertical destes perfis, a profundidade da camada de mistura (MLD) foi estimada segundo Kara et al. [2000].

4.2 Coleta, processamento e análise de amostras por microscopia

Amostras de fitoplâncton foram coletadas em camada superficial para estimativa de densidade de células fitoplanctônicas e/ou tricomas de cianobactérias. Todas as amostras referentes ao cruzeiro realizado pelo projeto TALUDE-V (2012) foram

coletadas e analisadas segundo Detoni et al. [2016a]. Já para o conjunto de dados referente aos cruzeiros financiados pelo projeto “Bonito Listrado” de 2018, amostras superficiais foram coletas com auxílio de um recipiente plástico de 2L e homogeneizadas com auxílio de um Becker® plástico. Alíquotas de 150ml foram então acondicionadas em frascos de vidro ambâr e fixadas com uma solução 1-2 % de Lugol acidificado [UNESCO, 2010], com o objetivo de romper as vesículas de gás de células de cianobactérias e facilitar a contagem em microscopia. A identificação e a abundância de células e tricomas foram estimadas pelo método de Utermöhl [1958] e [UNESCO, 2010], em câmara de sedimentação com auxílio de um microscópio invertido (Axiovert.A1 ZEISS) com imagens obtidas por meio de uma câmera acoplada (AxioCam MRc). A câmara toda foi analisada para ocorrência de organismos maiores que 20-50 µm, e transectos ou campos aleatórios (mínimo de 30) para células menores. Um número mínimo de 100 espécimes foi contado para cada uma das categorias de tamanho para manter os níveis de erro inferido menores que 20% [Lund et al., 1958]. Todos os espécimes foram identificados a nível de espécie quando possível ou até o menor nível taxonômico. Os flagelados foram definidos a nível de classe. Para o cálculo de biomassa em carbono, os espécimes foram medidos e, para cada formato celular, uma fórmula geométrica similar ao formato foi aplicada [Hillebrand et al., 1999; Sun & Liu, 2003; Olenina et al., 2010]. Por fim, a biomassa em carbono para cada espécie/morfoespécie foi determinada por meio das equações de Montagnes & Franklin [2001] para diatomáceas, Montagnes et al. [1994] para dinoflagelados, Menden-Deuer & Lessard [2000] para outros taxa, exceto cianobactéria, para a qual foi utilizado o fator proposto por Carpenter et al. [2004]. Para ciliados, o modelo utilizado foi o proposto por Putt & Stoecker [1989]. A biomassa em carbono dos autotrófos/mixotrófos e a biomassa em CHL-*a* foram utilizados para cálculo da razão em C:CHL-*a*.

O status taxonômico de todas as espécies foi verificado com base no banco de dados online Algae Base page [Guiry & Guiry, 2018] e cruzados com dados de espécies potencialmente produtoras de toxinas pela Lista de Referência Taxonômica para Algas Nocivas da IOC-UNESCO [Moestrup et al., 2009].

4.3 Pigmentos lipossolúveis: CHL-*a* e carotenóides

A biomassa de CHL-*a* foi obtida por meio de um espectrofotômetro UV-Vis. Primeiramente, alíquotas de água da camada superficial foram coletadas utilizando um recipiente de plástico de 2 L, homogeneizadas com auxílio de um Becker® 200 mL, e então filtradas. Filtros GF/F (Whatmann®-porosidade 0.7mm) foram utilizados juntamente com aparelho de filtragem a vácuo Millipore® com diâmetro de *holder* de 25 mm e seringa de 100mL. O filtrado resultante foi acondicionado em papel alumínio, no escuro a -20^{o}C até o momento da extração. A fração de pigmentos lipossolúveis foi extraída utilizando metanol e clorofórmio tamponado com ($MgCl_2$) (1:3:2) [Folch et al., 1957], seguido de ultrassonificação (ILSHER – modelo UP100H) em 3 ciclos de 45 s (200W, 24 kHz) [Dey & Rathod, 2013] até a completa destruição do filtro. O extrato resultante foi então mantido no escuro à -20 ^{o}C durante 24 horas. Das duas fases consistentes da mistura, apenas a apolar foi analisada. A leitura foi conduzida em um espectrofotômetro Varian-50 UV-Vis em 665nm para CHL-*a* e 750 nm (para exclusão de qualquer interferência causada pela turbidez) e 416, 435, 485 nm para carotenóides. A concentração final foi expressa em µg L^{-1} seguindo as equações de Britton [1985, 2008].

4.4 Pigmentos hidrossolúveis: ficoeritrinas, ficocianinas e aloficocianinas

O procedimento de filtragem para amostras destinadas a quantificação de pigmentos hidrossolúveis foi o mesmo adotado para estimativas de CHL-*a*. As determinações de concentração também foram conduzidas em um espectrofotômetro Varian-50 UV-Vis. No entanto, o método de extração adotado foi adaptado e aplicado de acordo com Tandeau de Marsac & Houmard [1988, 2003]. Primeiramente, os filtros GFF Whatman® (0.7 porosidade) foram acondicionados em placas de petri (60 × 15mm), e então, imersos em 5mL de solução tampão de acetato de sódio (20 mM pH 5,5) juntamente com 0,5mL de solução de sulfato de streptomicina 1% (p/v). A extração mecânica foi desempenhada por meio do posicionamento das placas em um agitador orbital a 50-54 rpm durante 24 horas. O extrato resultante foi então levado à centrífuga por 1 min a 10000 rpm e 4^{o}C. As análises espectrofotométricas foram conduzidas a 565, 620, 650 e 750 nm utilizando o tampão como branco. A concentração final foi expressa em µg L^{-1} ou mg ml^{-1}, e calculada segundo as equações Bennet & Bogorad [1973] e Bryant et al., [1979].

4.5 Fração particulada de STX

Para a determinação de saxitoxinas foi aplicado o método cromatográfico adaptado de Rourke et al. [2008]. A etapa de filtragem seguiu os passos previamente mencionados, mas os filtros utilizados foram de nylon de 25 mm (porosidade nominal relativa a rede de fitoplâncton de 100µm), com volume total do filtrado entre 60 e 180 mL, que foi congelado e envolto em papel alumínio, mantido no escuro a -20ºC até o momento da análise. Para a extração, o material resultante do processo de filtragem foi imerso em uma solução 0,05 M de HCl, por no mínimo 24 horas seguido de extração mecânica em ultrassonificador, onde 3 ciclos de 30 segundos foram intercalados com 30 segundos de intervalo. A extração mecânica foi então seguida por uma etapa de centrifugação de 5 minutos a 6000 rpm. Após isso, o sobrenadante foi separado e filtrado em filtros de acetato de celulose, e por fim, analisado em HPLC. O cromatógrafo usado foi o modelo Shimadzu LC20 com um controlador CBM 20A, acoplado a um detector de fluorescência RF-10Axl (330 nm excitação e 390 nm emissão), duas bombas binárias LC-20AD e um forno para coluna CTO-20A com temperatura controlada a 35°C com utilização do software Labsolution 5.41.240. Já para a pós-derivatização, uma bomba quaternária LC-10AD de controle manual foi utilizada juntamente com um sistema de banho (Novatecnica, modelo NT 245) mantido a 80°C onde as mangueiras de Teflon permaneceram imersas. A coluna utilizada foi Agilent® Zorbax Bonus-RP C8 (150 × 4.60mm, 5µm). As amostras foram imersas na fase móvel específica (eluente) e, então, injetadas manualmente com um injetor Rheodyne, com um loop de 100 µL. A fase móvel ou eluente A (GTX 1-5) consistiu em uma solução de heptanosulfonato 11 mM e de ácido fosfórico 5,5 mM aferidos a um pH de 7,1. Já o eluente B (Neo-STX, dc-STX, STX) foi constituído de heptanosulfonato 11 mM e ácido fosfórico 16,6 mM também aferido a um pH de 7,1, onde acetonitrila 11,5% também foi adicionada. A taxa de fluxo foi mantida em 1 mL/min e o gradiente de eluição em 15 min. A derivatização pós-coluna foi alcançada utilizando uma solução de ácido periódico 7 mM e hidrogenofosfato dipotássico trihidratado 10 mM e estabilizada com ácido acético. Para a análise da fração particulada de STX, foram considerados os padrões dos análogos da GTX (GTX-1, GTX-2, GTX-3, GTX-4, e GTX-5) e 3 STX (Neo-STX, decarbamoylsaxitoxina (dc-STX) além da STX. Todas as análises foram conduzidas em triplicatas e a concentração final na amostra foi determinada pelo total de todas as variantes, levando em consideração a toxicidade de relativa de cada análogo com a concentração total em STX equivalente

(STX eq) [Oshima, 1995]. Todos os padrões e material de referência foram obtidos do National Research Council Canada (NRC). Os limites de detecção e quantificação do instrumento para STX foram estimados através da razão sinal/resíduo considerando pelo menos 3 a 10 vezes a razão do sinal (área do pico) pelo resíduo (linha de base). De maneira complementar, também foi considerado o método visual [ANVISA, 2003; Ribani et al., 2004]. O LD foi 0,01 µg L^{-1} e o LQ 0,09 µg L^{-1} e os brancos foram avaliados em triplicata.

4.6 Fração particulada de AD

Para a quantificação da fração particulada de AD, alíquotas de água de superfície foram coletadas utilizando um recipiente de plástico de 2L, homogeneizadas com auxílio de um Becker® 200 mL, então filtradas com auxílio de sistema de filtragem Millipore® 25 mm e filtros de nylon (porosidade referente a rede de fitoplâncton de 100µm), onde um volume de 60-180 ml de água foram filtradas, e a fração retida foi então embrulhada em papel alumínio e armazenada no escuro a -20 ^{o}C até o momento da análise. A fração particulada foi submetida a extração por meio da adição de solução de acetonitrila a 10% [Proença & Oliveira, 1999], e o resultante submetido a ultrassonificação em 3 ciclos de 1 minuto (1 min de intervalo), para rompimento total das células. O superaquecimento da amostra foi evitado por meio da imersão do recipiente em banho frio. Em seguida o material foi centrifugado durante 10 minutos a 5000 rpm, e o sobrenadante separado e filtrado em filtros de acetato de celulose (0,45µm de porosidade) para posteriores análises em Cromatografia Líquida de Alta Performance (HPLC) de acordo com Miguez et al. [1996].

A análise foi conduzida em um cromatógrafo Shimadzu LC20 equipado com um controlador CBM 20, e detector UV-DAD (Diode Array Detector) acoplado a uma lâmpada de deutério (D2) com emissão UV em 242 nm. Duas bombas binárias LC-20AD e uma coluna de forno CTO-20 a 40°C também foram utilizadas, em conjunto com o software Labsolution 5.41.240. A coluna analítica utilizada foi C-18 Phenomenex Luna (250×4.6 mm, 5µm). As amostras foram diluídas nas respectivas fases móveis (eluentes), que foram injetadas por meio de um injetor Rheodyne, com um loop de 20µL. Padrões comerciais específicos (NRC-CNRC® Canada) foram analisados e as concentrações estimadas, baseadas nas áreas e tempo de retenção dos picos obtidos com base nos mesmo parâmetros dos padrões comerciais. As fases móveis consistiram em duas soluções, uma de Milli Q acidificada com ácido trifluoroacético (TFA) 0,1% e acetonitrila acidificada

com o mesmo composto. O fluxo foi mantido em 1mL min^{-1}, em um tempo de total de corrida de 20 min com tempo de retenção entre 7 e 8 min. Todos os reagentes utilizados eram próprios para HPLC, bem como água ultrapura proveniente de um sistema tipo Milli Q. Os limites de detecção (LD) e de quantificação (LQ) do equipamento foram estimados através da razão sinal/resíduo considerando pelo menos 3 a 10 vezes a razão do sinal (área do pico) pelo resíduo (linha de base), e de maneira complementar também foi considerado o método visual [ANVISA, 2003; Ribani et al., 2004]. O LD foi 0,001 µg L^{-1} e o LQ 0,003 µg L^{-1} e os brancos foram avaliados em triplicata.

4.7 Análises estatísticas

Médias de CHL- *a* derivadas de fluorímetro (até 5 metros de profundidade) e de CHL-*a* quantificada através de espectrofotometria foram utilizadas para o teste de correlação de Spearman e regressão linear considerando o intervalo de confiança de 95% e $p<0,05$. Os mesmos procedimentos foram aplicados para as concentrações de toxina, sendo que um teste *t* (Student) de uma via foi considerado com intervalo de confiança de 95 % e $p<0,05$. Todos os modelos de regressão foram obtidos utilizando o programa GraphPad Prism 6®. Análises de agrupamento, análise de similaridades (ANOSIM), e porcentagem de contribuição para a similaridade (SIMPER) foram aplicados aos dados biológicos (abundância e biomassa fitoplanctônicas). O índice de similaridade de Bray-Curtis foi considerado para a construção de matrizes de similaridade utilizando o programa PAST-v.3® para os dados de biomassa e abundância, considerando a iteração de 1000 vezes no modelo *bootstrap*. Também foram determinados parâmetros de diversidade (Shannon H'), equitabilidade (Pielou-J), e dominância (D) utilizando o mesmo programa PAST-v.3®. Esses parâmetros foram comparados entre si, utilizando um teste não-paramétrico (Mann-Whitney, U) com intervalo de confiança de 95 % e $p<0,05$.

CAPÍTULO V: ARTIGOS CIENTÍFICOS

Artigo I submetido ao periódico *Toxicon [TOXCON-D19-00424]* aguardando parecer dos revisores

Plankton succession and their environmental drivers-an approach of toxic species and neurotoxin production over the west of South Atlantic

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Abstract

An assessment of major pigments and neurotoxins coupled with a description of phytoplankton community, within the coastal region of Rio de Janeiro State (Brazil) was carried out during winter and the following spring. Overall, six different stations were occupied for oceanographic conditions (with CTD casts). Filtered water samples were used to estimate chlorophyll *a* (CHL-*a*), carotenoids (CAR) and phycobiliproteins (PHY) by UV-Vis spectrophotometry, as well as quantification of Saxitoxins (STX) and

Domoic Acid (DA), through HPLC. Planktonic organisms were counted using sedimentation chambers of different volumes and an inverted microscope. Cluster analysis, SIMPER and ANOSIM were applied to phytoplankton data, along with diversity indexes, and non-parametric statistics to toxins and pigments. There was a significant difference between the winter and spring phytoplankton community, associated with the mixed layer (r^2= -0.626, $p<0.05$;) depth and temperature (r^2 = 0.641, $p<0.05$). Phytoplankton biomass and C:CHL-*a* revealed a higher production during the winter than in spring, being the potentially toxic genus *Pseudo nitzschia* responsible for 12.79% of autotrophic abundance (SIMPER output). Pigments showed slight increase in [CAR] during spring, while [PHY] remained at trace concentrations. Both [DA] and [STX] were quantified in winter and spring, but with significant differences only for [STX] between the sampling periods. Among 71 taxa, 11 were ascribed as potentially toxic with emphasis on [STX]-producing dinoflagellates and cyanobacteria, like *Alexandrium* sp., *Gymnodinium* spp. along with *Trichodesmium* spp. Season-related environmental variability might have been the major driving force modulating the mixed assemblage of species, that tended to correspond to different toxins.

Keywords: Tropical coastal region; Plankton succession; Phycotoxins composition; Pigments; Environmental variability.

1. Introduction

The southwestern Atlantic Ocean along the Brazilian coastline can be characterized as a region of constant hydrodynamics and water redistribution, mainly due to features such as eddies from Brazil Current (BC) (Silveira et al., 2000), intrusion of the South Atlantic Central Water (SACW) along the continental shelf and costal upwelling (Castro et al., 2006). In a spatial and temporal point of view (Castro et al., 2006), all of

these oceanographic features confer complex oceanographic processes on that area, especially as the plumes coming from Guanabara Bay and Sepetiba affects the dynamic of the inner Brazilian Continental Shelf (Soares & Moller Jr et al., 2001; Coelho et al., 2010). For instance, the plume of Guanabara Bay spreads its waters as low salinity fronts up to 120 km offshore (Moser et al., 2016).

Those circulation processes that occur along the Brazil coastline favor a drop of salinity on the superficial layer of the water column, mainly during summer in the Cape Frio (Stevenson, 1998; Souza & Robinson, 2004). At the imminent arriving of the spring-summer season, the superficial waters in this region gradually became warmer, slightly saline and more stratified as a result of the [BC] intrusion as the current flows towards south in the offshore region (Campos et al., 1994).

All of those conditions constrain variations to the local biota. Since primary producers usually structure and define food webs, it is important to know what influences this trophic level, whose biomass is controlled by atmospheric, oceanic and geomorphological variables and biological factors (Coelho-Souza et al., 2012). The biotic interactions within the plankton community in an environment as dynamic as this, in general, are ruled by competition for resources, mainly nutrients in low concentrations (Moser et al., 2014), favoring the development of pico- and nanoplanktonic members of the community (Ciotti et al., 2007; Hanson et al., 2007). Balance between diversity and biomass typically presented by this community can change due to modifications in the water column structure through seasonal shifts. For instance, intrusion of colder and nutrient-rich waters, or flow of riverine plumes, can increase the contribution and representation of larger (high biovolume) microphytoplankton, such as diatoms and dinoflagellates (Tenenbaum et al., 2006; Ribeiro et al., 2016; Gonçalves-Araujo et al., 2018).The studies already mentioned state

the occurrence and adjustment of several species in these mesotrophic layered waters, so explaining the organismal strategies to maximize competition in this place, is essential to realize how they cope with the environmental variability (Silva, 2007). For these purposes, planktonic organisms developed tools to increase competition capacity in the ecosystem and the temporal-spatial dynamics of it.

