POTENCIAL BIOTECNOLÓGICO DA MACROALGA *ULVA FASCIATA*: DO CICLO PRODUTIVO À EXTRAÇÃO DE POLISSACARÍDEO

Tiphane Andrade Figueira

Tese de Doutorado apresentada ao Programa de Pós-graduação em Biotecnologia Vegetal e Bioprocessos, CCS, da Universidade Federal do Rio de Janeiro, como parte dos requisitos necessários à obtenção do título de Doutor em Biotecnologia Vegetal e Bioprocessos.

Orientadores: Alex Enrich Prast
Vinícius Peruzzi de Oliveira

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POTENCIAL BIOTECNOLÓGICO DA MACROALGA *ULVA FASCIATA*: DO CICLO PRODUTIVO À EXTRAÇÃO DE POLISSACARÍDEO

Tiphane Andrade Figueira

Setembro/2020

Orientadores: Alex Enrich Prast
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Programa: Biotecnologia Vegetal e Bioprocessos

Espécies do gênero *Ulva* se destacam por seu potencial biotecnológico em setores como biocombustível, alimentação e fármacos. Isso se dá principalmente por sua presença cosmopolita, rápida taxa de crescimento, elevado conteúdo de carboidratos e a presença do biopolímero ulvana. Nesse contexto, o objetivo desse estudo foi ampliar o conhecimento do potencial biotecnológico da macroalga *Ulva fasciata*, desde a seleção de população e análises fisiológicas até a obtenção e caracterização de polissacarídeo de interesse biotecnológico. Em uma primeira etapa (capítulo 1) avaliou-se o efeito do fosfato na fisiologia e produção de carboidrato pela macroalga *U. fasciata* de duas regiões distintas, Arraial do cabo, submetida ao fenômeno de ressurgência e Niterói, região sem amplas variações ambientais, cultivadas em diferentes concentrações de fosfato. Os indivíduos de Niterói apresentaram menor conteúdo de carboidratos no tratamento com maior concentração de fosfato (46% massa seca), enquanto os indivíduos oriundos de Arraial do Cabo apresentaram maior produção de carboidratos em todos os tratamentos (71% massa seca). Indivíduos coletados nessa região foram utilizados na etapa subsequente para avaliação, *in vitro*, da eficiência de remoção de nitrogênio e fósforo de água enriquecida e caracterização da ulvana extraída da biomassa produzida (capítulo 2). Ao final do período experimental os indivíduos cultivados em água do mar enriquecida removeram 100% do nitrogênio (amônio + nitrato) e 22% do fosfato. Indivíduos controle (cultivados em água não enriquecida) apresentaram taxa de crescimento significativamente menor ($p < 0{,}1$) e forte redução no teor de fósforo tecidual, indicando seu uso para sustentar o crescimento em condições de depleção de nutrientes. A biomassa gerada após esse experimento foi utilizada para obtenção de

ulvana. A ulvana extraída da biomassa cultivada e da biomassa coletada diretamente do ambiente natural são constituídas principalmente de ramnose, ácido idurônico, ácido glucurônico, sulfato e, no caso da ulvana do ambiente natural, xilose, conforme mostrado pelas técnicas de caracterização de Espectrometria no Infravermelho com transformada de Fourier (FT-IR) e Ressonância Magnética Nuclear (RMN), sendo similar à ulvana reportada na literatura. O conhecimento da ecofisiologia da *Ulva* e dos desafios envolvidos na sua produção é fundamental para que a fronteira biotecnológica entre a pesquisa e indústria possa ser transposta. Os resultados aqui apresentados ampliam esse conhecimento, servindo de referência para estudos futuros de escalonamento da produção de *Ulva fasciata* e seus diversos produtos.

Palavras-chave: Biotecnologia. Fósforo. Macroalgas. Nitrogênio. Polissacarídeo sulfatado. Sustentabilidade. Ulvana

Abstract of Thesis presented to CCS/UFRJ as a partial fulfillment of the requirements for the degree of Doctor of Science (D.Sc.)

BIOTECHNOLOGICAL POTENTIAL OF THE MACRO ALGAE *ULVA FASCIATA*: FROM THE PRODUCTION CYCLE TO POLYSACCHARIDE EXTRACTION

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September/2020

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Department: Plant Biotechnology and Bioprocesses

Species of the genus *Ulva* presents great biotechnological potential in sectors such as biofuel, food and pharmaceuticals. This is mainly due to its cosmopolitan distribution, rapid growth rate, high carbohydrate content and the presence of the biopolymer ulvan. In this context, the goal of this study was to expand the knowledge of the biotechnological potential of the macroalgae *Ulva fasciata*, from population selection and physiological analyzes to obtain and characterize polysaccharides of biotechnological interest. In a first step (chapter 1), the effect of phosphate on the physiology and carbohydrate production by the macroalga *U. fasciata* from two different regions, Arraial do cabo, submitted to the upwelling phenomenon and Niterói, a region without wide environmental variations, cultivated in different concentrations of phosphate was evaluated. Individuals from Niterói had lower carbohydrate content in the treatment with higher phosphate concentration (46% dry mass), while individuals from Arraial do Cabo had higher carbohydrate production in all treatments (71% dry mass). Individuals collected in this region were used in the subsequent step to assess, *in vitro*, the efficiency of removing nitrogen and phosphorus from enriched water and characterizing the ulvan extracted from the produced biomass (chapter 2). At the end of the experimental period, individuals cultivated in enriched sea water removed 100% of nitrogen (ammonium + nitrate) and 22% of phosphate. Control individuals (cultivated in non-enriched water) showed a significantly lower growth rate ($p < 0.1$) and a reduction in the content of tissue phosphorus, indicating its use to sustain growth under nutrient depleted conditions. The biomass produced after this experiment was used to obtain

ulvan. Ulvan extracted from cultivated biomass and biomass collected directly from the natural environment consists mainly of rhamnose, iduronic acid, glucuronic acid, sulfate and, in the case of ulvan from the natural environment, xylose, as shown by Infrared Spectrometry characterization techniques with Fourier transform (FT-IR) and Nuclear Magnetic Resonance (NMR), being similar to the ulvan reported in the literature. Knowledge of *Ulva* ecophysiology and the challenges involved in its production is essential so that the biotechnological frontier between research and industry can be overcome. The results presented here expand this knowledge, serving as a reference for future studies on the production schedule of *Ulva fasciata* and its various products.

Keywords: Biotechnology. Phosphorus. Macroalgae. Nitrogen. Sulfated polysaccharide. Sustainability. Ulvan.

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Introdução

Introdução geral

Ecossistemas marinhos são fontes extremamente ricas de bioprodutos, muitos dos quais com características físico-químicas não encontradas em organismos terrestres (Kijjoa and Sawangwong, 2004; Smit, 2004). Esta diversidade biológica origina compostos químicos únicos, com potencial para desenvolvimento de fármacos (Smit, 2004; Wang et al., 2017), cosméticos (Ariede et al., 2017; Li et al., 2018), suplementos nutricionais (Ramesh et al., 2015; Wells et al., 2016) e agroquímicos (Craigie, 2010; Cury et al., 2011). Atualmente, tem-se na literatura cerca de 35 mil artigos relacionados a compostos de origem marinha, com 8 mil moléculas isoladas, sendo 25% delas extraídas de algas (Stiger-Pouvreau and Zubia, 2020).

Dentre as macroalgas marinhas com potencial para a produção de insumos com fins biotecnológicos encontram-se espécies pertencentes ao gênero *Ulva* Linaeus. Por estarem sujeitas ao regime de maré essas algas são capazes de tolerar longos períodos de dissecação, variações de luz e temperatura (Holzinger et al., 2015; Teichberg et al., 2009). Além disso, essas algas são r-estrategistas, nitrófilas e possuem elevada relação superfície:volume, proporcionando a *Ulva* vantagens competitivas que as permitem rápido crescimento em ambientes ricos em nutrientes (Cui et al., 2019). Sustentar tal metabolismo requer atividade enzimática com alto custo energético, nutrida principalmente por nitrogênio e fósforo (Martínez et al., 2012; Roleda and Hurd, 2019). Essa ampla disponibilidade e o rápido crescimento são requisitos para espécies alvos em estudos biotecnológicos, principalmente para obtenção de compostos extraídos da biomassa tecidual.

O produto de maior potencial econômico da *Ulva* é o polissacarídeo ulvana (Shao et al., 2014; Tran et al., 2017). A ulvana é um heteropolissacarídeo sulfatado presente na parede celular, representando em torno de 29% do peso seco da alga (Konasani et al., 2018). Ele é formado por dois dissacarídeos repetidos, ácido ulvanobiuronico tipo A e tipo B, e constituído principalmente de ramnose, ácido idurônico, xilose, ácido glucurônico e sulfato (Kidgell et al., 2019; Tziveleka et al., 2019).

Dentre as aplicações da ulvana está o uso da ramnose em produtos "anti-idade" (Alves et al., 2012) e do ácido idurônico, não encontrado em outros polissacarídeos

marinhos, que atua na síntese química de análogos da heparina (Konasani et al., 2018). De modo geral, a ulvana vem apresentando resultados promissores principalmente no setor de fármacos, cosméticos e agricultura. Nesse cenário, caracterizações moleculares e comparações entre ulvanas extraídas de ambientes naturais e de laboratório podem estabelecer uma nova forma de exploração de produto.

ambientes mais ou menos afetados pela poluição antropogênica

Diante das informações acima, esta tese descreve desde o passo inicial do cultivo de *Ulva* fasciata em laboratório até a caracterização do polissacarídeo extraído da biomassa produzida cujos resultados foram publicados (Anexo A). O presente estudo encontra-se organizado em 2 capítulos que demonstram parte do processo de obtenção de produtos biotecnológicos.