Moreover, the environment favors this process, selecting different adaptations forefront different resources available (Margalef, 1978; De Castro & Moser, 2012). As a strategy to cope with constant environmental changes, distinct phytoplankton species developed different strategies of physiological control to fulfill some determined functions (e.g. niche) in the ambient (Polechová & Storck, 2018).A widespread presence of toxins in many groups of freshwater and marine phytoplankton along with formation of secretions and aggregation for example(Hallegraeff, 1995), are forms of quick response to environmental variations (Garcia et al., 2004).

Even though the worldwide discussion about the toxic phytoplankton and toxin quantification have been increasing , works within the South Atlantic Ocean about this subject still are scarce in comparison with other regions (Noga & Gomes,2018).Still, phytoplankton related works have been increasing since 1990 (Nabout et al.,2011) especially to understanding phytoplankton attributes and how they impair and are impaired by the surrounding environment. One of those attributes are disturbance tolerance, and usually it can permit species to take advantage of the constant turbulent transport to reach different layers, not only to achieve other levels of irradiance but also to overcome nutritional limitation (Smayda & Reynolds, 2003). Other members of the plankton can present yet different approaches when it comes to cope with environmental variability, like rapidly colonization of the environment when the conditions are

favorable, or on the contrary, resistance to environment stress such as light or nutrient limitation (Smayda, 2002).

In the case of cyanobacteria, besides being tolerant to nutritional stress (Kumar Saha et al., 2003), the cell itself develops structures that permit certain regulation of the organism position in the vertical profile within the water column through the presence of air vacuoles (Bergamn et al., 2013). Apart from the capacity of sustaining itself within surface layers, cyanobacteria are capable of synthesizing accessory pigments like phycobiliproteins (Tandeau de Marsac & Houmard, 1988; Tandeau de Marsac, 2003). These pigments allow photosynthesis in a wider range of wavelengths, optimizing the energy caption under low or high light periods (Roy et al., 2011). Besides the different types of pigments, phytoplankton species are also capable of toxin synthesis and at least a hundred species of marine phytoplankton [only ~2% of all species accounted (Sournia, 1995)] can produce phycotoxins. In a broad sense, phycotoxins can be classified according to their chemical structure, and consists of ten groups: azaspiracids (AZA), brevetoxins (BTX), cyclic imines (CI), domoic acid (DA), okadaic acid (OA), pectenotoxins (PTX), saxitoxins (STX), yessotoxins (YTX), palytoxins (PITX) and tetrodotoxins (TTX), each one of them with different ecological and toxicological impacts on marine environments and produced by a plethora of diatoms, cyanobacteria and dinoflagellates (Farabegoli et al., 2018).

In Brazil the trigger to investigate the occurrence of those organisms and toxins produced by them, are usually toxin linked events and mollusk farming production (Mafra Jr et al.,2019).Even so, toxin reports in Brazilian marine waters include the occurrence of several toxins, mostly produced by dinoflagellates (Noga & Gomes,2018), like [OA], [STX] and some dinophysistoxins [DTX].

In the other hand, recently several authors across the South Atlantic Ocean have described the DA-producers *Pseudo-nitzschia* and *Nitzschia* (Proença & Oliveira, 1999; Villac et al., 2005; Olguín, 2006; Almandóz, 2007; Guinder, 2018), which have a wide geographic distribution (Hasle, 2002). Nonetheless, there still are a very few studies that quantify the [DA] content and intend to pose the toxin production on an environmental context, especially in this undersampled Brazilian ocean region (Costa et al., 2019). Other potentially STX-producers, *Trichodesmium* spp., have got their spatio-temporal distribution and ecological relationships studied in this region (Detoni et al., 2016), but there is a need for more comprehensive studies that try to combine many approaches such as the identification of a great part of the phytoplankton community and the discrimination of the types of toxins present within that community. Considering what has been mentioned so far, the main objectives of the present study are (1) the characterization of major pigments, kinds of toxins and phytoplankton assemblages collected during two contrasting environmental periods (winter and spring) from southeastern, coastal sites of the Rio de Janeiro state (Brazil), (2) the relation between the phytoplankton assemblages and the kinds of toxins, particularly the quantification of [DA] and [STX], and (3) the association of these biological features with some oceanographic variables.

2. Material and methods

2.1 Study Area

The study was developed over two expeditions to the evaluation of the shoals of *Katsuwonus pelamis* (Linnaeus, 1758) across the southeastern Brazilian shelf. Cruises took place in shallow coastal waters of the state of Rio de Janeiro, where three sampling

stations were occupied during two seasonal periods: winter (WS1, WS2 and WS3; from 24th–25th June), and spring (SS1, SS2 and SS3; from 24th–25th September) of 2018, specifically for phytoplankton samplings (Fig. 1).

2.2 Environmental parameters

Environmental parameters were measured with a CTD (Conductivity, Temperature and Depth) SEABIRD® profiler system, and additional data of dissolved oxygen (DO) and fluorescence with (WetLab Eco-RTD®) sensors attached to the system. Profiles of temperature and salinity were used to obtain the potential density of seawater, and the MLD (mixed layer depth) calculated based on the vertical density profiles (Kara et al., 2000).

2.3 Phytoplankton samples

Total phytoplankton samples were collected with 2L plastic flasks at the surface water, homogenized trough manual mixture with a 200 mL Becker® flask and then conditioned in 150 ml glass amber bottles for further estimates of abundance of cells and/or trichomes of cyanobacteria. These samples were fixed with acidified Lugol's solution (1–2%) (UNESCO, 2010), in order to collapse the gas vesicles of cyanobacteria and to estimate appropriately the abundance of their trichomes. Cell (and trichomes) identification and abundance were estimated using the inverted microscope (Axiovert.A1 ZEISS) technique (Utermöhl, 1958; UNESCO, 2010) and with images from a camera Axio Cam MRc. In general, the whole chamber was inspected for organisms larger than 20–50 µm, and transects or fields were analyzed to estimate the abundance of the smaller species. For each size fraction, at least 100 specimens (cells or colonies/trichomes) were counted to maintain the error less than 20% (Lund et al.,

1958). All specimens were measured and identified to species level as far as possible, except for the flagellates that were considered by size and/or assembled in taxonomic higher categories (very often as class). Biovolume (cell or colonies/trichomes) was estimated by applying geometric formulae to each corresponding shape/form (Hillebrand et al., 1999; Sun & Liu, 2003; Olenina et al., 2010). After that, cell/trichome carbon biomass was determined using the equations due to Montagnes & Franklin (2001) for diatoms, Montagnes et al. (1994) for dinoflagellates Menden-Deuer & Lessard (2000) for other taxa except cyanobacteria, whose data were transformed using the factor proposed by Carpenter et al. (2005) and ciliates, according to Putt & Stoecker (1989).Using the carbon biomass data derived from both autotrophic and mixotrophic species, and the CHL- *a* , que calculated the ratio C. Taxonomical status of all species was checked according to the Algae Base page (Guiry & Guiry, 2018) and toxin-producing taxa were verified and cross-checked using the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Moestrup et al., 2009).

2.4 Biomass: chlorophyll *a* and carotenoids quantification

Chlorophyll *a* biomass was quantified using a UV/VIS spectrophotometer. The water samples were homogenized and filtered onto GFF 25 mm glass fiber filters (Whatman – 0.7 pore size), using a Millipore® 25mm plastic holder filtration system coupled to a 100 mL syringe. Filters were then wrapped in aluminium foil and then, stored in the dark at -20º C until the extraction. Buffered ($MgCl_2$) chloroform and methanol (1:3:2) (Folch et al., 1957) were used to extract [CHL-*a*] and [CAR] from the filters by ultrasonication (ILSHER – model UP100H) in 3 cycles of 45s (200W, 24 kHz) (Dey & Rathod, 2013) until entire disruption of the filters and, lastly stored in the dark at -20ºC during 24 hours. Since the extraction performed consisted in 2 phases, centrifugation

was not required and only the apolar phase represented by the chloroform/methanol mixture were analyzed. The reading was conducted in a Varian-50 UV-Vis-Spectrophotometer at 665nm and 750 nm for [CHL-*a*] (the latter wavelength to exclude any remaining turbidity effect) and 416, 435, 485, and 750 nm for the quantification of carotenoids; the final concentration was expressed according to Britton (1985, 2008) in µg L^{-1}.

2.5 Quantification of phycobiliproteins

The same filtration protocols adopted for [CHL-*a*] were inflict for [PHY]. The measurements were conducted in the same equipment as the other pigments, but the extraction method was adapted from Tandeau de Marsac & Houmard (1988)(Tandeau de de Marsac & Houmard,1988). A 5 mL of buffer sodium acetate (20 mM pH 5,5) and 1 % (w/v) streptomycin sulfate 0,5 ml were added to extract phycobiliproteins from GFF glass fiber filters (Whatman – 0.7 pore size). The mechanical method of the extraction was conducted by placing the filters with the reagent in Petri dishes 60×15 mm in an orbital shaker 50–54 rpm for 24 hours. After that, samples were centrifuged for 1 minute at 10000 rpm at 4 ^{o}C. Spectrophotometric absorbances were taken at 565, 620, 650 and 750 nm, and buffered sodium acetate 20 mM was used as blank. Final phycobiliprotein concentration were obtained using the equations from Bennet & Bogorad (1973) and Bryant et al. (1979), and the values expressed in µg L^{-1}.

2.6 pSTX analysis

Saxitoxin (STX) or paralytic toxins (PSP – Paralytic Shellfish Poisoning) were determined according to Rourke et al. (Rourke et al., 2008). Samples of water were filtered as previously mentioned above, but into a 25 mm mesh filter fiber at volumes

ranging from 60–180 mL and the retained fraction was frozen until processing for particulate Saxitoxin (pSTX) analysis. Those materials were extracted from de silk filter immersed in a 0.05 M HCl solution for at least 24 hours. Total cell disruption was achieved using ultrassonification (three 30-second cycles with 30-second intervals). The material was then centrifuged for 5 minutes at 6000 rpm. The supernatant was separated and filtered on cellulose acetate filter for analysis on High Performance Liquid Chromatography (HPLC).

The [HPLC] analysis for [pSTX] was performed on a Shimadzu LC20 chromatography consisting of a CBM 20A controller, an RF-10Axl Fluorescence detector (330 nm excitation and 390 nm emission), two LC-20AD binary pumps and a CTO-20A column oven controlled at 35°C using Labsolution 5.41 software. 240. For post-derivatization reactions, a manually controlled LC-10AD quaternary pump and a bath system (Novatecnica, model NT 245) with a temperature controlled at 80°C were used, immersing the Teflon hoses. The analytical column used for the analyses was Agilent® Zorbax Bonus-RP C8 (150 × 4.60mm, 5μm).

Samples diluted in the specific mobile phase (eluent) were injected manually by a Rheodyne injector, with a 100 μL loop. The eluent A [GTX1-5] consisted of 11 mM heptanesulfonate and 5.5 mM phosphoric acid at pH 7.1. Eluent B [Neo-STX, dc-STX, STX] consisted of 11 mM heptanesulfonate and phosphoric acid 16.6 mM at pH 7.1 and contained 11.5% Acetonitrile. Flow rate was 1 mL/min and gradient elution of 15 min. The column eluents were derivatized with 7 mM periodic acid and di-Potassium hydrogen phosphate trihydrate 10 mM and stabilized with acetic acid. For the total [pSTX] analyses, analogues of gonyautoxin standards (GTX-1, GTX-2, GTX-3, GTX-4, and GTX-5) and three [STX] neosaxitoxin (Neo-STX), decarbamoylsaxitoxin, and [dc-STX] were used in addition to the saxitoxin [STX] standard. Control experiments

(mobile phase and 0.05 N hydrochloric acid) were also performed in triplicates. All standard analyses were done in triplicate. The final concentration of the sample was determined by the total of all variants, taking into account their relative toxicity, and calculating the total concentration of equivalent [STX] according to (Oshima, 1995). Standards and reference material certificates were obtained from the National Research Council Canada (NRC).

The detection (DL) and quantification (QL) limits of the instrument for [pSTX] were estimated from the signal-to-noise ratio, considering at least 3 and 10 times the signal ratio (peak area) by the baseline (noise) and in a complementary way, the visual method was considered (ANVISA, 2003; Ribani et al., 2004). The [DL] [pSTX] was 0.01 µg L^{-1}, and [QL] [pSTX] was 0.09 µg L^{-1} and blanks in triplicate were evaluated.

2.7 pDA analysis

As primarily described in (Costa et al., 2019), surface water aliquots were filtered through a mesh filter fiber at volumes ranging from 60–180 mL, and the retained fraction was frozen until processing for domoic acid particulate (pDA) analysis. These particulate fractions were extracted with 10% Acetonitrile solution (Proença & Oliveira, 1999). Total cell disruption was achieved using ultrassonification (Three 1 minute cycles with 1-minute intervals). Excessive heating of the extract was avoided by flushing the vials in ice water. Subsequently, this material was centrifuged for 5 minutes at 5000 rpm. The supernatant was separated and filtered on cellulose acetate filter for analysis on [HPLC] according to (Miguez et al., 1996). [HPLC] analysis was performed on a Shimadzu LC20 chromatography consisting of a CBM 20A controller, a UV-DAD (Diode Array Detector), with a Deuterium (D2) lamp that emits UV radiation at 242 nm, two LC-20AD binary pumps and a CTO-20A column oven controlled at 40°C

using Labsolution 5.41 software. 240. The analytical column used for the analyses was column C-18 Phenomenex Luna (250×4.6 mm, 5µm).

Samples diluted in specific mobile phase (eluent) were injected manually by a Rheodyne injector, with a 20µL loop. The specific commercial standard was analyzed (NRC-CNRC® Canada) and the concentration estimated based on the area and the retention time of the obtained peak as compared to the area and peak retention time for the commercial standard. The mobile phases consisted of Acetonitrile acidified with Trifluoroacetic acid (0.1% TFA) and Milli-Q water acidified in the same manner. The flow was 1 mL min^{-1}. The running time was 20 minutes and retention time was between 7 and 8 minutes, all [HPLC] analytical grade reagents and ultrapure water type Milli-Q system. The detection (DL) and quantification (QL) limits of the instrument for [pDA] were estimated from the signal-to-noise ratio, considering at least 3 and 10 times the signal ratio (peak area) by the baseline (noise) and in a complementary way, the visual method was considered (ANVISA, 2003; Ribani et al., 2004). The [DL] was 0.001 µg L^{-1}, and [QL] was 0.003 µg L^{-1} and blanks in triplicate were evaluated.

2.8 Statistical analysis

Means of [CHL-*a*] measured by fluorometer until the depth of 5 m and [CHL-*a*] measured in the spectrophotometer were applied into one-tailed non-parametric Spearman correlation and linear regression test with 95% of confidence intervals, at the significant level of $p<0.05$. The same was made to toxin concentrations where one-way Student (*t*) test was applied with 95% of confidence intervals, at the significant level of $p<0.05$. All these regression models and correlations were carried out with a licensed GraphPad Prism 6® where a matrix was also built, using biological data and physical

parameters to correlate using a Spearman correlation analysis. Cluster analysis, ANOSIM and SIMPER approaches were applied with the biological data. It was adopted the Bray-Curtis similarity index for construction of similarity matrices and boostrap (9999 runs) using PAST-v.3® software for abundance and biomass data restricted to autotroph and mixotroph species. Also, there were determined the diversity (Shannon-H'), Equitability (Pielou-J) and dominance (D) parameters with PAST-v.3®. These biological parameters, and concentrations of each type of toxin as well, were compared with one each other by means of non-parametric Mann-Whitney test (*U*) test with 95 % confidence intervals, at significant level of $p<0.05$.The mean of the first 5 m for temperature, salinity and [DO] for each period, was also submitted to one tailed parametric Student (*t*) test along with [MLD] values, and posteriorly compared to the mean cell density and carbon biomass of the toxic taxa using a Spearman correlation and linear regression test with 95% of confidence intervals.

3. Results

3.1 Environmental parameters

Winter stations were located close (southward) to the Guanabara Bay and Sepetiba River hydrologic system and spring stations, closer to the Cabo Frio region (northward) that is characterized by its upwelling system. During both winter and spring stations (WS and SS; Table I), sea surface temperature (SST) ranged from 21.9^{o}C to 23^{o}C, and sea surface salinity from 34.5 to 35.7. Mixed layer depth (MLD) was deeper in the winter (23–49m) than in the spring (9–10m) (Table I), with a formation of the seasonal thermocline in this latter period (Fig. 2) reflecting the significant difference ($p<0.05$) for these parameters between winter and spring. During the winter period the water column was almost entirely mixed down to the seafloor (Table I),but water column stratification

was closely related to the salinity (r^2 = 0.978, $p<0.05$).Fluorometer-derived phytoplankton biomass spanned from 0.26–1.64 (WS1-3 stations) to 0.33–0.42 (SS1-3), which was closely (r=0.81) related to the CHL-*a* values obtained with the spectrophotometric method (Fig. 3).Also it was moderately associated with salinity (r^2 = 0.579, $p<0.05$),.while there was an inverse correlation to temperature (r^2 = -0.611, $p<0.05$). There was no formation of deep chlorophyll maximum (DCM) during the winter period, but during the spring stratified water column lead to [DCM] formation around 15 m (Fig. 3). Average [DO] concentrations were slightly higher in spring (5.91–5.93 mg L^{-1}) than in winter (5.63–5.83 mg L^{-1}) (Table I) and unlike fluorescence data, were closely correlated to temperature (r^2 = 0.703, $p<0.05$) but weakly related with sum autotroph biomass (r^2 = 0.470, $p<0.05$). Furthermore, water oxygen saturation reached a maximum value of 92.26% at 4m (WS2) and minimum value of 65.72% at 1m (WS1) during winter, while there were similar differences in springtime: minimum of 68.77% at 42m and maximum value of 99.59% at 9m (SS1) (Fig. 2).