O primeiro capítulo (artigo 1) intitulado "The effects of phosphate on physiological responses and carbohydrate production in *Ulva fasciata* (Chlorophyta) from upwelling and non-upwelling site" trata da influência da concentração de fosfato no meio de crescimento sobre parâmetros fisiológicos como taxa de crescimento, concentração de fósforo no tecido, fotossíntese e produção de carboidrato de *Ulva fasciata* oriunda de regiões distintas. A hipótese geral testada foi:

Indivíduos de uma mesma espécie originários de diferentes regiões de coleta respondem de forma distinta às diferentes concentrações de fosfato.

O segundo capítulo 2 intitulado "Nitrogen and phosphorus uptake and effects in *Ulva fasciata* physiology and extracted ulvan" trata de uma avaliação dos efeitos da disponibilidade de compostos nitrogenados e fosfatados em parâmetros fisiológicos da macroalga *Ulva fasciata* e do potencial de remoção desses compostos da água. Sua hipótese geral foi:

A Ulva fasciata é um eficiente agente na remoção de nitrogênio e fósforo dissolvidos na água e seu cultivo em meio enriquecido não altera a estrutura do polissacarídeo ulvana

Base teórica da pesquisa

Macroalgas

Macroalgas marinhas são organismos autotróficos fotossintetizantes, talófitos que possuem em comum o pigmento clorofila-*a*. Esses organismos são predominantemente bentônicos, servindo como berçário e refúgio para diversos grupos de animais. Além disso, são importantes produtores primários nos ecossistemas costeiros, sendo responsáveis por 5 a 10% da produção primária

marinha global(Hurd et al., 2014).

As macroalgas utilizam uma variedade de nutrientes para seu crescimento sem os quais não é possível completar seu ciclo reprodutivo (Hurd et al., 2014). Esses elementos são divididos em macronutrientes (H, Mg, S, K, Ca, C, B, N e P), micronutrientes (Zn, Fe, Cu, Mn) e vitaminas (biotina, tiamina e cianocobalamina). Dentre esses, nitrogênio e fósforo são limitantes para o crescimento das algas sendo utilizados na síntese de proteínas, nos ácidos nucleicos, na composição do aceptor químico NADPH, da molécula adenosina trifosfato (ATP) entre outros (Raven, 2013; Ribeiro et al., 2016; Roleda and Hurd, 2019).

Características ecofisiológicas da *Ulva*

O gênero *Ulva* descrito por Linnaeus em 1753 pertence a ordem Ulvales, família Ulvophyceae, e atualmente possui em torno de 100 espécies reconhecidas (Hayden et al., 2003; Matsumoto and Shimada, 2015). Esse gênero de algas verdes é encontrado tanto em ecossistemas de água doce quanto marinho (Dawes, 2016), e suas espécies podem apresentar talo tubular monostromático (por exemplo, *Monostroma grevillei*) ou foliáceo distromático (por exemplo, *Ulva fasciata*)(Wichard et al., 2015). O acesso a ferramentas de estudos moleculares revolucionou a identificação de espécies de *Ulva*, já que a diversidade, morfologia simples e grande plasticidade fenotípica do gênero dificulta sua identificação. Esses estudos apontaram que os gêneros *Ulva*, *Enteromorpha* e *Chloropelta* não são entidades evolutivas distintas e, portanto, não devem ser consideradas gêneros separados (Hayden et al., 2003).

Espécies de *Ulva* são bentônicas e encontradas na região do infralitoral. Por estarem sujeitas ao regime de maré essas algas são capazes de tolerar longos períodos de dissecação, variações de luz e temperatura (Holzinger et al., 2015; Teichberg et al., 2009). A *Ulva* é cosmopolita, sendo encontrada de ambientes polares a tropicais (Figura1). Espécies oportunistas como a *Ulva* apresentam elevadas taxas de absorção de nutrientes, necessários para sustentar seu crescimento rápido e maturação reprodutiva, sintetizando aminoácidos, proteínas e outras moléculas orgânicas ricas em nutrientes (Martínez et al., 2012). Sustentar tal metabolismo requer uma máquina enzimática energeticamente cara, alimentada principalmente por nitrogênio e fósforo (Martínez et al., 2012; Roleda and Hurd, 2019).