3.2 Plankton community: mainly the photoautotrophs and toxin-producers

In overall, there were 50 (71) taxa identified and counted in winter (spring) (*Supplementary Material Table S1*). From all those planktonic organisms the pennate diatom *Pseudo-nitzschia* spp. were the main contributors to the cell density in winter (54,286 cells L^{-1}) (Table II), whereas unidentified heterotrophic flagellates attained 55,730 cells L^{-1} in spring. A patch of *Trichodesmium* spp. was observed in spring, with an abundance of 543,467 trichomes L^{-1}. Besides these potentially *harmful algal bloom* (HAB) species, other potential HAB species were ascribed for the studied periods, especially from the phyla Ochrophyta (class Bacillariophyceae, in particular those DA

producers) and Miozoa (class Dinophyceae – for example, genera *Alexandrium* and *Gymnodinium*; STX producers) (Table II). In fact, Miozoa/Dinophyceae contributed for a great part of the species counted, mainly in the springtime.

A cluster analysis (CA) followed by an analysis of similarities (ANOSIM) highlighted a significant difference between winter and spring plankton community composition in terms of cell abundance (Fig.4A).Also, with an analysis of percentage similarity (SIMPER) the global average dissimilarity was 56.15%, excluding the spring sample S11 for representing an outlier (*Trichodesmium* spp. bloom). In wintertime, the [CA] dendrogram based on carbon biomass data gave similar clusters as the same dendrogram based on cell abundance. The centric diatoms *Thalassiosira* spp. and *Paralia sulcata* were the major contributors in the phytoplankton community. While, in spring period, *Pseudo-nitzschia* spp. (potential DA producers) were the most abundant organisms, especially in the samples S01(SS1) and S09(SS3) (Fig. 4A, Table II). Similarly, in terms of carbon biomass, there was significant difference between winter and spring periods (Fig. 4B), with a global average dissimilarity of 56.65%. In that latter case, the large (>50µm in GALD), centric diatoms *Dactyliosolen* sp. and *Rhizosolenia* spp. played a major role in terms of carbon biomass.

The major phytoplankton taxa, in terms of cell abundance, contributed fairly equal to the dissimilarity between the winter and spring periods, as following: *Pseudo-nitzschia* sp. (12.79%), *Leptocylindrus* spp. (12.18%) and *Phaeocystis* sp. (11.35%) (Fig. 5A). *Pseudo-nitzschia* sp. and *Phaeocystis* sp. were more abundant in winter, while *Leptocylindrus* spp. were predominant in spring. Although they were not considered as toxigenic species, *Dactyliosolen* sp. (17.23%), *Leptocylindrus* spp. (16.38%) and *Rhizosolenia* spp. (8.94%) were main contributors in carbon biomass levels (Fig.5B),

influencing the difference between the winter and spring. In overall, there was little variation in Shannon-H' index between winter (1.95–2.55) and spring (2.24–2.92), but with significant differences (Mann-Whitney test, $p<0.05$). Also, all the biological parameters [Shannon-H', Pielou-*J* and dominance (D)] were affected by the high cell abundance/biomass of diatoms in winter (mainly) and spring sampling periods (Fig. 5), excluding the outlier condition of the patch of *Trichodesmium* spp. in springtime.

3.3 Chla-Carbon Biomass ratio: community physiological state and toxin production

High surface [CHL-*a*] concentration was determined in winter (particularly at WS1) in comparison to spring (Fig.6). In reflection to this condition, the autotrophic carbon biomass C/CHL-*a* ratio averaged 89 (44-142) during winter with a (only one) high ratio of 130 during the spring. As temperature correlated positively with autotrophic (r^2 = 0.641, $p<0.05$) and heterotrophic carbon biomass (r^2 = 0.643, $p<0.05$), also correlated well with total carbon biomass (r^2 = 0.621, $p<0.05$). On the other hand, water density (sigma-*t*) seemed to affect negatively total carbon biomass (r^2 = -0.603, $p<0.05$) as well the autotrophic carbon biomass (r^2 = -0.626, $p<0.05$) and heterotrophic carbon biomass (r^2 = -0.641, $p<0.05$).

Also, carotenoids (CAR) were more noticeable during the spring (Fig. 6) and trace amounts of phycobiliproteins (PHY) were found during both cruises but attained 0.02 µg L^{-1} only in spring (SS3), at surface samples. These [PHY] were related to cyanobacteria (as *Trichodesmium* spp.) in the springtime or other picocyanobacterial not quantified during the study. For the sample related to the *Trichodesmium* spp. bloom observed in spring, only [CHL-*a*] (2.61 µg L^{-1}) and [CAR] (2.13 µg L^{-1}) were determined . Also, pSTXeq from this *Trichodesmium* spp. bloom was equal to 0.96 µg L^{-1} STX-eq, in comparison with 0.59 µg L^{-1} p STX-eq measured at SS3 (Fig. 7). There

were observed higher values of STX-eq in WS1 (3.06 µg L^{-1}) and WS2 (3.59 µg L^{-1}). Thus, there were significant differences between winter (3.36 µg L^{-1}) and spring (1.26 µg L^{-1}) in terms of average pSTX-eq (Mann-Whitney test), excluding the value of the *Trichodesmium* spp. patch.

Although STX eq concentrations varied over the two sample periods, and more dramatically in the *Trichodesmium* spp. bloom, the identified analogs [STX] were the same throughout the study. Taking into consideration the molecular weight, the analogs appear chromatographically in the following order: the gonyautoxins [GTX] 4,1 and 5 and later, the more toxic saxitoxin [STX].However, the analog percentages to STX (Fig. 7A) were more susceptible to fluctuations. They showed a relatively constant contribution of [STX] (40.26-41.85%) over the sample period, but with a tendency of about 11% for GTX-4 and 5 to decline for the less toxic analogue GTX- 1.

Like the bloom of *Trichodesmium* spp. was sampled only during spring, it was not possible to compare the composition of analogs for this data set. Although there was a clear difference in the composition of analogs in the stations. *Trichodesmium* spp. as with dominant taxa, the composition of analogs was mainly [GTX-4] (59.37%) rather than [STX], showing a clear influence of taxonomic composition not only on saxitoxin concentrations but on the composition of different analogs.

As presented in Costa et al. (2019), average [pDA] values were almost a thousand-fold lower than [pSTX] values during winter and fifteen times in the spring, although [pDA] was found both in winter and spring (Fig.7B). Unlike the pattern observed for [pSTX], [pDA] were higher in spring than in winter. Costa et al. (2019) stated also that these [pDA] values were close to the detection limit (DL) of the method and, in consequence, there was no significant difference between the [pDA] values of sampling periods.

4. Discussion

4.1 Environmental framework

All samples were carried out within the shelf waters close to the city of Rio de Janeiro, Brazil characterized by a Tropical Water mass (TW) (Möller Jr et al., 2008). Generally, this water mass is warm, saline, and with low concentrations of dissolved inorganic nutrients (Braga & Niencheski, 2006). Despite our study did not present any nutrient dataset, other reports have already recorded low values of nitrate, ammonium, nitrite, phosphate and silicate near our study region, mostly during the spring (Braga, 1999 and references therein). It has previously appeared that the distribution of [TW] seemed to be irregular in the coastal zone next to our study area, which can constrain the intrusion of (nutrient-rich) slope water into that region. Thus, it might have somehow to contribute to the low surface concentration of inorganic nutrients mostly during the spring and lower levels of salinity (Fig. 2).

This pattern reflected on the correlation matrix where sigma-*t* and fluorescence (see Results section) were mostly linked to salinity, not temperature, which unlike some other tropical regions is a usual feature of the Brazilian coastal zone (Estrada et al., 2016). Also, this feature seems to be common to this region (Takanohashi et al., 2015; Ribeiro et al., 2016), therefore, no disturbances or great circulation-related events may have impacted the water column structure of the study area that time. So we can only suggest that the phytoplankton community and its physiological state, as we have sampled, might correspond to their natural state of growth, abundance, biomass and toxin production.

Another evidence of that state were the [DO] levels, that showed a low range of variability considering the seasonal difference .These small differences in [DO] concentration could be associated both with variations in turbulence, but also with

changes in ocean ventilation, solubility and the biological pump more active in the winter (Volk &Hoffert, 1985). In that latter sampling period, the [DO] levels could be also related to wind drive solubility and water surface temperature. Higher [SST] do not favor oxygen solubility, so during the spring the maximum values of [DO] are present in the base of the [ML](~9) and the minimum in the deeper layer (42m), so under a more stratified water column regime, the [DO] concentrations are directly correlated to the temperature structure and the formation of the early thermocline, decreasing slowly towards the bottom. Now, during the winter this profile is reversed, where the minimum and maximum values are within the first 5 m of the water column, and the minimum values occurs in the first meter and rapidly increases towards the 5 m meter layer reaching maximum concentrations, indicating a major influence of the colder water temperature in this process when the thermocline is absent (Zaker et al., 2007).Overall the [DO] were strongly correlated to the temperature structure ($r^2 = 0.703$), but not so much with the total autotroph biomass ($r^2 = 0.470$), indicating that the physical factor (as temperature) might have surpassed the phytoplankton O_2 production. Since the surface biomass [CHL-*a*] levels were relatively high in winter, we suggest that the effects of a deep mixed layer might have made available dissolved nutrients for phytoplankton growth. However, under an expected,-theoretical light limitation, in contrast to spring conditions, when there may have the entrainment of the plankton community within the upper layers, it favors the exposure to the light but under a progressed condition of nutrient limitation up to summer (Doney, 2006; Grittings et al., 2018).

This seasonal pattern was greatly reviewed, when two different environmental scenarios should be presented: an enhancement in phytoplankton biomass during the winter (Ocean type I) versus a drop in that phytoplankton parameter in spring/warmer periods (Ocean type II) (Chiswell et al., 2015). In both cases, the combination of a critical

turbulence (winter) and the onset of stratification (spring) should drive phytoplankton growth, depending upon the depth of [MLD] or the development of a shallow weak stratification that appears once deep-mixing ceases in the end of winter (Chiswell et al., 2015). That environmental scenario should favor an intermediate phase of low turbulence between the mixing (winter) and stratified (spring) periods, i.e., there may have place a difference between surface blooms and deep integrated phytoplankton biomass, as it may seem occurred in our study. Here, an enhancement in surface phytoplankton biomass observed during the winter (Ocean type I) and a supposedly intermediate phase when the surface [CHL-*a*] biomass was low and a [DCM] was formed (see Fig. 3), being the whole process controlled by turbulence/mixing.

Being a major environmental driver during the winter, turbulence seems to favor the water and nutrient renovation, and play an essential role in returning the nutrients from the levels and layers where they tend to accumulate (Margalef, 1997 and references therein). The turbulence usually can keep throwing the phytoplankton community to deeper layers, thus, favoring ruderal species (e.g., mostly diatoms) and promoting niche differentiation among each and every phototrophic microorganism, particularly with respect their harvesting light complex (Stomp et al., 2004). Likewise, this kind of association has been attributed to nutrient-enriched winter waters provided by the mixing process within the water column as comparable as an upwelling region where nutrient-rich waters are forced into the OML (Ocean Mixed layer) and brought into the photic zone (Kantha & Clayson, 2003). As it was observed deeper (lower) [MLD] in winter (spring) within those shelf waters near the Rio de Janeiro, we suggest that a potential process responsible for the high (winter) phytoplankton biomass at surface waters (5-m water column for fluorescence-derived biomass; Fig. 3) could have linked to the nutrient-rich water due to renovations. Several authors have already pointed out

that the shelf waters close to the Rio de Janeiro did not appear to show any pattern in temperature-salinity spatial distributions, indicating the influence of upwelling process and riverine/estuarine plumes from the Guanabara Bay and Sepetiba River (Ikeda et al., 1974; Guenther &Valentin, 2008; Moser et al., 2016). Otherwise, upwelling events would be usually restricted to summer periods (Coelho-Souza et al., 2012). The drop in salinity observed in this study (see Fig. 2) could indicate an intrusion of continental waters coming from the Guanabara Bay; also, a [DCM] layer showed in the springtime (seen in fluorescence-derived phytoplankton biomass) highlighted the interrelationship between light and nutrients effects upon the development and biomass accumulation of phytoplankton (e.g. Margalef, 1997).

4.2 Biological parameters of phytoplankton species, mainly toxin-producers

Our observation of considerable species richness and relatively high [CHL-*a*] biomass during winter periods has been mentioned in middle latitudes environments (Macêdo et al., 2009). On the other hand, a drop-in diversity index must have to be associated with the shifts of major contributors to cell abundance and carbon biomass within phytoplankton community. As the autotrophs corresponded to a major part of the total biomass ($r^2 = 0.995$), the heterotrophic carbon biomass contribution did not seem to be enough to characterize the trophic state of the studied period. Otherwise, the C:CHL-*a* ratio of the later (spring) sampling time appeared to evince an autotrophic community stressed (Jakobsen & Markager, 2016). Thus, we can suggest that this community would decay to a heterotrophic one up to the summer, where the heterotrophic biomass can surpass the autotrophic one (Dodds & Cole, 2007).

Evidence of the nutrient depletion and physiological stress can also be evaluated once the C: CHL-*a* ratio is considered, since the high value estimated for spring (130). This kind of result may reflect less [CHL-*a*] production during the springtime. As far as we know, works discussing C:[CHL-*a*] still are lacking on the on the South Atlantic region, but models of the North Pacific (Taylor et al.,1997) and data from the North Atlantic and North Sea (Li et al.,2010, Jakobsen & Markager ,2016) showed that in addition to be greatly variable (20-160 or more within studies). This C:CHL-*a* ratio can be affected by a number of factors, where the nutrient availability is generally critical. For instance, as the community moved towards the maximum growth state, generally related to nutrient enrichment (winter), more [CHL-*a*] was produced and the ratio was kept relatively low (89,minium of 44).In so far as the plankton community slowly became nutrient depleted (spring),probably by N , the ratio was shifted upwords indicating a refractory C stock. These physiological changes would turn into changes in pigment composition and, of course, toxin production (especially in [DA] taking into account the great contribution of *Pseudo-nitzschia* spp.).

In terms of types of main pigments, [CAR] was slightly higher in spring period, relating to a photoacclimation or nutrient limitation as these compounds are linked to the photoprotection of photosystem II (Roy et al., 2011). Among [CAR], we can suggest that zeaxanthin would be a major component in springtime as well myxoxanthophyll and β, β-carotene (Lima et al., 2019), since *Trichodesmium* spp. would be susceptible to high ambient light (Domonkos et al., 2009). The use of spectrophotometry did not discriminate every kind of pigment, but we can also suggest that peridinin would be indicative of *Gymnodinium* spp. in our samples as seen previously in a close study area (Lima et al., 2019). In overall, the great contribution of diatoms would be expressed in

terms of fucoxanthin, diadinoxanthin and diatoxanthin (other CAR as estimated coarsely here).

As phycobiliproteins attained higher concentrations in spring, we can indicate that cyanobacteria were greatly responsible for it, derived from *Trichodesmium* spp. Otherwise, picocyanobacteria (e.g. *Synechococcus* and *Prochlorococcus*) and cryptophytes are important phycobilin producers. In our study, unfortunately, there were not used appropriate methods for the quantification of picoplankton cells. Nonetheless, high light availability in spring might have increased the biosynthesis of phycobiliproteins to fulfill the so-called "green gap" of the [CHL-*a*] optimizing the photosynthetic efficiency of these organisms (Hong Zhao et al., 2011). Also, if nutrient uptake during the winter were progressively exhausting their availability towards the spring, the water surface would easily become limited mostly by nitrogen (Legendre & Rassoulzadegan, 1995). That would quickly reduce both the quantity and activity of PSII mostly in diatoms (Liefer et al., 2018), which were the major contributors to the phytoplankton community in our study.

For instance, the N metabolism effects on phytoplankton have been associated with the production of Domoic Acid (DA]), when the N limitation seemed to correspond to increasing levels in [DA] by the potential toxin-producer diatoms *Pseudo-nitzshia* and *Nitzshia* (Pan et al., 1998; Pan et al., 2001). Likewise, presumed lower nutrient concentrations in spring could trigger the ecophysiological strategy of maintaining N intracellular content through the [DA] synthesis (Bates, 1998; Bates et al.2018, Pan et al., 1998, 2001). Specifically, low winter concentrations in [pDA] added to low C:CHL-a ratio express that the phytoplanktonic community, particularly diatoms as *Pseudo-nitzschia* spp., would not be stressed by N limitation or P limitation, and toxin production per cell would be in much varying concentrations (Pan et al., 1998). Also, it

is known that higher levels of the biomass seem to dilute the toxin effect, i.e., the cell toxin increases towards the senescence phase (Godinho et al., 2018), so it is possible that [DA] have defined environmental triggers, linked to decrease in nutrient availability and N stock when the biomass is lower, rather than an allelopathic reaction to co-occurring toxic and nontoxic species and/or grazers in that case.