Nitrogênio e fósforo no metabolismo da *Ulva*

Amônio ($NH4^+$) e nitrato (NO_3^-) são as principais fontes de nitrogênio geralmente disponíveis no ambiente marinho (Shuuluka et al., 2012), sendo o amônio a forma preferencialmente absorvida pela *Ulva* devido a menor quantidade de energia necessária para sua assimilação e maior vantagem metabólica para a assimilação

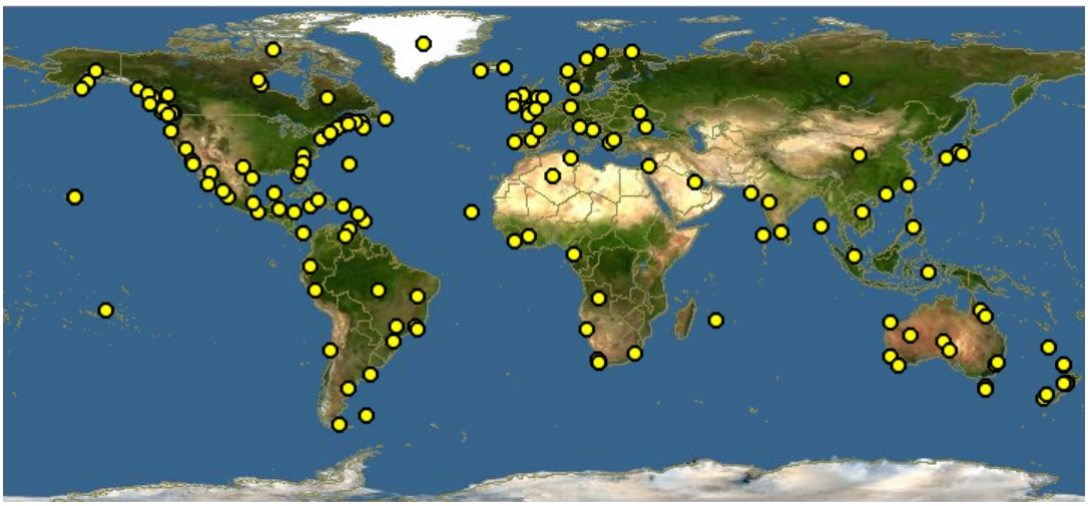

Figura 1: Distribuição global de *Ulva* lactuca. Fonte: Global information facility.

fotossintética, já que o nitrato precisa ser reduzido a amônio pela enzima nitrato redutase antes que possa ser metabolizado (Li et al., 2016; Reidenbach et al., 2017). Li et al. (2016) observaram que *U. pertusa* cultivada em meio com adição de amônio apresentou as maiores taxas de crescimento com eficiência de absorção de 98% do nitrogênio no terceiro dia, enquanto no tratamento com nitrato a taxa de crescimento não foi significativamente diferente do controle (água do mar sem adição de nitrogênio). Estudos mostram que dentre as macroalgas, as clorófitas apresentam a maior tolerância a concentrações de amônio que podem ser tóxicas para outras algas (Reidenbach et al., 2017; Shuuluka et al., 2012). Algas de rápido crescimento como a *Ulva* têm maior demanda por nitrogênio do que algas perenes de crescimento lento, principalmente para manter suas elevadas taxas de crescimento e teor de N no tecido (Ale et al., 2010; Shuuluka et al., 2012).

O fósforo está presente nas macroalgas na forma de lipídios, açúcares, nucleotídeos, polifosfatos e fosfoproteínas (Martínez-Aragón et al., 2002; Runcie et al., 2004). Embora a absorção e requisitos por fósforo seja menor do que por nitrogênio, sendo mais dependente das taxas de crescimento e das concentrações críticas de fósforo na alga, a falta desse elemento é fator limitante tanto na absorção de nitrogênio quanto no crescimento da *Ulva* (Pedersen et al., 2010). Zhou et al. (2015) e Hong et al. (2011) mostraram que mesmo em água rica em nitrogênio e com temperaturas ideais – de 15 a 25^{o}C - o crescimento da *U. pertusa* na recorrente floração que ocorre na China desde 2008 é limitado pela disponibilidade de fósforo na água. Além disso, a disponibilidade de fósforo no meio influencia diretamente no metabolismo fotossintético facilitando a fosforilação, acelerando o transporte de elétrons e promovendo a síntese de rubisco no ciclo de Calvin (Li et al., 2016).

Potencial biotecnológico

justificativa

Com o mundo enfrentando sérias questões econômicas e ambientais como a redução nas reservas de combustíveis fósseis, crescimento da população mundial e mudanças climáticas, pesquisadores vêm explorando matérias-primas alternativas, entre elas as macroalgas. Dentre os gêneros pesquisados, a *Ulva* tem mostrado excelentes resultados em diversos setores. Na indústria alimentícia é usada em alimentos funcionais por seu elevado teor de vitaminas (A, B, C e E) e minerais (K, Mg, Mn, Ca, Zn, Cu, Fe) e como fonte alternativa de proteína (7.9 – 18.6 % peso seco) (Khairy and El-Shafay, 2013; McDermid and Stuercke, 2003). Como forragem animal a *Ulva* pode impactar positivamente a saúde dos animais, reduzindo a emissão de gases por ruminantes, aumentando a resistência de peixes a doenças e melhorarando a qualidade dos produtos, por exemplo reduzindo o teor de gordura intramuscular em galinhas e o teor de colesterol dos ovos (Elizondo-González et al., 2018; Fleurence, 2016; Rjiba-Ktita et al., 2016). Na produção de biocombustíveis de terceira e quarta geração, a *Ulva* se destaca pelo elevado teor de carboidratos e maior produção de biomassa por área (Korzen et al., 2015; Torres et al., 2019).

O cultivo de *Ulva* teve início na década de 1970 nos Estados Unidos para geração de biometano e como parte do projeto US Ocean Food and Energy Farm de exploração e desenvolvimento de alimentos, combustíveis e fertilizantes a partir de organismos marinhos (Ghadiryanfar et al., 2016). Hoje seu cultivo comercial está estabelecido em países como Japão, como alternativa para coleta em bancos naturais e na África do Sul para alimentação de abalone (FAO, 2018). A produção de *Ulva* é uma das apostas do setor de aquacultura, dada sua distribuição geográfica e capacidade de remover compostos nitrogenados e fosfatados do meio (Brundu and Chindris, 2018).

Os primeiros trabalhos avaliando a eficácia do cultivo de macroalgas para atenuar os impactos do aporte excessivo de nitrogênio e fósforo no ambiente marinho foram publicados na década de 1970 e desde então diversos estudos têm demonstrado a eficácia desta abordagem (Cohen and Neori, 1991; Wu et al., 2015; Xiao et al., 2017). A eficiência de remoção de nitrogênio pela *Ulva* gira em torno de 69-98 % (Brundu and Chindris, 2018; Li et al., 2016) e entre 25 – 64% para fosfato (Elizondo-González et al., 2018; Nardelli et al., 2018). A eficiência de remoção desses compostos pela *Ulva* também foi reportada em efluentes sanitários parcialmente misturados com água salina, mostrando sua versatilidade (Sode et al., 2013; Tsagkamilis et al., 2009).

O uso da biomassa de ulva oriunda de ambientes impactados...

O cultivo de *Ulva* em ambientes impactados pelo excessivo aporte de nitrogênio e fósforo se apresenta como uma oportunidade para produção de biomassa de valor agregado e gestão circular desses nutrientes, retornando-os para o ciclo tecnológico através da coleta da biomassa gerada. Um exemplo são os cultivos multi-tróficos

coletada

(produção simultânea de organismos de diferentes níveis tróficos) onde a *Ulva* pode atuar em dois níveis, sendo cultivadas ao redor ou dentro dos tanques dos animais (por exemplo camarão ou salmão), removendo parte do nitrogênio e fósforo dissolvido na água e posteriormente utilizada como suplemento alimentar dos mesmos (Elizondo-González et al., 2018; Qiu et al., 2017).

Ulvana

Dentre os produtos obtidos a partir da biomassa da *Ulva*, o polissacarídeo ulvana é o que apresenta maior potencial econômico. A ulvana é um heteropolissacarídeo sulfatado presente na parede celular da *Ulva*, representando em torno de 29% do peso seco da alga (Konasani et al., 2018). Ele é formado por dois dissacarídeos repetidos, ácido ulvanobiuronico tipo A e tipo B (→4)-β-D-GlcA-(1→4)-α-L-Rha 3S-(1→ e →4)-α-L-IdoA-(1→4)-α-L-Rha 3s(1→, respectivamente) (Figura 2). A ulvana é constituída principalmente de ramnose (16.8 - 45.0 %), ácido idurônico (0.7 - 9.1 %), xilose (2.1 - 12.0 %), ácido glucurônico (6.5 - 19.0 %) e sulfato (14. 5 - 23.2 %) (Kidgell et al., 2019; Tziveleka et al., 2019; Yaich et al., 2017).