On the other hand, saxitoxins (STX) production has been directly linked to the increasing phytoplankton biomass (Band-Schmidt et al., 2019), despite changes in the C: [CHL-*a*] ratio. In our sampling periods, the [STX] content has been ascribed to dinoflagellates (*Gymnodinium* spp. and *Alexandrium* sp.) as well to *Trichodesmium* spp., however these taxa had contributed less than diatoms in cell/trichome abundance, for example. As far as we know, there have been carried out few multi-approach (microscopy, toxin analysis) studies on HAB/toxigenic species along the Brazilian coastline as our present study, although some important works fairly concentrated in the southernmost part of that region (Proença et al., 2001; Odebrecht et al., 2002). Unfortunately, we were not able to apply molecular biology techniques, as they are considered more promising in the identification of toxigenic species regardless their abundance level in the aquatic systems (Bush et al., 2016).

Nonetheless, some [STX] analogues determined in our study period were related to a few dinoflagellate genera, taking into account that its relative contribution (even in non-bloom condition and low cell abundance) should reflect in increased toxin levels, if all these genera were lumped together for toxin analysis (Guinder et al., 2018; Band-Schmidt et al., 2019).For instance, as *Gymnodinium* spp. occurred in all samples that gave some STX-eq values, we can indicate a coarse ratio equal to 32 pg STX-eq per *Gymnodinium* cell, which would be three- (10.9) to ten-fold (3.7 pg cell^{-1}) higher than field samples linked to *Gymnodinium catenatum* in three Latin America countries

(Argentina, Mexico and Uruguay; Band-Schmidt et al., 2019), but close to the toxin content per cell (29 pg cell^{-1}) reported from cultured cells in Brazil, likewise (Proença et al., 2001). In this way, we can suggest that a geographic influence would be linked to the toxin content per cell. Overall, we assumed that all *Gymnodinium* species were STX-producers and even with any presumed counting errors of phytoplankton cells and/or other caveats in filtering samples or toxin quantification. Stations (WS1-2 and SS2-3) where saxitoxins were also detected showed a similar profile [GTX-1,4,5] and [STX], which reflected on the determination in terms of STX eq.

Since other studies registered this toxigenic species in Brazil, but phycotoxins quantification is limited, compar toxin profile and analogue content within this region is difficult. Nevertheless, analogue percentage and composition registered here suggest a high toxicity, as the most potent analogues [STX](Wiese et al.,2010) represented a major portion of the toxin content(see Fig.7A).Considering the toxin environmental profiles herein, and the lack of isolated cultures, we can only suggest similarities with other environmental samples from Argentina and Uruguay (Band-Schmidt et al.,2019).And even the slightly differences in analogues percentages are usually linked to temperature changes, as well as bacterial an enzymatic biotransformation (Wiese et al.,2010). Furthermore, *Trichodesmium* spp. patch observed in springtime also presented [STX] and three analogues [GTX-1,4,5], but with a more expressive representation of [GTX-4] (59,37%) mostly because of the organism cell density and contribution, already reported in Detoni et al (2016). Likewise, other works had also reported the occurrence of [GTX-3] and [dc-STX] in bloom of *T. erythraeum* only (Proença et al., 2009).

Taking into account the high variability in toxicity from field and experimental samples mentioned above, we could point out that the dinoflagellates *Alexandrium* sp. and

Gymnodinium spp. were strong candidates as STX-producing species in winter (Fig. 7) whereas *Trichodesmium* spp. added to this contribution in springtime. Since [STX] toxicity by a unique species is usually low (Gárate-Lizárraga et al., 2006), the species richness observed in our study should indicate that many potentially toxin-producer species could contribute to moderate [STX] levels in field samples. For example, those dinoflagellates that played a major role in synthesizing [STX] comprise only 10% of the species (~200) coupled with toxigenic events in many environments (Smayda & Reynolds, 2003). Also, we may suggest that the low [pDA] levels found in our sampling periods would be associated with physiological and ecological responses [as presented in (Costa et al., 2019)] as well with the relatively low diversity of diatom species that produce [DA] (see Table II and *S1*), thereby low biodiversity could mean low field toxin concentration.

Even though there were significant differences between winter and spring toxin [STX] concentration, we could not ascertain what physical/chemical parameter would be linked to toxin levels, instead we can affirm that a combination of water column structure and salinity variation is behind this difference. On the contrary, some biotic features such as co-occurring toxic or non-toxic species and grazers have been suggested to affect the level of toxicity and toxin content in phytoplankton community (Fernández-Herrera et al., 2016; Band-Schmidt et al., 2017; Bif et al., 2019). Still there will be necessary further ecological studies in order to understand the complex biotic interactions within phytoplankton community and their implications over the production and fate of phycotoxins. In fact, our work quantified only two kinds of neurotoxins [STX and DA], produced only by a significant percentage of the toxin producers, but many other potential toxin-producer taxa were identified (see Table *S1*, supplementary material) such as *Phaeocystis* sp. and *Amphidinium* sp. (production of hemolysins;

Moestrup, 2009); *Dinophysis* spp. and *Prorocentrum* spp. (for example, Okadaic Acid); *Gonyaulax* spp. (yessotoxin, YTX).Besides the already listed , *Alexandrium* sp. and *Gymnodinium* spp.can also produce other toxic compounds (Tamele et al., 2019).Beyond that, toxin producing dinoflagellates are usually linked to toxic events and biota impacts even in very low cell density $<10^3$ cell L^{-1}, that is, natural or not bloom state (Reguera et al.,2014). For instance, besides showing a wide variability of toxin profile and occurrence worldwide, *Dinophysis* and *Prorocentrum* spp., usually linked to DSP's, were only reported twice before in Brazilian marine waters, both in shellfish farming (Mafra Jr.,2013 and references therein). Considering its importance not only for production but also for risks to local marine biota, it is imperative to know more about the coastal zone species in Brazil.

In our study, *Trichodesmium* spp. bloom observed/sampled only in the spring showed a minor STX concentration (~ 0.96 µg L^{-1}), albeit in the similar range reported previously along the southeastern Brazilian shelf and slope (Detoni et al., 2016, Bif et al., 2019). Since the identification of trichomes was only conducted to genus level, this relative low concentration could be linked to the occurrence of relatively low density of toxigenic species, most likely *T. erythraeum* (Proença et al., 2009; Narayana et al., 2014) and *T. thiebautii* (Hawser et al., 1992), both reported to the Brazilian coast, along with three other: *T. clevei*, *T. hildebrandtii*, and *T. radians* (Bif et al., 2019). Although these works reported *Trichodesmium* spp. occurrence along with [STX] quantification, there were any assays with isolated strains in order to quantify the [STX] concentration per each *Trichodesmium* species and determine how many of them indeed would or would not synthesize [STX].

In short, relatively high cell/trichome abundance at surface waters would be linked to the ability of phytoplankton species (e.g. *Trichodesmium* spp., diazotrophic diatoms) to

grow in nutrient-limited, warm and stratified water column due to its own physiology and morphology adaptations. Considering the model proposed by Reynolds (Reynolds, 1988) and more recently revisited by Alves-de-Souza et al. (Alves-de-Souza et al., 2008) of three functional groups including colonist, ruderal and stress-tolerant (C-R-S) strategists, *Trichodesmium* spp. would be S-strategist species, with low growth and low surface/volume ratio that use their air vesicles to maintain themselves at surface layers, and dominate oligotrophic, stratified waters and under high light conditions (spring conditions). On the other hand, diatoms were the major contributors during both periods sampled (see Fig. 5), and would be ruderal-strategists (in winter, mainly) when the mixed layer was deeper. In the case of dinoflagellates, the dynamic of species assemblages would be basically linked to the kind of environment, which could select against specific life forms (Smayda &Reynolds, 2001). Excluding the SS1 station, the other ones were placed in the coastal zone (see Fig. 1) that comprised a type-I (nearshore) and a type-II (coastal) ambient, with abundance of gymnodinioids and prorocentroids, respectively (Fig. 5, Table II). This association ambient-life form indicated that dinoflagellates would not depend upon a single uniform or basic condition on ecological zones and, instead, they would have a diverse habitat preference (Smayda & Reynolds, 2003).

5. Concluding remarks

We showed that the coastal region close to Rio de Janeiro -Brazil harbor a diverse community, represented mostly by diatoms, and especially during winter by toxic ones. This environmental characteristics and kinds of pigments [CHL-*a*, CAR and PHY] and toxins (mainly, STX) were related to the variations in water column stratification. Also, those biological parameters were coupled to the phytoplankton community composition,

mainly the major contribution of pennate (e.g. *Pseudo-nitzschia* spp. in winter) or large centric diatoms (e.g., *Rhizosolenia* spp.) and dinoflagellates, in springtime; as well, higher [CAR] in spring could be associated with a nutrient depletion or photo stress evidenced by an increase in the C:CHL-*a* ratio. Neurotoxins [DA and STX], even at low concentrations, appeared to be ubiquitous within the study area, which raises concerns about endangerment of the local biota, specially by dinoflagellates species, that can cause impact even in low cell density. The differences between winter and spring could be linked to shifts in kind of environmental stressors: light (winter) and nutrients (spring), for diatoms (DA concentrations) at least. This information is essential to understand the dynamics of phytoplankton and its physiological state, as well as the toxins produced by it in the water column. Yet there is a need for thorough studies encompassing the entire euphotic layer, at least, in order to describe and relate the toxin production and fate with the dynamics of physical driven phytoplankton succession.

Acknowledgements

The authors would like to thank the environmental dataset, the logistical support and sampling by the staff of "Bonito Listrado" Project: Lauro Antônio Saint Pastous Madureira, Stefan Cruz Weigert and Marcelo Peres de Pinho. The "Bonito Listrado" Project fills a compensatory measure that is established by the Chevron Company's responsible conduct adjustment term, carried out by the Federal Public Prosecutor of the Rio de Janeiro State (Brazil) by means of the Brazilian Biodiversity Fund – FUNBIO. At last but not least, we thank the endorsement of the GlobalHAB/IOC-SCOR to this work.

Funding

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES . C.CWerlang is granted by a MSc fellowship (CAPES) number [88882.182296/2018-01]. M.S de Souza and L.D.F Costa are both granted by PNPD/MEC-CAPES fellowship numbers [88882.314601/2019-01] and [88882.314596/2019-01] respectively , and J.S.Yunes a productivity fellowship of Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq number [311402/2017-8].

Artwork (figures) with Captions

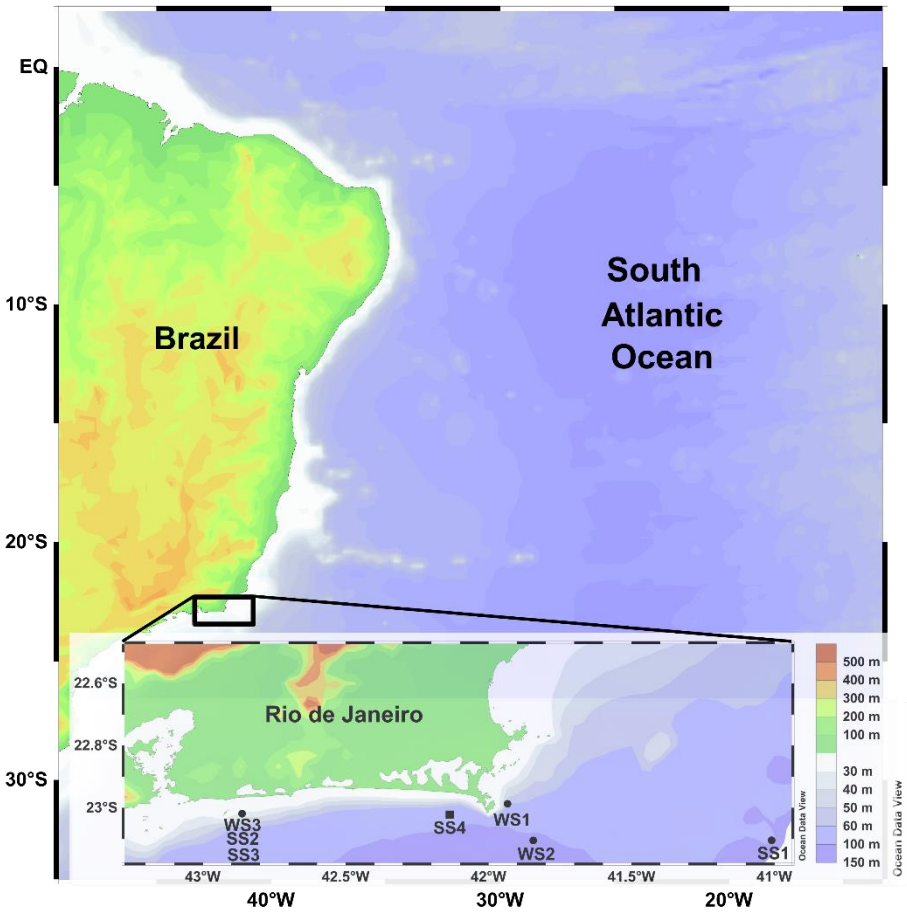

Fig. 1. Study area in the coast region of Rio de Janeiro state (South Atlantic Ocean). Labels on the map mean winter stations (WS1, WS2 and WS3) and spring stations

(SS1, SS2 and SS3). Station SS4 (represented by a square) corresponds to a *Trichodesmium* spp. Bloom founded during Spring and color bar represents ocean bathymetry and land topography.

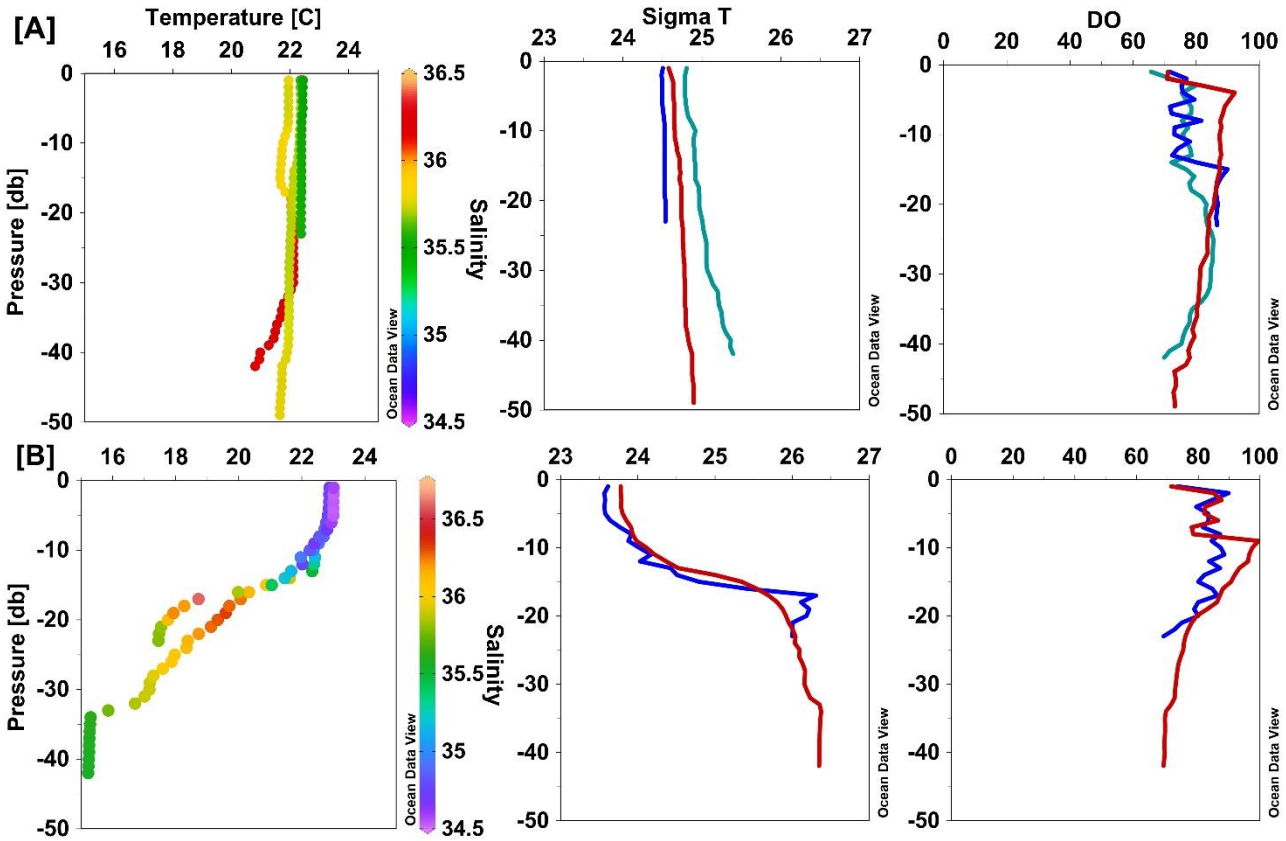

Fig. 2. Temperature (ºC), Sigma-t and dissolved oxygen (DO %) vertical profiles for the winter stations [A] and the spring stations [B].