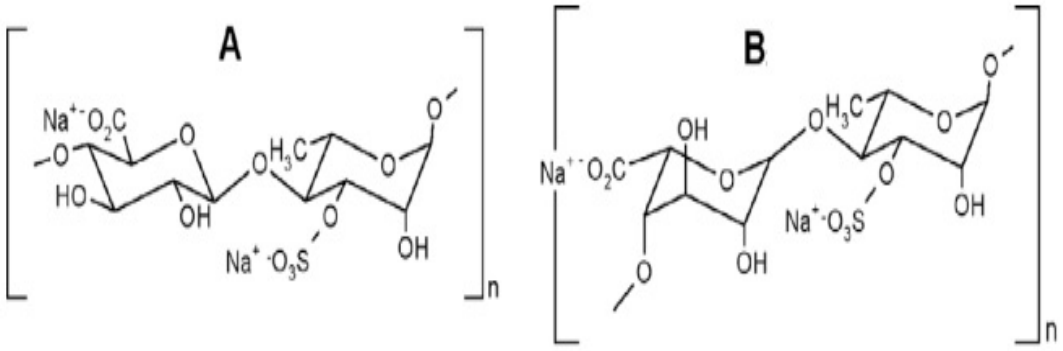

Figura 2: Estrutura da ulvana. (a) ácido ulvanobiurônico tipo A (b) ácido ulvanobiurônico tipo B (Adaptado de Robic et al. (2009)).

A ulvana se destaca pela sua versatilidade e presença dos açúcares raros ramnose e ácido idurônico (Konasani et al., 2018; Tziveleka et al., 2019). A ramnose é amplamente utilizada em produtos anti-idade (Alves et al., 2012). Já o ácido idurônico, não encontrado em outros polissacarídeos marinhos, atua na síntese química de análogos da heparina (Konasani et al., 2018).

A ulvana vem apresentando excelentes resultados principalmente no setor de fármacos, cosméticos e agricultura. Thanh et al. (2016) reportam significativa citotoxicidade da ulvana contra carcinoma hepatocelular, câncer de mama e câncer cervical. Em concentrações de 100µg/mL de ulvana a viabilidade de células cancerígenas chegou a zero nos três casos. Li et al. (2018) avaliaram a atividade antioxidante de ulvana com diferentes pesos moleculares e reportaram que todos os polissacarídeos avaliados apresentaram atividade, com os melhores resultados sendo

da ulvana com maior peso molecular e teor de ácido urônico (ácido idurônico mais ácido glucucurônico).

Na agroindústria, Araújo et al. (2014) mostraram que folhas de maçã *Malus domestica* aspergidas com ulvana apresentaram queda de 50% na severidade da doença causada pelo fungo *Colletotrichum gloeosporioides*. Estudo proposto por del Rocío Quezada-Rodríguez and Fajer-Ávila (2016) mostrou que a ulvana pode aumentar a resposta imune da tilápia (*Oreochromis niloticus*) quando alimentadas com ração enriquecida com ulvana em concentrações entre 0.1 até 1% kg^{-1} de ração. Muitos outros estudos e patentes dos usos de ulvana podem ser encontrados, mostrando o potencial desse polissacarídeo (Adrien et al., 2017; Briand et al., 2010; Yaich et al., 2017). Por essas características únicas a ulvana é comercializada por até €319 euros 100 mg (https: //www.elicityl-oligotech.com/, acessado em 11/12/2019).

Produção integrada

A maior conscientização dos consumidores em relação às matrizes produtivas tradicionais e os impactos do consumo no meio ambiente têm levado indústrias a buscar produtos e matrizes mais sustentáveis, eficientes e a custos reduzidos (Torres et al., 2019). Um dos pilares dessa nova abordagem é a economia azul, onde a promoção do crescimento econômico se dá pelo uso sustentável e eficaz dos recursos oceânicos (Prabhu et al., 2020). Nesse cenário, a produção de macroalgas tem sido estimulada com o surgimento de startups e incentivos governamentais como *Water framework* e *Biorefinery International Energy Agency* (IEA) (Balina et al., 2017; Magnusson et al., 2016).

Porém um dos principais desafios para o estabelecimento de mercados para produtos de macroalgas é a viabilidade econômica da sua produção. Surgiu então o conceito de produção integrada, que inspirada nas refinarias de petróleo, extrai todos os diferentes constituintes da biomassa de forma sequencial (Mata et al., 2015). Essa forma de produção otimiza o uso de recursos, utilizando a maior parte da biomassa para extração produtos com maior valor agregado, reduzindo a produção de rejeitos e aumentando a competitividade do setor (Seghetta et al., 2016; Torres et al., 2019). Prabhu et al. (2020) realizaram a extração integrada de seis produtos diferentes (sal, amido, lipídio, ulvana, proteína e celulose) a partir de *U. ohnoi*, aproveitando 90% da biomassa inicial. Magnusson et al. (2016) obteve sais ricos em minerais (Ca, Fe, K, Mg, Na, P) e menor relação Na:K, a partir de biomassa de *U. ohnoi* e *U. tepida*. Além disso, os autores extraíram ulvan (19% peso seco) e observaram um aumento de 20 - 50% no potencial energético (18 MJ kg^{-1}) e de 11-14% no teor de proteínas (27,4% peso seco) da biomassa residual.