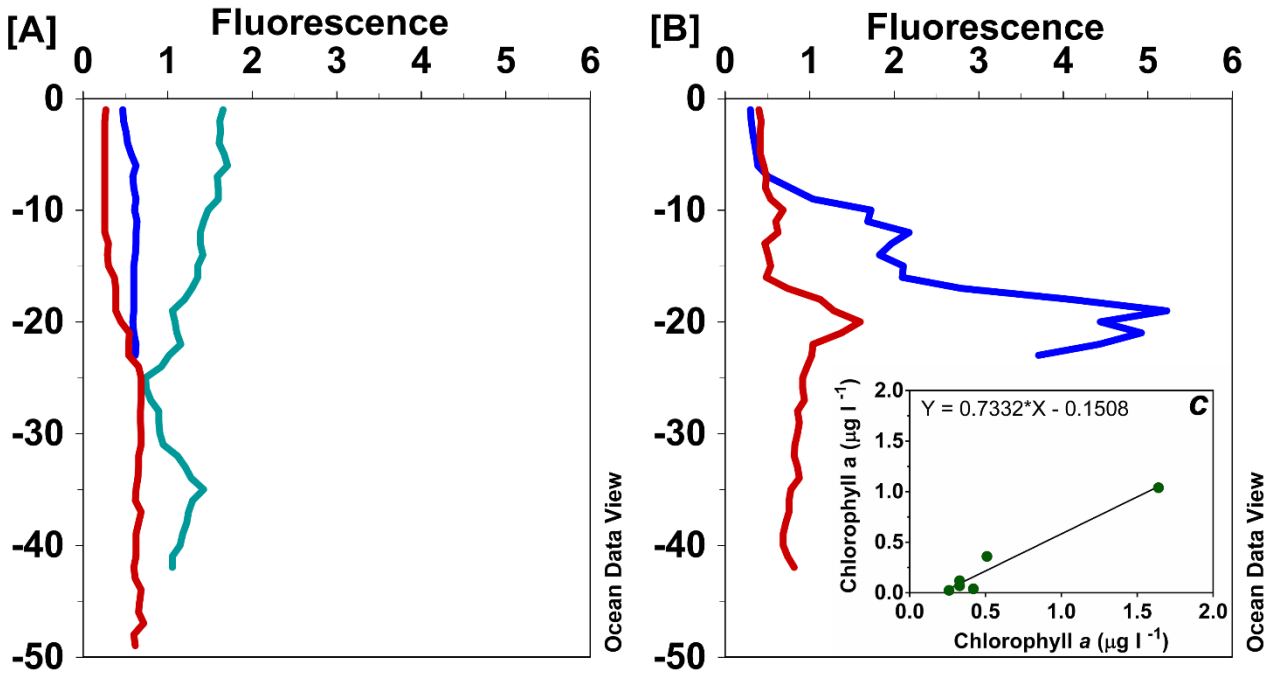

Fig. 3. Phytoplankton biomass derived from fluorometer sensor (µg L^{-1}): [A]refers to winter sampling stations and [B] refers to spring sampling stations, showing [DCM] below 15 m during springtime. Inset c: It refers to Spearman correlation and linear regression between x= phytoplankton biomass derived from fluorometer sensor and y= chlorophyll *a* measured by spectrophotometer. Both Spearman and linear regression tests showed $p < 0.05$ ($r^2 = 0.96$ and Spearman $r = 0.81$).

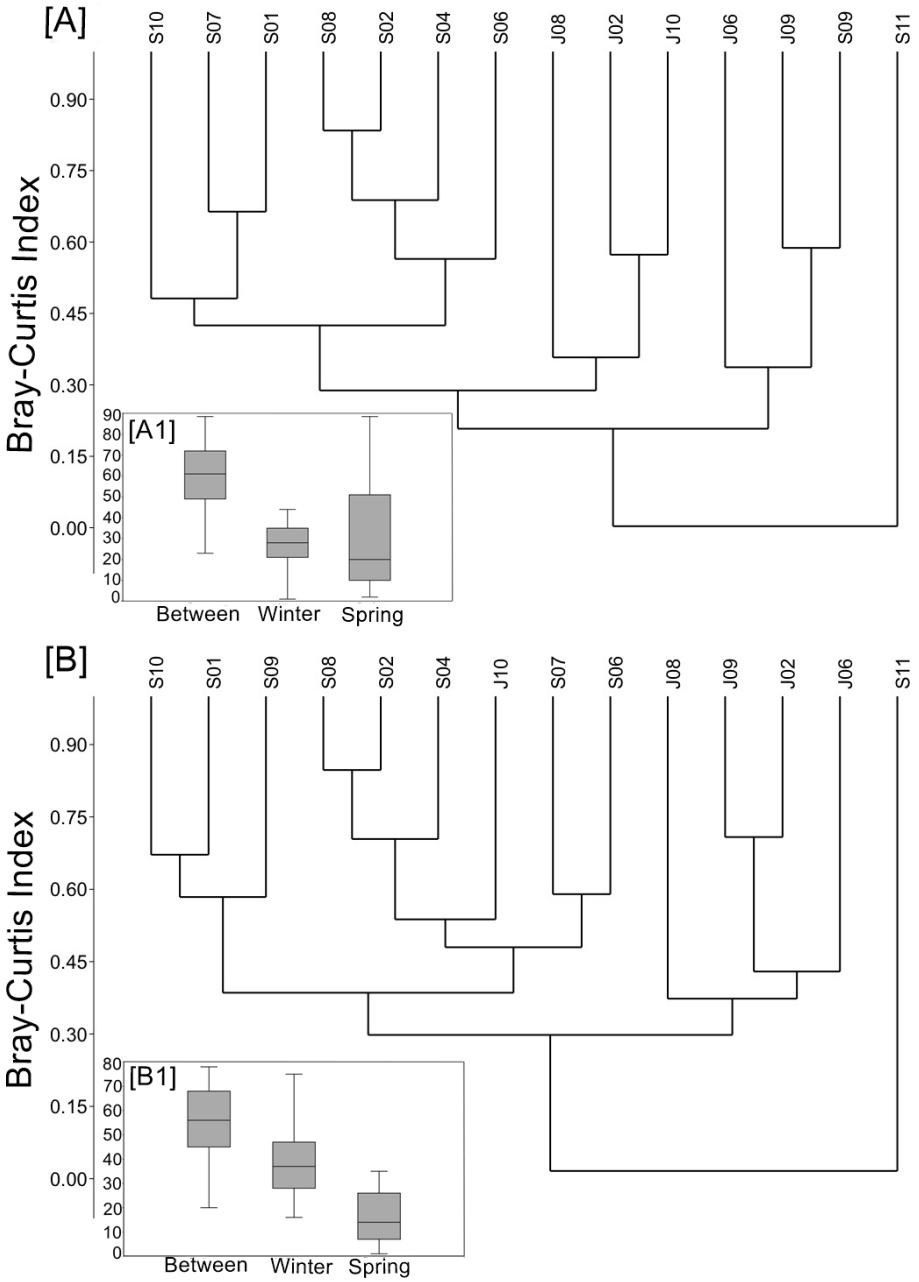

Fig. 4. Dendrogram based on Bray-Curtis Index (A-B) and ranked ANOSIM results (A1 and B1). S = samples represent Spring season, J = samples represent Winter Season. A = Cluster Analysis and ANOSIM with abundance data, B = Cluster Analysis and ANOSIM with biomass data.

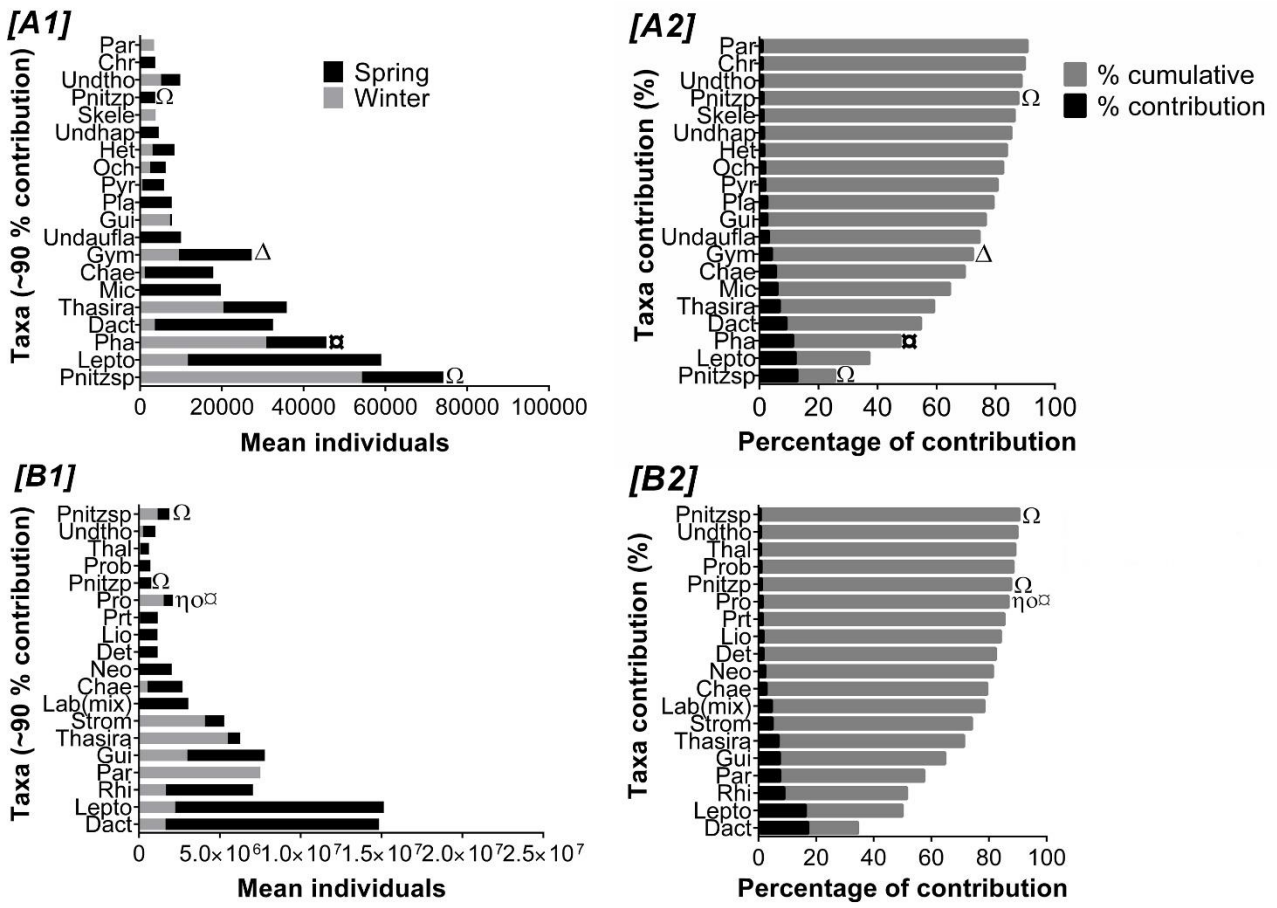

Fig. 5. Percentage similarity analyses (SIMPER) of A1 = contribution of each taxa for the difference in abundance both in winter and spring sampling periods, and A2 = contribution percentage of each taxa and cumulative contribution. B1 = contribution of each taxa for the difference in biomass both in winter and spring sampling periods and B2 = contribution percentage of each taxa and cumulative contribution. Symbols indicate toxic genus/species that are listed in supplementary Table S1 along with taxa cod. Names.

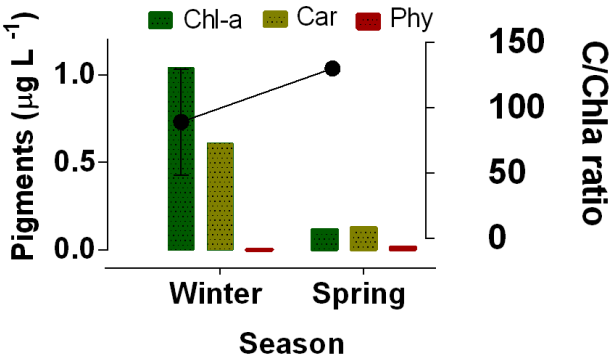

Fig. 6. Pigment concentration (bars) and Carbon biomass: CHL-a ratio (line and dot) during the winter and spring sampling periods. *Only values within the 20-160 range were included, values above that were considered as artifacts one.

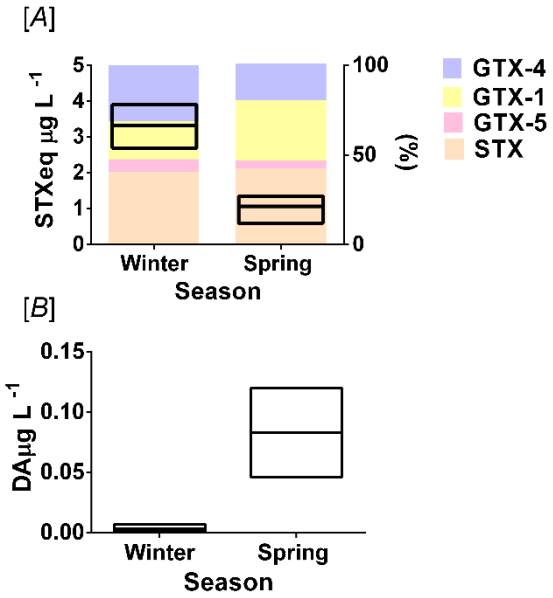

Fig. 7. Mean, maximum and minimum particulate [STX] (A) including analogues percentage and particulate [DA] (B) concentrations during the winter and spring cruises.

Tables with Captions

Table I. Mean values and (±) standard deviation of environmental parameters [temperature (ºC), salinity, MLD (mixture layer depth in m), fluorescence (average biomass within the first subsurface 5-m, in µg L^{-1}) dissolved oxygen (mg L^{-1} in approximate value) and bottom depth (m)] during austral winter (WS1–3) and spring period (SS1–3) of 2018, in the coastal region of the state of Rio de Janeiro. **Trichodesmium* spp. bloom spot during the spring cruise, but with no CTD data available. **Standard deviation bellow 0.01.

Environmental Parameters	**WS1**	**WS2**	**WS3**	**SS1**	**SS2**	**SS3**	**SS4***
Temperature	21.9**	22.3±0.01	22.4±0.01	22.8±0.02	23.0±0.01	23.0±0.01	_
Salinity	35.7 ± 0.01	35.6 ± 0.01	35.5 ± 0.01	34.7 ± 0.01	34.5 ± 0.03	34.5 ± 0.03	_
MLD	42	49	23	10	09	09	_
Fluorescence (biomass)	1.64 ±0.03	0.26 ± 0.00	0.51 ± 0.04	0.42 ± 0.26	0.33 ± 1.65	0.33 ± 1.65	_
OD	5.63 ± 0.30	5.83 ± 0.37	5.67 ± 0.44	5.92 ± 0.44	5.93 ± 0.30	5.93 ± 0.30	_
Bottom Depth	52	55	31	54	31	31	77

Table II. List of potentially toxic phytoplankton taxa identified during the Winter and Spring Cruises with mean, minimum and maximum values. Δ = PSPs producers, o = DSPs, Ω = ASPs, ¤ = hemolisyn, ɤ = yessotoxin and homoyessotoxin, ŋ = neurotoxins.

* means that those toxins were quantified during this study. Taxon code indicates code names used in the statistical analyses. $ = Values for *Trichodesmium* spp. do not take into account the number of trichomes and colonies found in (spring) patches.

		Winter (cell•L $^{-1}$)	**Spring** (cell•L $^{-1}$)$^{\$}$
Taxa	**Taxon cod.**		
Cyanobacteria			
Trichodesmium spp. Ehrenberg ex Gomont, 1892 ***Δ**	Trich	0(0-0)	132 (0-320)
Haptophyta			
Phaeocystis sp. Lagerheim, 1893 ¤	Pha	30857 (0-112380)	14650 (0-53040)
Dinophyta/Miozoa			
Alexandrium sp. Halim, 1960 *oΔ**	Ale	190 (0-952)	76 (0-612)
Amphidinium sp. Claperède & Lachmann, 1859 ¤	Amp	190 (0-952)	0 (0-0)
Dinophysis spp. Ehrenberg, 1839 º	Din	0 (0-0)	28 (0-100)
Gonyaulax spp. Diesing, 1866 ɤ	Gon	190 (0-952)	18 (0-60)
Gymnodinium spp. (mixotroph) Kofoid & Swezy 1921 Δ*	Gym	9523 (3809-18095)	17846 (1872-28848)
Prorocentrum spp. Ehrenberg, 1834 ŋo¤	Pro	2857 (952-4761)	2041 (504-6573)
Ochrophyta/Bacillariophyceae			
Nitzschia spp. A.H. Hassall, 1845 Ω*	Nitz	857 (0-1905)	745 (0-1632)
*Pseudo-nitzschia pungens (*Grunow ex Cleve) G.R.Hasle, 1993 *Ω	Pnitzp	0 (0-0)	3709 (1280-8976)
Pseudo-nitzschia spp. H. Peragallo, 1900 *Ω	Pnitzsp	54285 (34286-80952)	19933 (13872-28152)

Table *S1*. List of all plankton taxa identified during the both surveys (Winter and Spring), with mean, minimum and maximum values (in that order). Symbols at some taxa indicate potential toxin production (except for mucous or any other harmful effects) according to the IOC-UNESCO list of HAB (Moestrup,2009). Δ = PSPs producers, o = DSPs, Ω = ASPs, ¤ = hemolisyn, ɤ = yessotoxin and homoyessotoxin, ŋ = neurotoxins. * means that those toxins were quantified during this study. Taxon code indicates code names used in the statistical analyses. $ = Values for *Trichodesmium* spp. do not consider the number of trichomes and colonies found in (spring) patches.

		Winter (cell L $^{-1}$)	**Spring** (cell L $^{-1}$)$
Taxa	**Taxon cod.**		
Cyanobacteria			
Trichodesmium spp. Ehrenberg ex Gomont, 1892 *Δ	Trich	0(0-0)	132(0-320)
Unidentified filaments	Und	190(0-952)	234(0-1354)
Euglenozoa			
Euglenophyceans	Eug	190(0-952)	342(0-2200)
Chlorophyta			
Polytoma sp. Ehrenberg, 1831 (heterotroph)	Pol(htr)	0(0-0)	3724(0-12189)
Other chlorophyceans	Och	2476(0-10476)	3780(0-15349)
Prasinophyta			
Micromonas sp. Manton & Parke, 1960	Mic	0 (0-0)	19750(0-57785)
Pyramimonas spp. Schmarda, 1849	Pyr	571(0-2857)	5304(0-13543)
Cryptophyta			
Chroomonas sp. Hansgirg, 1885	Chr	0(0-0)	3724(0-10835)
Plagioselmis sp. Butcher ex G.Novarino, I.A.N.Lucas & S.Morrall, 1994	Pla	0(0-0)	7730(0-16252)
Unidentified cryptophytes	Undcry	1523(952-2857)	0(0-0)
Haptophyta			
Phaeocystis sp. Lagerheim, 1893 ¤	Pha	30857(0-112380)	14650.(0-53040)
Umbilicosphaera sibogae (Weber Bosse) Gaarder, 1970	Umb	761(0-3809)	0(0-0)
Unidentified haptophytes	Undhap	0(0-0)	4570(0-36567)
Several Phyla			
Unidentified autotrophic flagellates	Undaufla	0(0-0)	10044(0-23475)
Telonemia			
Telonema subtile Greissmann, 1913 (heterotroph)	Tel(htr)	0(0-0)	3160(0-6772)
Dinophyta/Miozoa			
Alexandrium sp. Halim, 1960 oΔ*	Ale	190(0-952)	76(0-612)
Amphidinium sp. Claperède & Lachmann, 1859 ¤ (mixotroph)	Amp	190(0-952)	0(0-0)
Amphisolenia bidentata B.Schröder, 1900	Amps	0(0-0)	3(0-20)
Cochlodinium sp. F.Schütt, 1896 (mixotroph)	Coc	0(0-0)	328(60-800)
Dinophysis spp. Ehrenberg, 1839 º (mixotroph)	Din	0(0-0)	28(0-100)
Gonyaulax spp. Diesing, 1866 ɤ	Gon	190(0-952)	18 (0-60)

Gymnodinium spp. Kofoid & Swezy 1921 Δ* (mixotroph)	Gym	9523(3809-18095)	17846(1872-28848)
Gyrodinium falcatum Kofoid & Swezy 1921	Gyr	190(0-952)	0(00)
Gyrodinium spp. Kofoid & Swezy, 1921 (heterotroph)	Gyr(htr)	2380(0-7619)	3189(1060-5916)
Heterocapsa spp. Stein, 1883	Het	3047(0-5714)	5360(0-13543)
Katodinium spp. B.Fott, 1957 (heterotroph)	Kat(htr)	952(0-1904)	982(80-2180)
Levanderina fissa Moestrup, P.Hakanen, G.Hansen, Daugbjerg & M.Ellegaard, 2014	Lev	190(0-952)	0(0-0)
Oxytoxum spp. Stein, 1883	Oxy	571(0-1904)	82(0-408)
Polykrikos sp. Bütschli, 1873 (heterotroph)	Poly(htr)	95(0-476)	0(0-0)
Pronoctiluca spinifera (Lohmann) Schiller, 1932 (heterotroph)	Prn	0(0-0)	18(0-140)
Prorocentrum spp. Ehrenberg, 1834 ŋo¤	Pro	2857(952-4761)	2041(504-6573)
Protoperidinium spp. Bergh, 1881 (heterotroph)	Prt	190(0-952)	478(180-1080)
Pyrocystis sp. Wyville-Thompson, 1876	Pyro	0(0-0)	5(0-20)
Pyrophacus sp. F.Stein, 1883	Pyrp	0(0-0)	58(0-120)
Scrippsiella trochoidea Alech ex A.R.Loeblich III, 1965	Scr	0(0-0)	849(80-2080)
Torodinium robustum Kofoid & Swezy, 1921	Tor	380(0-952)	30(0-80)
Tripos spp. Bory, 1823	Tri	0(0-0)	45(0-120)
Unidentified thoracosphaeraleans	Undtho	5142(1905-9524)	4689(960-9564)
Unidentified thecates	Undthe	0(0-0)	5(0-40)
Ochromonas sp. Vysotskii [Wysotzki, Wyssotzki], 1887	Och	1714(0-8571)	0(0-0)
Ochrophyta/ Chrysophyceae			
Other chrysophyceans	Othchry	761(0-2857)	0(0-0)
Meringosphaera sp. Lohmann, 1903	Mer	0(0-0)	677(0-4063)
Ochrophyta/Bacillariophyceae			
Asteromphalus spp. Ehrenberg, 1844	Ast	0(0-0)	596(0-1304)
Bacteriastrum hyalinum Shadbolt, 1854	Bac	0(0-0)	8(0-60)
Chaetoceros spp. Ehrenberg, 1844	Chae	1142(0-5714)	16842(4724-26522)
Climacodium frauenfeldianum Grunow, 1868	Cli	0(0-0)	235(0-720)
Corethron pennatum Castracane, 1886	Cor	0(0-0)	20(0-160)
Coscinodiscus spp. Ehrenberg, 1839	Cos	0(0-0)	115(0-816)
Cylindrotheca closterium (Ehrenberg) Reimann & J.C.Lewin, 1964	Cyl	380(0-1905)	1441(0-3672)
Dactyliosolen sp. Castracane, 1886	Dact	3619(0-6667)	28924(11440-43248)
Detonula pumila (Castracane) Gran, 1900	Det	0(0-0)	590(0-2480)
Fragilariopsis sp. Hustedt, 1913	Fra	571(0-2857)	0(0-0)
Guinardia spp. H.Peragallo, 1892	Gui	7333(3811-14286)	453(0-2280)
Hemiaulus spp. Heiberg, 1863,	Hemi	571(0-1905)	359(0-1428)
Leptocylindrus spp. Cleve, 1889	Lepto	11714(2857-21905)	47349(18480-78336)
Lioloma elongatum (Grunow) Hasle, 1997	Lio	0(0-0)	153(0-400)
Lyrella spp. [Karaeva], 1978	Lyr	95(0-476)	30(0-160)
Meuniera membranacea (Cleve) P.C.Silva, 1996	Meu	0(0-0)	260(0-1060)
Neocalyptrella robusta (G.Norman ex Ralfs) Hernández-Becerril & Meave del Castillo 1997	Neo	0(0-0)	5(0-40)
Nitzschia spp. A.H. Hassall, 1845 Ω*	Nitz	857(0-1905)	745(0-1632)
Paralia sulcata (Ehrenberg) Cleve, 1873	Par	3428(0-17143)	0(0-0)
Pleurosigma/Gyrosigma W.Smith, 1852	Ple	190(0-952)	58(20-140)
Proboscia alata (Brightwell) Sundström, 1986	Prob	0(0-0)	103(0-240)
Pseudo-nitzschia pungens (Grunow ex Cleve) G.R.Hasle, 1993*Ω	Pnitzp	0(0-0)	3709(1280-8976)
Pseudo-nitzschia spp H. Peragallo in H. Peragallo & M. Peragallo, 1900 *Ω	Pnitzsp	54285(34286-80952)	19933(13872-28152)
Rhizosolenia spp. Ehrenberg, 1843	Rhi	380(0-1905)	888(340-1604)
Skeletonema costatum complex (Greville) Cleve, 1873	Skele	3714(1429-5714)	65(0-520)
Thalassionema spp. Grunow ex Mereschkowsky, 1902	Thal	952(0-4762)	1151(220-3096)
Thalassiothrix sp. Cleve & Grunow, 1880	Tharix	0(0-0)	5(0-40)
Thalassiosira spp. Cleve, 1873	Thasira	20380(952-40000)	15511(708-42512)
Trieres chinensis (Greville) Ashworth & E.C.Theriot, 2013	Tries	0(0-0)	3(0-20)
Unidentified nano-centrics	Undnacen	0(0-0)	1749(0-7223)
Unidentified naviculaceans	Undnavi	1428(952-2381)	2957(140-8065)
Cercozoa/Thecofilosea			
Ebria tripartita (J.Schumann) Lemmermann, 1899 (heterotroph)	Ebr(htr)	380(0-952)	0(0-0)

Several phyla			
Other heterotrophic flagellates	Othfla(htr)	666(0-1905)	55730(29795-97511)
Phylum Ciliophora			
Dadayiella ganymedes Lim, 2017 (heterotroph)	Dada(htr)	0(0-0)	8(0-60)
Didinium sp. Meunier, 1910 (heterotroph)	Didi(htr)	0(0-952)	28(0-100)
Eutintinnus spp. Kofoid & Campbell, 1939 (heterotroph)	Euti(htr)	190(0-952)	8(0-40)
Laboea strobila Lohmann, 1908 (mixotroph)	Lab(mix)	0(0-0)	80(0-440)
Lohmanniella sol Leegaard, 1915 (heterotroph)	Loh(htr)	95(0-476)	0(0-0)
Mesodinium rubrum Leegaard, 1915	Mesd	95(0-476)	165(0-816)
Rhabdonella sp. Brandt, 1906 (heterotroph)	Rhabd(htr)	0(0-0)	20(0-80)
Salpingella acuminata Kofoid & Campbell, 1939 (heterotroph)	Salp(htr)	190(0-952)	63(0-120)
Strobilidium spp. Schewiakoff, 1892 (heterotroph)	Strob(htr)	1904(0-3810)	2233(428-6023)
Strombidium spp. Claparède & Lachmann, 1859 (mixotroph)	Strom	2000(0-5714)	1688(340-3508)
Tintinnopsis sp. Stein, 1867 (heterotroph)	Tint	0(0-0)	20(0-160)
Unidentified scuticociliates (heterotroph)	Undscu(htr)	476(0-2381)	28(0-160)

Artigo II

Screening *Trichodesmium* (Ehrenberg ex Gomont, 1892) abundance, phycobiliproteins and saxitoxins along the Southwest Brazilian Shelfbreak

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Abstract: The cyanobacteria from the genus *Trichodesmium* are studied worldwide for its role in the biogeochemical global N cycle, and primary production mostly on the tropical and sub-tropical environments. Members of this genus are also known for bloom formation capacity and saxitoxins production, as well as a significant amount of phycobiliproteins. In this work, we aimed to quantified saxitoxins and phycobiliproteins, this for the first time, and try to link them with *Trichodesmium* occurrence over the Brazilian continental slope. To fulfill these goals a cruise from 23º S to 28º S, along with 37 water sampling stations where water column physical properties were measured with a seabird CTD were used, along with phytoplankton counted on inverted microscope. Using filtered water samples phycobiliproteins were quantified on a UV-VIS spectrophotometer, and saxitoxins with HPLC. Results showed

that *Trichodesmium* occurred throughout the entire study area with a variation 1.78 trichome L^{-1} close to the middle part of the section , maximum values of 6099.75 trichome L^{-1} .However, linear regression showed that *Trichodesmium* has little to do with phycobiliprotein concentrations (r^2=0.0125).Saxitoxins concentration were kept low (<1 µg L^{-1}) in almost the whole area, except for tree stations where saxitoxins reached a mean of 9.2 µg L^{-1}, but no *Trichodesmium* trichomes were found. These results indicate that, altough *Trichodesmium* (primarily *T.erythraeum*) produces phycobiliproteins it is probable that other members of the phylum Cyanobacteria that belongs to the pico-plankton, were responsible for the values of this study .On the contrary, saxitoxins seems to be mostly linked to *Trichodesmium* occurrence but in lower amounts, and some other groups most likely dinoflagellates.

Key-words: Cyanobacteria, Water soluble pigments, Spectrophotometry, Phycotoxins, HPLC.

1.Introduction

The genus *Trichodesmium* is usually planktonic and instead of isolated cells, consists of trichomes, that are, extensions(filaments) formed by various cells (Komárek & Anagnostidis, 2005). Since the genus was first recognized as a valid taxonomic identity (Gomont ,1892), studies have shown a trichome arrangement typical of this group. Usually, trichomes are arranged in fascicles or radially, presenting rounded colonies, cylindrical or slightly attenuated as well as straight or curved, with gas vesicles always present (Sant'Anna et al., 2018). Morphometrics suggested by Komárek & Anagnostidis (2005) separated the genus in 11 different species, and those, 9 are exclusive from marine environments.

Althought its species number is relatively low, it is widely distributed throughout the world oceans (Bergman et al., 2013). It also plays an important role in several biogeochemical cycles (Wu et al., 2018). *Trichodesmium* involvement on the global Nitrogen (N) cycle is well established in the literature (Capone et al., 1997), and acts as a provider of new N for the Ocean water column. Besides, it is intrinsically tied to the Carbon (C) and cycle trough photosynthesis, and recently associated with the iron (Fe) retention and remineralization linked to colony formation (Bif & Yunes, 2017).

Another ecologically important characteristics of the genus is the capacity of phycotoxin production. Up to date, freshwater species of this genus are not linked to toxic events or phycotoxin production, but in marine environments, some species have been responsible for bloom formations, and ecosystem contamination associated with microcystins and saxitoxins production (Long & Carmichael, 2003). Of these two classes of phycotoxins, saxitoxins are frequently associated with *Trichodesmium* occurrence (Shunmugam et al., 2017), and its capability of adaptation forefront oligotrophic conditions, competition or light limitation.

To cope with light variation, usually in the superficial layer, *Trichodesmium* can produce specific photosynthetic pigments besides chlorophyll *a* (Kupper et al., 2009). Like other cyanobacteria, *Trichodesmium* is capable of, in some conditions, synthesize specific accessory pigments. The so-called phycobiliproteins are water soluble protein-like pigments, capable of absorbing light on a myriad of wave lengths. Thus, allowing the organism to harvest light energy in sites other than close to the red spectrum (Tandeau de Marsac, 2008) These properties provide an important advantage, which is maximum photosynthetic efficiency in low light or even on photo stress situations (Roy et al., 2011), since situations like this occur frequently in middle latitudes.

Together, phycobiliprotein and saxitoxins quantification can provide precise information about *Trichodesmium* occurrence on the ocean and its physiological state. There are a variety of factors that regulate the secondary pathways responsible for phycobiliprotein production (Pagels et al., 2019) and saxitoxins synthesis (Cusick & Sayle, 2013). Generally, factors like pH, temperature, salinity, nutrient and light intensity are linked with changes in phycobiliprotein production (Maurya et al., 2014). In the case of saxitoxins, besides those variables, its production can also be affected by other ecological factors like co-occurrent organisms, competition (Bif et al., 2019) or even geographical location (Band-Schmidt et al., 2019).

In the South Atlantic Ocean close to the Brazilian coast, factors linked to *Trichodesmium* occurrence have been well stablished over a few studies (Proença et al., 2009; Detoni et al.,2016a; Detoni et al., 2016b; Bif et al.,2019). On the other hand, studies that aimed to understand phycobiliprotein and saxitoxins production by those organisms are still scarce. For example, it is known at least since the 2000's, that *Trichodesmium* occurs on a large portion of the Brazilian coast (Proença et al., 1999).Factors linked to its occurrence vary widely, but in general are related to Brazil's Current warm temperatures, higher salinity and low nutrients, that allows the register of this organism from Recife (northeastern) to the Rio Grande do Sul (southeastern) (Rorig et al., 1998).

Although the occurrence and distribution of the *Trichodesmium* itself are relatively well known (Detoni et al.,2016a), saxitoxin quantification on natural samples was not done yet until 2009. Even so, the toxic effect of the organism was associated with toxin production only in ongoing bloom, usually in the coast (Proença et al., 2009) and more recently over the continental slope (Detoni et al.,2016b). *Trichodesmium* occurrence on

this region and potential toxicity, has been linked to deleterious effects on the marine ecosystem, and can lead to a decrease on local microplankton richness (Bif et al.,2019).

Regardless, previous works did not take into account the potential toxicity of *Trichodesmium* trichomes that were not in bloom state. So even in low concentration, *Trichodesmium* trichomes could be associated with high values of saxitoxin content. So one of the objectives of this study is to quantify saxitoxin content associated with *Trichodesmium* trichomes in not bloom state. And also include the first quantification of phycobiliproteins linked to the occurrence of *Trichodesmium* to oceanographic characteristics on the Brazilian continental slope from 28 ° S to 23 ° S.

2.Material and methods

2.1 Study Area

Samples were collected within the scope of the "TALUDE V" project between the Santa Marta Cape 28° S and Frio Cape 23° S (Fig.8). During the spring period (June) of 2012, 37 stations were performed abord of the oceanographic vessel "Atlântico Sul" of the Federal University of Rio Grande (FURG), across the Brazilian continental slope, most often following the 2000 m isobath.

2.2 Physical parameters of the sea water

Environmental data were collected using a CTD system (Conductivity,Temperature and Depth sensors) SEABIRD®. The resulting vertical profiles of temperature and salinity were used to calculate the potential density and mixed layer depth (MLD), based on the derivatives from the profiles adapted from Glover & Brewer (1988) and Kara et al. (2000).Water column stratification was calculated using Brünt Väisäla's (rad2 s-2) frequency and mean for the first 25 meters. All the data generated was already discussed on Lima et al., (2019).

2.3 *Trichodesmium* thichome counts and colonies quantification

To analyze the *Trichodesmium* spp abundance as well colony formation, water samples were taken of the superficial layer from 37 stations (Detoni et al.,2016a). Approximately 10 L of sea water were flush into a 5 µm phytoplankton mesh, and then carefully washed up with filtered seawater (Whatman ® 0.7 pore size fiber filters) sea water. The washed product containing the retained material, was transferred to 150ml amber glass flasks. The samples were fixed in a 4 % formalin solution. To express the final trichome concentration or density, a 66.67 (related to the volume filtratered) concentration factor was used after the trichome and colonies count.