A exploração integrada dos recursos da biomassa oferece uma oportunidade para fortalecer setores como agricultura, pesca, energia e indústria química, observando

princípios da economia circular, como por exemplo, na produção de biogás, onde o resíduo gerado pode ser utilizado como fertilizantes de solo (Akila et al., 2019). Além disso, essa abordagem reduz as pressões ambientais e pode ser uma fonte de renda para população costeira (Prabhu et al., 2020).

Objetivos

Objetivo geral

Verificar o potencial biotecnológico da macroalga *Ulva fasciata* através do acompanhamento do ciclo produtivo, desde a seleção do local de origem cujos indivíduos apresentem maior produtividade, avaliação da eficiência de remoção de compostos nitrogenados e fosfatados, culminando na obtenção e caracterização de polissacarídeo de interesse biotecnológico.

Objetivos específicos

- Avaliar a relação entre diferentes concentrações de fosfato e a produção de carboidrato por *Ulva fasciata* de duas regiões distintas;
- Avaliar respostas fisiológicas e eficiência de remoção de nitrogênio e fósforo pela macroalga *Ulva fasciata* cultivada em água enriquecida;
- Comparar a estrutura de ulvana extraída de *U. fasciata* coletada em ambiente natural e cultivada *in vitro* através de técnicas de caracterização de polissacarídeos.

Referências Bibliográficas

Adrien, A., Bonnet, A., Dufour, D., Baudouin, S., Maugard, T., and Bridiau, N. (2017). Pilot production of ulvans from *Ulva sp.* and their effects on hyaluronan and collagen production in cultured dermal fibroblasts. *Carbohydrate Polymers*, 157:1306–1314.

Akila, V., Manikandan, A., Sukeetha, D. S., Balakrishnan, S., Ayyasamy, P. M., and Rajakumar, S. (2019). Biogas and biofertilizer production of marine macroalgae: An effective anaerobic digestion of *Ulva* sp. *Biocatalysis and Agricultural Biotechnology*, 18:101035.

Ale, M. T., Mikkelsen, J. D., and Meyer, A. S. (2010). Differential growth response of *Ulva lactuca* to ammonium and nitrate assimilation. *Journal of Applied Phycology*, 23(3):345–351.

Alves, A., Sousa, R. A., and Reis, R. L. (2012). A practical perspective on ulvan extracted from green algae. *Journal of Applied Phycology*, 25(2):407–424.

Araújo, L., Gonçalves, A. E., and Stadnik, M. J. (2014). Ulvan effect on conidial germination and appressoria formation of *Colletotrichum gloeosporioides*. *Phytoparasitica*, 42(5):631–640.

Ariede, M. B., Candido, T. M., Jacome, A. L. M., Velasco, M. V. R., de Carvalho, J. C. M., and Baby, A. R. (2017). Cosmetic attributes of algae - a review. *Algal Research*, 25:483–487.

Balina, K., Romagnoli, F., and Blumberga, D. (2017). Seaweed biorefinery concept for sustainable use of marine resources. *Energy Procedia*, 128:504–511.

Briand, X., Cluzet, S., Dumas, B., Esquerre-Tugaye, M.-T., and Salamagne, S. (2010). Ulvans as activators of plant defense and resistance reactions against biotic or abiotic stresses. US Patent 7,820,176.

Brundu, G. and Chindris, A. (2018). Nutrients uptake and growth of *Ulva lactuca* (linnaeus, 1753) in grey mullet (*Mugil cephalus*) wastewater versus natural estuarine water. *Chemistry and Ecology*, 34(6):495–505.

Cohen, I. and Neori, A. (1991). *Ulva lactuca* biofilters for marine fishpond effluents. i. ammonia uptake kinetics and nitrogen content. *Botanica Marina*, 34(6).

Craigie, J. S. (2010). Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology*, 23(3):371–393.

Cui, J., Zhang, J., Monotilla, A. P., Huo, Y., Shi, J., Zhao, X., Kang, X., and He, P. (2019). Assessment of blooming *Ulva* macroalgae production potential in the Yellow Sea, China. *Phycologia*, 58(5):535–541.

Cury, J. C., Araujo, F. V., Coelho-Souza, S. A., Peixoto, R. S., Oliveira, J. A. L., Santos, H. F., Dávila, A. M. R., and Rosado, A. S. (2011). Microbial diversity of a Brazilian coastal region influenced by an upwelling system and anthropogenic activity. *PLoS ONE*, 6(1):e16553.