Sedimentation chambers were used to count *Trichodesmium* spp. using the method proposed by Utermöhl (1958). To achieve a better estimative, a 100 × dry lent was used during the counting process on an inverted microscope. Before the counting process even began, 1 ml of acetic acid was dripped on the samples, in order to collapse the gas vesicles inside the *Trichodesmium* cells, thereby resulting on a better sedimentation process (Cronberg et al. 2004). The whole chamber was inspected for trichome presence, and the total trichome number was a sum of free trichomes and trichomes arranged in colonies. The number of trichomes per colony was considered as equivalent of a 200 trichomes col $^{-1}$ (Carpenter,1983).

2.4 Phycobilissome content: phycobiliprotein quantification

Water samples were filtered into glass fibers filters with volumes ranging from 1.2 L to 2.2 L, and the retained material stored in the dark with liquid nitrogen onboard. Phycobilissome content was measured by means of spectrophotometry using a Varian-50 UV-Vis spectrophotometer. The extraction protocol was adapted from Tandeau de

Marsac & Houmard (1988). A 5 mL of buffer sodium acetate (20 mM pH 5,5) and 1 % (w/v) streptomycin sulfate 0,5 ml were added to GFF glass fiber filters (Whatman® – 0.7 pore size) placed on a 60×15 mm Petri dish, to extract phycobiliproteins. The mechanical method of the extraction was conducted by placing the Petri dishes with the reagent in an orbital shaker 50–54 rpm for 24 hours. Residual turbidity or any impurity left on the extract was removed by centrifugation for 1 minute at 10000 rpm at 4 ºC. Spectrophotometric absorbances were taken at 565, 620, 650 and 750 nm, and buffered sodium acetate 20 mM was used as blank. The final concentration for the three primary phycobiliproteins was obtained using the equations from Bennet & Bogorad (1973) and Bryant et al. (1979), and the values expressed in mg ml^{-1}.

2.5 Saxitoxins quantification: HPLC quantification for the particulate fraction (pSTX)

Saxitoxins (STX) content on the particulate fraction were determined according to Rourke et al. (Rourke et al., 2008). Water samples from the superficial layer were filtered onto (0.7 µm) glass fiber filters GF/F (Whatman®). Filtered volume ranged from 1 to 2 L of seawater and the retained fraction was frozen into liquid nitrogen until processing for particulate saxitoxin (pSTX) analysis. Toxins were extracted from the filters by immersing them in a 0.05 M HCl solution for a minimum of 24 hours. Mechanical extraction was performed using ultrasonication (three 30-second cycles with 30-second intervals). The resulting extract was submitted to 5 minutes at 6000 rpm centrifugation process. After, the separated supernatant was filtered on a cellulose acetate filter (Whatman 0.45-pore size) for analysis on HPLC.

The pSTX analysis was performed on a Shimadzu LC20 chromatography consisting on : a CBM 20A controller, an RF-10Axl Fluorescence detector (330 nm excitation and 390 nm emission), two LC-20AD binary pumps and a CTO-20A column oven

controlled at 35°C using Labsolution 5.41 software. 240. The analytical column used for the analyses was Agilent® Zorbax Bonus-RP C8 (150 ×4.60mm, 5µm).

Since the utilized method needed a derivatization step, a manually controlled LC-10AD quaternary pump and a bath system (Novatecnica, model NT 245) with a temperature controlled at 80°C was used, immersing the Teflon hoses.

Which sample was diluted in the specific eluent (mobile phase) and injected manually using a Rheodyne injector, with a 100 µL loop. The commercial standards were then eluted on the mobile phase A [GTX1-5] consisting on: 11 mM heptanesulfonate and 5.5 mM phosphoric acid at pH 7.1. Mobile phase B [Neo-STX, dc-STX, STX] consisted of 11 mM heptanesulfonate and phosphoric acid 16.6 mM at pH 7.1 and contained 11.5% Acetonitrile. Flow rate was kept at 1 mL/min and gradient elution of 15 min. Derivatization reaction with 7 mM periodic acid and di-Potassium hydrogen phosphate trihydrate 10 mM and stabilized with acetic acid. For the total pSTX analyses, analogs of gonyautoxin standards (GTX-1, GTX-2, GTX-3, GTX-4, and GTX-5) and three STX [neosaxitoxin (Neo-STX), decarbamoylsaxitoxin, and dc-STX] were used in addition to the saxitoxin STX standard. Toxins final concentration on the sample was determined by the sum of total toxins evaluated, taking into account their relative toxicity, and calculating the total concentration of equivalent STX according to (Oshima, 1995). Standards and reference material certificates were obtained from the National Research Council Canada (NRC).

Detection (DL) and quantification (QL) limits of the instrument for pSTX were estimated according to ANVISA, (2003) and Ribani et al., (2004) considering peak area and signal-to-noise ratio with final values of 0.01 µg L^{-1} for DL, and 0.09 µg L^{-1} for QL.

To assess DL and QL at least 7 and 10 readings from the same known sample. Control samples (blanks n=3) were read in triplicate.

2.5 Statistics

Logarithms were used to determine the relationship between phycobiliproteins and trichomes. To perform the linear regression that would follow, the absolute number of phycobiliproteins and trichomes were first converted to natural logarithm and then, a one-tailed non-parametric Spearman correlation and linear regression test with 95% of confidence intervals, was used at the significant level of $p<0.05$.

3.Results

3.1 Oceanographic conditions

As the environmental framework was published elsewhere and already discussed by Lima et al. (2019), oceanographic conditions showed a progression of an environmental condition dominated by Subtropical Shelf Water (STSW) closer to Cape of Santa Marta (Fig.8) to Shelf Water (SW) moving northward, and Tropical Water (TW) closer to Frio Cape (see Lima et al.,2019).During this cruise, only those 3 water masses were identified on the surface layer (first 1 meter depth).Also according to Lima et al., (2019), SST had a mean of 22.4 ºC and SSS of 36.1 of salinity. This data showed a clear division between a south quadrant, dominated for colder and less salty water from STSW, and a north quadrant, dominated for warmer and saltier waters from TW. As for the mixture layer, it was kept at a mean of 15 m but with a wide deviation of 13. Global stability of the water column in all stations sampled during this Spring cruise reached a mean of $1.0 \times 10^{-5} rad^2\ s^{-2}$ showing relatively low stability.

3.2 *Trichodesmium* cell counts and related phycobiliprotein production

Taxonomical identification of trichomes could only achieve a genus level, indicating that the *Trichodesmium* species are unequally distributed along the studied region. This distribution contributed in different amounts to the total trichome concentration in each station. *Trichodesmium* trichome and colonies counts displayed a mean of 281 trichome L^{-1} with minimum value of 1.78 trichome L^{-1} close to the middle part of the section and, maximum values of 6099.75 trichome L^{-1} (Fig. 9A) at the southmost part close to Santa Marta Cape. Although free trichomes varied between 1.20 and 4695 trichome L^{-1} it also presented with higher concentrations in the south quadrant, slowly decreasing towards the middle part of the section. Some stations did not show any *Trichodesmium* trichome or colonies but were included on the mean calculated for the entire cruise. Some stations were devoided of detectable phycobiliproteins (see Fig. 9A). This scenario reflected on the relation between phycobiliprotein and trichome counts, that showed to be weakly dependent ($r^2 = 0.0125$). Besides, the linear regression (Fig.9B) demonstrated which during this study, phycobiliprotein quantification were not conditioned to *Trichodesmium* occurrence.

3.3 Saxitoxins quantification and analog composition

From the 37 samples submitted to analysis, 21 presented saxitoxins concentrations within the DL and QL (Fig.10A). Phycotoxins composition for all samples included 6 saxitoxin analogs, GTX-2, GTX-4, GTX-5, GTX-3, Neo-STX, dc-STX occurred, but STX does not appeared in our analysis. Only its analogues were perceived. Considering only stations that presented *Trichodesmium* occurrence (trichomes and colonies),the average concentration presented was 0.29 µg L^{-1} STX eq, with a minimum of 0.087 and maximum of 2.61 µg L^{-1}.In the other hand, the 21 samples that presented STX, five

could not be linked to *Trichodesmium* occurrence, because the cell count did not reveal any *Trichodesmium* trichomes. In this case, the concentration values were grouped together and excluded from the average concentrations from the stations that presented *Trichodesmium* cells. Total STX concentration reached the highest values registered during this study with a mean of 9.2 µg L^{-1} STX eq, ranging from 0.094 to 47.96 µg L^{-1} STX eq. Apart from station 3 (closer to Santa Marta Cape) that showed the highest values where *Trichodesmium* appeared on count samples (2.61 and µg L^{-1}), STX concentration attained to values lower than 1 µg L^{-1} all stations from the southmost cross-section , and middle part of the section. Stations 30,35 and 36 (Fig. 10B) not only stand out from the others whit high values (47.96;4.76 and 2.56 µg L^{-1} STX eq respectively), but presented the occurrence of Neo-STX and dc-STX, while in the rest of the stations only GTX-2,3,4,5 occurred, showing a clear division between the southmost part of the section.

4.Discussion

4.1 Environmental conditions and *Trichodesmium* occurrence

Besides from *Trichodesmium* related phycobiliprotein production and its link to saxitoxins level over the Brazilian continental slope, this study discussed how the distribution of trichomes and colonies was influenced by at least tree water masses from 23º S to 28º. (Fig.8). According to Lima et al. (2019) and references therein, water mass allocation influenced greatly the phytoplankton distribution. Especially in the Southwestern Atlantic Ocean, irregular distribution of the Brazilian Current has this influence, thereby affecting the diversity, species composition and ecology of phytoplankton. This effect can be observed in a great part of the continental slope and outer shelf region (Detoni et al.,2016a).

As for *Trichodesmium* distribution during this study, microscopy revealed a pool of species, but no clear identification could be made. Considering other works that assembled microscopy data from the same region, at least three species can be considered from the four that were previously registered in Brazilian waters. The water column conditions favor the occurrence of *T.thiebautii, T erythraeum* and *T.hildebrantii* (Bif et al., 2019) mostly where the TW is present (northward) on the study area. This pattern of distribution suggests a preference of the organism for oligotrophic, warm stratified waters linked to BC, that is, in fact well documented for great part of the study area (Brandini & Kutner, 1986; Rodrigues et al., 2014).

Apart from the sole oligotrophic conditions that favor nano and pico-plankton organisms (Veldhuis.,2013), *Trichodesmium* physiology greatly contributes to this scenario. A typical cell from this genus presents gas vesicles, so buoyancy capacity allows the organism to stay in the upper water column. In addition, *Trichodesmium* can fix atmospheric N_2 (Capone,1997), have no natural predators, a low growth rate and like mentioned in the introduction section of this paper, a singular photosynthesis apparatus. All of these characteristics show the physiological advantage of living distributed over these stratified oligotrophic waters, but with sufficient concentrations in phosphate (Lima et al., 2019).

4.2 Phycobiliprotein quantification and *Trichodesmium* related production

Studies that involved phycobiliprotein quantification are scarce, and no records of phycobiliprotein quantification could be found for this part of the Atlantic Ocean, so data check and pattern establishment is difficult. However, studies about this particular set of pigments in cyanobacteria show that low concentrations (Fig.9A) are expected in natural samples (Tandeau de Marsac, 2008). Part of the condition responsible for this

presentation within natural samples, is the water-soluble nature of the pigments itself. So the compounds are exposed mostly to dissolution in the water column. Another factor associated with low concentrations found in this study is light degradation. Since phycobiliproteins are susceptible to damage by overheat, they tend to have a short life after the excited state, further hampering its quantification (Roy et al., 2011).

This state reflected on the relationship between *Trichodesmium* trichome occurrence and phycobiliprotein concentration (Fig.9B). Since these cyanobacteria is wide spread throughout the study area, it is only logical that *Trichodesmium* (independent from species) would be the prime responsible for phycobiliprotein production in this region. As shown in Fig.9B, that was not the case. The linear regression showed no co-dependency between the two variables, indicating that some other group that produces those specific pigments could be responsible for the pattern saw in this study. Data acquired by Lima et al. (2019), trough HPLC-CHEMTAX analyses, showed a contribution of cryptophytes (close to Frio Cape), and major contributions of *Prochlorococcus* and other members of the phylum cyanobacteria like *Synechococcus* (closer to Santa Marta Cape). Being this three groups already listed as phycobiliprotein producers (Doust et al., 2004), and abundant throughout the area, it is safe to say that, instead of the sole *Trichodesmium* contribution first theorized, those other organisms are responsible for the values found during this study.

Since no other methods besides light microscopy were used to access phytoplankton composition, and most of all, this study was focused on *Trichodesmium*, no other groups could be linked to phycobiliproteins, especially pico-planktonic groups like *Synechococcus* and *Prochlorococcus.* But, the use of pigment base phytoplankton evaluation trough HPLC could quantify those groups (Lima et al.,2019). This indicates

that in those oligotrophic waters, this small (> 2 µm) cell, with low surface-volume ratio phytoplankton produces these pigments as a strategy for energetic fuel (Scheer, 2003) to cope with nutrient or light intensity or quality. Considering its part on the photosynthesis, primarily in organisms that present phycobilisomes like cyanobacteria, the phycobiliproteins can work as an adaptation due to more intense irradiance during the spring (Lima et al.,2019), working as a funnel, allowing efficient light-harvesting production even in higher irradiance (Roy et al., 2011). Nutrient limitation, mostly by N, can also be linked to phycobiliproteins concentrations, that can work as an N storage (large number of amino acid) or N source, depending on the environment state, and phycobilin degradation (Hong Zhao et al., 2011).

4.3 Saxitoxin production and *Trichodesmium* related quantification over the continental slope

Considering other works that linked saxitoxin to *Trichodesmium* occurrence over the same region, concentration interval seems to be within values already reported (Proença et al., 2009; Detoni et al., 2016a; Bif et al., 2019), except in situations where slicks were present. In this study no slick were sampled and saxitoxin concentrations remain in low amounts, < 1µg L^{-1}. Even so, the toxicity of the saxitoxin alone, or its analogs in low amounts, can have a high impact in co-occurrent phytoplankton (Bif et al., 2019) and the local biota in general. Another point to be observed is that, before this study, saxitoxin quantifications linked to *Trichodesmium* in the Southwestern Atlantic was registered primarily in slicks (Proenca, 2009) that were thought to be the principal source of saxitoxins.

Although, what we came across was evidence that even in low amounts, *Trichodesmium* cells were able to produce recordable proportions of those phycotoxins. Tough in low

amounts, the sole presence and analog composition of this STX in the water column in this particular region can indicate, not only the register of the toxin production itself, but relationships with species composition and geographical location (Band- Schmidt et al., 2017).

As discussed in the past section of this article, we were not able to identify species, but considering previous works the only species knowingly proved to produce saxitoxins, and the analogs found where trichomes were present is *T.erythraeum* (Proença et al., 2009).Since this is the only marine species yet clearly responsible for reported saxitoxin production, it is likely that within the cell count presented in this study only some of them produced saxitoxin. Though we cannot state the proportion of toxin producing cells to the non-toxic ones, but it is clear that even lower concentration of toxin-producing cells, contributed to the concentration of saxitoxin quantified. Meaning higher toxicity, but with a probable unique source.

All stations that showed STX concentration (Fig.10A) coupled with *Trichodesmium* presence, demonstrated its own pattern of analogs .Presence of less toxic analogs within the gonyautoxin groups were the major contributors to those findings, meaning that is safe to say: *Trichodesmium* cells were responsible for the appearance of GTX 2-5 throughout in entire section and stations presented in (Fig. 10A).Curiously in the stations where STX were quantified but *Trichodesmium* was not, STX concentrations reached the highest levels (Fig .10B) along with the presentation of a differentiated analogs, like in dc-STX and Neo-SXT. Only one other group of STX producers could be responsible for those values: dinoflagellates. According to Lima et al. (2019) dinoflagellates made in second on ranked biomass contribution within the STSW (station 30) and TW region (stations close to Frio Cape). Therefore, in addition to

tracking STX production by *Trichodesmium*, it is possible that in our results we also quantified saxitoxin produced by dinoflagellates, most probable by organisms from the genus *Gymnodinium* or *Alexandrium* since they occur in that area (Moser et al., 2012) and are mapped worldwide, mostly because have shown high toxicity even in low cell density(Wang, 2008).

We can also point out that, in stations where *Trichodesmium* was not present (30,35 and 36), the analogs presented a different pattern from the rest of the stations. This reinforces the idea that other organisms, most probable dinoflagellates were playing a not yet known, but important part over the STX production in this region. Lastly, we did not count other components of the phytoplankton, and therefore the inference of STX production besides *Trichodesmium* itself is difficult. Even though, it can be safe to say that no other candidates to that position as a producer could be considered. So we can indicate the two groups already mentioned as responsible for the other pattern of analogs shown in this study, like dc-STX and Neo-STX (Persich et al., 2006; Menezes et al., 2018) also connected to the highest values of STX.

5.Concluding remarks

As a conclusion this study we argued that apart from any methodological or sampling limitations, we were able to access the *Trichodesmium* cell counts and conclude that oceanographic conditions like, a less stable water column with a shallower mixture layer and oligotrophic waters, provide a favorable environment for the occurrence of this organisms, almost throughout the entire study with a difference between sectors northward and southward. Phycobiliproteins showed not be closely related to *Trichodesmium* occurrence indicating interference of other groups like cryptophytes or

other cyanobacterial genera from the pico-plankton, like *Synechococcus* or *Prochlorococcus.* It was also made clear that STX production was kept in different patterns and concentrations in stations were *Trichodesmium* occurred (GTX 2-5) ~ 0.29 µg L^{-1} and were did not (dc -STX and Neo-STX) ~ 9.2 µg L^{-1} , showing that despite *Trichodesmium* related production, there are other organisms involved on this dynamic, most probable dinoflagellates from the genus *Gymnodinium* and *Alexandrium*.

Acknowledgments

This study would not be possible without the support of the crew of FURG's R V "Atlântico Sul" and we also thank specially to the researcher Dr.Amália Detoni for the collection of samples and managing of the database. Funded was provided by Chevron Brasil Upstrem Frade Ltda and ship supplies (diesel) was provided by the Brazilian Inter-Ministerial Commission for the Resources of the Sea (CIRM). This study is a contribution of the Brazilian National Institute of Science and Technology - INCT-Mar COI funded by CNPq Grant Number 610012/2011-8. This work also received funds from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES. C.CWerlang is granted by a MSc fellowship (CAPES) number (88882.182296/2018-01). M.S de Souza and L.D.F Costa are both granted by PNPD/MEC-CAPES fellowship numbers (88882.314601/2019-01) and (88882.314596/2019-01) respectively , and J.S.Yunes a productivity fellowship of Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq number (311402/2017-8).

Artwork (figures) with Captions

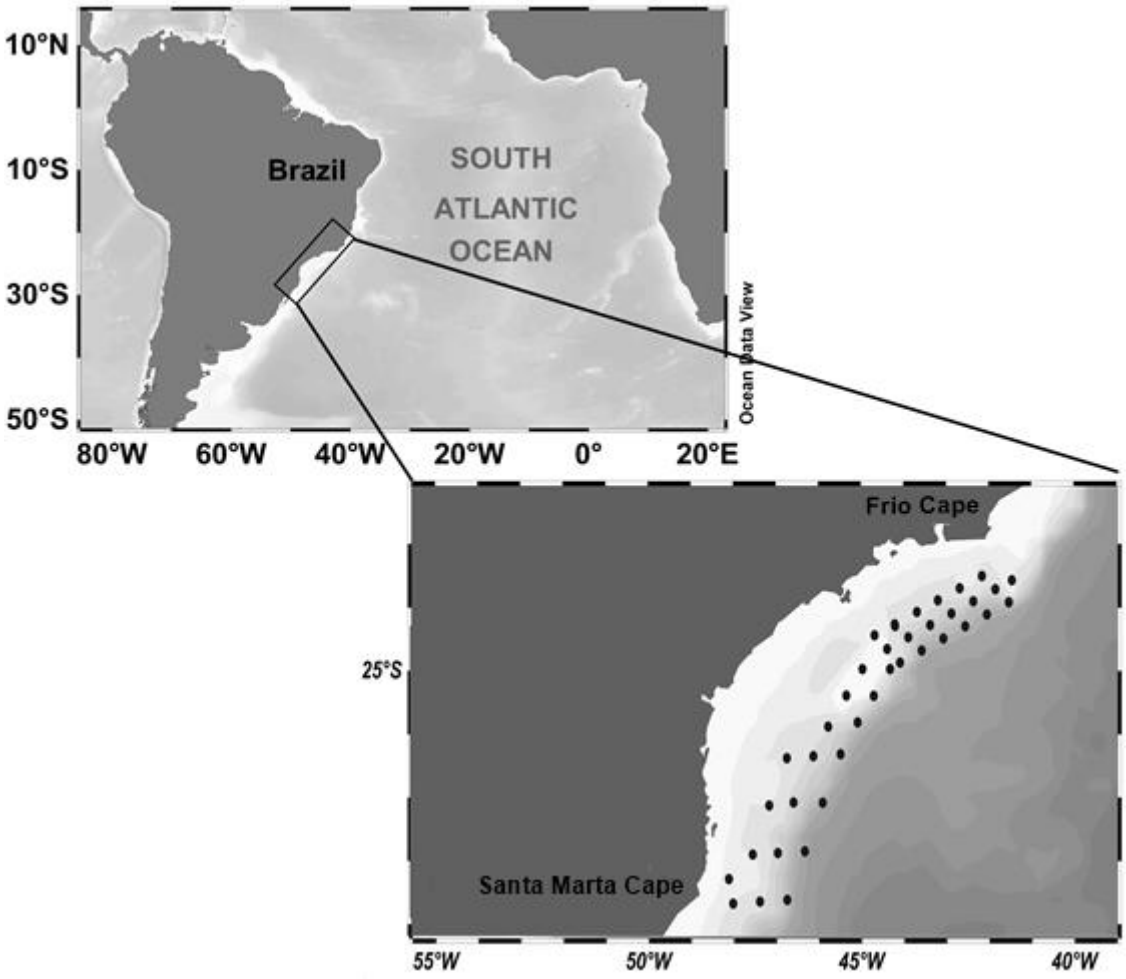

Fig.8. Study area representing the section between Santa Marta Cape (28°S) and Frio Cape (23°S) during 11–24 June of 2012 following the Brazilian continental slope under the umbrella of the project "TALUDE-V".

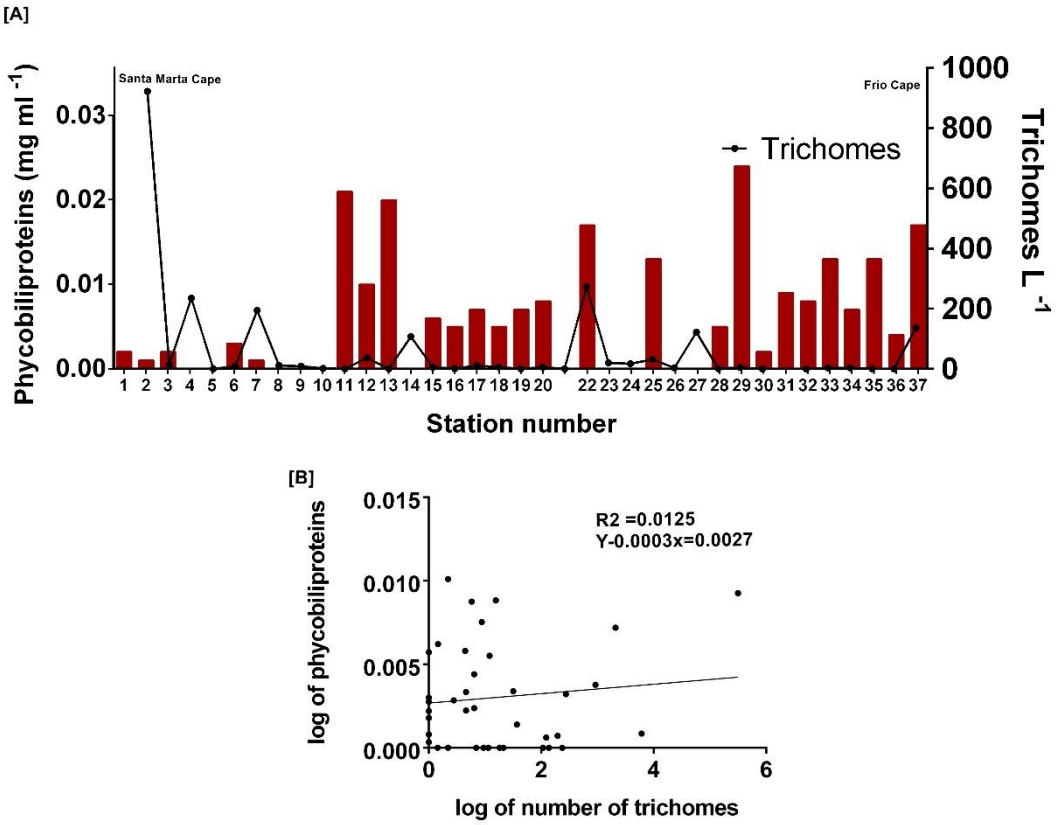

Fig.9. [A] Phycobiliprotein concentration and number of trichomes of *Trichodesmium* in 37 stations across the Brazilian southeastern slope, under the umbrella of the TALUDE-V project (11–24 June, 2012). [B] Linear regression between log of trichomes final concentration and phycobiliproteins where $r^2 = 0.0125$.

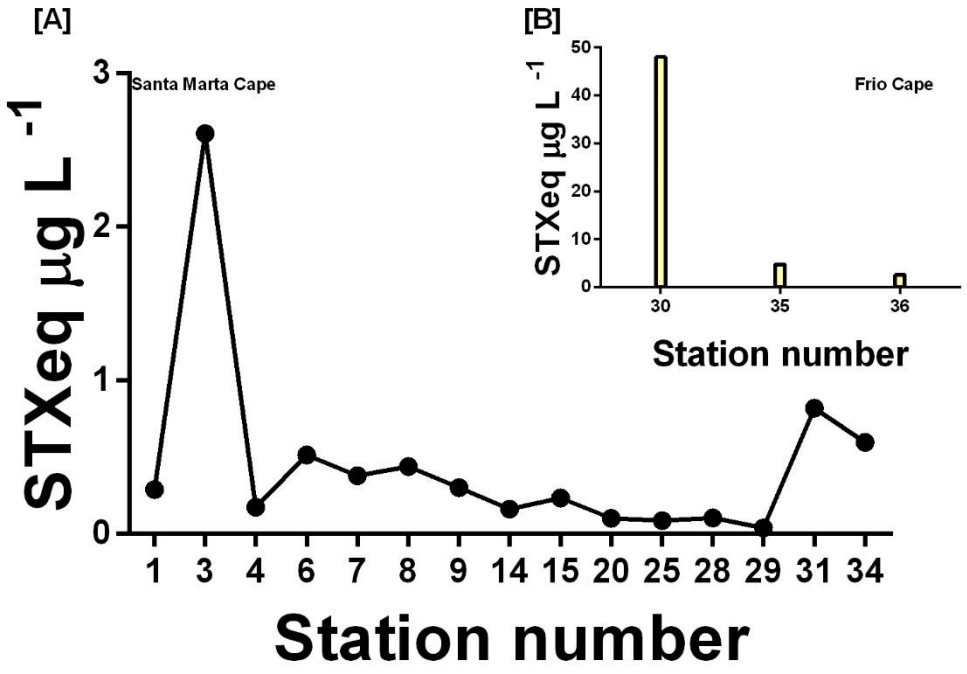

Fig.10. Saxitoxin concentration across the Brazilian southeastern slope, under the umbrella of the TALUDE-V project (11–24 June, 2012). [A] represents stations with occurrence of *Trichodesmium* spp. and [B] corresponds to stations where saxitoxins attained higher values but *Trichodesmium* spp. were absent.

CAPÍTULO VI: SÍNTESE DA DISCUSSÃO E CONCLUSÕES

Considerando o presente conjunto de dados disponível (nos dois manuscritos inclusos), foi possível determinar a concentração de diferentes pigmentos e pelo menos uma toxina (AD) ao longo da costa do Rio de Janeiro e talude continental brasileiro (STX) do Cabo de Santa Marta até Cabo frio. Quanto à região costeira próxima ao Rio de Janeiro, representada pelos cruzeiros realizados no âmbito do projeto "Bonito Listrado", ficou claro que este local apresenta comunidades fitoplantônicas muitos diversas, ainda que com o predomínio de diatomáceas. Esse fenômeno parece ser independente das estações do ano amostradas. Mesmo assim, houve clara predominância de diatomáceas potencialmente toxigênicas (*Nitzschia* e *Pseudo-nitzschia*) durante o inverno. Também foi possível notar que as mudanças nas características ambientais (por exemplo, a estratificação da coluna d'água ou profundidade da camada de mistura), contrastando aqueles períodos de inverno e primavera, parecem influenciar a concentração de STX e pigmentos, principalmente carotenóides. Esses parâmetros foram relacionados com a composição da comunidade fitoplanctônica, descrita em termos de abundância de células e biomassa em carbono. Houve uma comunidade dominada por diatomáceas penadas (*Pseudo-nitzschia*) durante o inverno, até outra dominada por diatomáceas cêntricas e cilíndricas (*Rhizosolenia*, por exemplo) e juntamente com dinoflagelados, na primavera. Essas mudanças podem estar ligadas a estressores ambientais, tais como o decaimento na concentração de nutrientes ou foto-oxidação, sendo este último devido ao aumento da luminosidade (especialmente na primavera e, provavelmente, até o verão). Esse cenário foi visualizado pelos valores da razão C:CHL-*a*.

As neurotoxinas (STX e AD) avaliadas parecem ser onipresentes ao longo da região sul-sudeste e, logicamente, suas concentrações devem estar ligadas com as diferenças relativas de composição fitoplanctônica: inverno com o predomínio de *Pseudo-nitzschia* e primavera com o aumento relativo de dinoflagelados e *Trichodesmium*. Sendo assim, mesmo em baixas concentrações, essas toxinas foram mensuradas em todas as estações, tanto no inverno quanto na primavera. Uma vez que os dinoflagelados são considerados os maiores produtores de STX no mar e podem causar dano a biota, mesmo em baixa densidade celular, a região sul-sudeste brasileira pode continuar como alvo de futuros estudos sobre a concentração e produção de variadas toxinas (além das quantificadas nessa dissertação) e suas implicações ecológicas e econômicas.

Já quanto às amostras provenientes do cruzeiro realizado apenas durante a primavera, no âmbito do projeto "TALUDE-V", foi possível avaliar apenas a distribuição de *Trichodesmium,* relacionada a variáveis oceanográficas e com a determinação da concentração de ficobiliproteínas e STX. Apenas o gênero *Trichodesmium* foi quantificado (ver Detoni et al., 2016b, e dados cedidos pela primeira autora). Mesmo assim, evidenciou-se a distribuição de *Trichodesmium* spp. associadas a três massas d'água e à CB. A abundância de *Trichodesmium* spp. variou conforme a distribuição da ASP e AP na porção mais ao sul, e segundo a AT na porção mais ao norte da área abrangida pelo projeto "TALUDE-V". Foram estimadas baixas concentrações de ficobiliproteínas e, aparentemente, não seriam relacionadas apenas com a abundância de *Trichodesmium* spp., corroborando outros estudos publicados que apontaram a contribuição relativa de cianobactérias picoplanctônicas (*Synechococcus* e *Prochlorococcus*) e, possivelmente, criptofíceas. Indicou-se que *Trichodesmium* spp. devem ter sido os principais responsáveis pela concentração de STX, considerando a sua maior abundância relativa em certas estações de coleta. De maneira inversa, nas estações mais próximas a Cabo Frio, houve maiores concentrações de STX, mas sem a presença de *Trichodesmium* spp. Portanto, sugere-se que ainda que *Trichodesmium* produza STX, outros organismos (potencialmente dinoflagelados) sejam mais importantes para os níveis de STX e seus derivados.

Por fim, considerando as contribuições e as limitações dessa dissertação, é possível propor que futuros projetos ponderem sobre o papel da sucessão/diferenças de composição na comunidade fitoplânctônica. Tudo isto em um contexto de frequência e abundância de organismos produtores de toxinas e seus potenciais efeitos sobre a biota marinha. Como não foi possível a coleta de amostras durante um ciclo sazonal completo, também seria interessante avaliar a influência das (todas) estações do ano sobre a variação da comunidade fitoplanctônica e, consequentemente, na concentração de seus pigmentos e toxinas. Outra perspectiva seria a avaliação da comunidade fitoplanctônica (pigmentos e toxinas) ao longo da camada eufótica, considerando tanto a produção primária e de toxinas, pelo menos até a base da camada de mistura, já que diferenças na proporção entre os organismos podem levar a diferenças na concentração de toxinas. Por isso, encorajar a identificação de espécies potencialmente tóxicas (caso de diatomáceas penadas e dinoflagelados), a criação desses registros, e o estabelecimento de cultivos desses organismos para ensaios laboratoriais seriam propostas a ser bem mais exploradas

futuramente. Frente às dificuldades em se obter dados de razão C:CHL-*a* ao longo do Atlântico Sul, sugerimos futuros estudos que abordem a quantificação da produção primária aliada com estimativas de CHL-*a* e biomassa em carbono, que possuem potencial para a compreensão da dinâmica/ciclo do carbono, incluindo a dinâmica de carbono refratário e seus efeitos ambientais sobre a estrutura e dinâmica de comunidades fitoplanctônicas.

Considerando a importância das comunidades fitoplanctônicas na estruturação dos ecossistemas marinhos a nível global, é imperativa a produção de conhecimento sobre esse tema. No entanto o estado atual da ciência brasileira e o constante corte em verbas têm prejudicado a capacidade dos pesquisadores brasileiros de manter estudos robustos considerando os custos e logística para embarques oceanográficos. Além disso, é um desafio diário manter tanto os custos das análises em laboratório quanto o ritmo necessário para a produção científica de qualidade. O corte de financiamento pelo governo federal assombra os pesquisadores desde 2016, quando 44% do financiamento foi congelado. A cota para 2017 foi de 3,2 bilhões de reais, o mais baixo dos últimos 12 anos [Angelo, 2017], e até 2019 o orçamento foi ainda menor, onde apenas 2,9 milhões foram destinados à pesquisa [Angelo, 2019]. Além disso, os congelamentos e cortes de bolsas de estudo também desestabilizaram os pesquisadores no Brasil [Andrade, 2019; Mega, 2019]. Esse cenário coloca em risco não só a ciência como ferramenta de produção de conhecimento e solução de problemáticas que afetam a sociedade como um todo, mas também a produção de conhecimento sobre a biodiversidade brasileira. Portanto abordar temas como a estruturação das comunidades fitoplanctônicas e a diversidade desses organismos, pode se tornar uma tarefa ainda mais árdua em um futuro próximo, cabendo a sociedade como um todo e os pesquisadores vindouros a difícil tarefa de reestabelecer a educação e ciência brasileira, como importante ponto de investimento governamental nos anos a seguir.

CAPÍTULO VII: REFERÊNCIAS BIBLIOGRÁFICAS

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