Dawes, C. (2016). Macroalgae systematics. In *Seaweed in Health and Disease Prevention*, pages 107–148. Elsevier.

del Rocío Quezada-Rodríguez, P. and Fajer-Ávila, E. J. (2016). The dietary effect of ulvan from *Ulva clathrata* on hematological-immunological parameters and growth of tilapia (*Oreochromis niloticus*). *Journal of Applied Phycology*, 29(1):423–431.

Elizondo-González, R., Quiroz-Guzmán, E., Escobedo-Fregoso, C., Magallón-Servín, P., and Peña-Rodríguez, A. (2018). Use of seaweed *Ulva lactuca* for water bioremediation and as feed additive for white shrimp *Litopenaeus vannamei*. *PeerJ*, 6:e4459.

FAO (2018). *The state of world fisheries and aquaculture 2018 : meeting the sustainable development goals*. Food and Agriculture Organization of the United Nations, Rome.

Fleurence, J. (2016). Seaweeds as food. In *Seaweed in Health and Disease Prevention*, pages 149–167. Elsevier.

Ghadiryanfar, M., Rosentrater, K. A., Keyhani, A., and Omid, M. (2016). A review of macroalgae production, with potential applications in biofuels and bioenergy. *Renewable and Sustainable Energy Reviews*, 54:473–481.

Hayden, H. S., Blomster, J., Maggs, C. A., Silva, P. C., Stanhope, M. J., and Waaland, J. R. (2003). Linnaeus was right all along:*Ulva* and *Enteromorpha* are not distinct genera. *European Journal of Phycology*, 38(3):277–294.

Holzinger, A., Herburger, K., Kaplan, F., and Lewis, L. A. (2015). Desiccation tolerance in the chlorophyte green alga *Ulva compressa*: does cell wall architecture contribute to ecological success? *Planta*, 242(2):477–492.

Hurd, C. L., Harrison, P. J., Bischof, K., and Lobban, C. S. (2014). *Seaweed Ecology and Physiology*. Cambridge University Press.

Khairy, H. M. and El-Shafay, S. M. (2013). Seasonal variations in the biochemical composition of some common seaweed species from the coast of Abu Qir Bay, Alexandria, Egypt. *Oceanologia*, 55(2):435–452.

Kidgell, J. T., Magnusson, M., de Nys, R., and Glasson, C. R. (2019). Ulvan: A systematic review of extraction, composition and function. *Algal Research*, 39:101422.

Kijjoa, A. and Sawangwong, P. (2004). Drugs and cosmetics from the sea. *Marine Drugs*, 2(2):73–82.

Konasani, V. R., Jin, C., Karlsson, N. G., and Albers, E. (2018). Ulvan lyase from *Formosa agariphila* and its applicability in depolymerisation of ulvan extracted from three different *Ulva* species. *Algal Research*, 36:106–114.

Korzen, L., Pulidindi, I. N., Israel, A., Abelson, A., and Gedanken, A. (2015). Marine integrated culture of carbohydrate rich *Ulva rigida* for enhanced production of bioethanol. *RSC Advances*, 5(73):59251–59256.

Li, H., Zhang, Y., Han, X., Shi, X., Rivkin, R. B., and Legendre, L. (2016). Growth responses of *Ulva prolifera* to inorganic and organic nutrients: Implications for macroalgal blooms in the southern Yellow Sea, China. *Scientific Reports*, 6(1).

Li, W., Jiang, N., Li, B., Wan, M., Chang, X., Liu, H., Zhang, L., Yin, S., Qi, H., and Liu, S. (2018). Antioxidant activity of purified ulvan in hyperlipidemic mice. *International Journal of Biological Macromolecules*, 113:971–975.

Magnusson, M., Carl, C., Mata, L., de Nys, R., and Paul, N. A. (2016). Seaweed salt from *Ulva*: A novel first step in a cascading biorefinery model. *Algal Research*, 16:308–316.

Martínez, B., Pato, L. S., and Rico, J. M. (2012). Nutrient uptake and growth responses of three intertidal macroalgae with perennial, opportunistic and summer-annual strategies. *Aquatic Botany*, 96(1):14–22.

Martínez-Aragón, J., Hernández, I., Pérez-Lloréns, J., Vázquez, R., and Vergara, J. (2002). Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 1. phosphate. *Journal of Applied Phycology*, 14(5):365–374.

Mata, L., Magnusson, M., Paul, N. A., and de Nys, R. (2015). The intensive land-based production of the green seaweeds *Derbesia tenuissima* and *Ulva ohnoi: biomass and bioproducts*. *Journal of Applied Phycology*, 28(1):365–375.

Matsumoto, K. and Shimada, S. (2015). Systematics of green algae resembling *Ulva conglobata*, with a description of *Ulva adhaerens* sp. nov. (Ulvales, Ulvophyceae). *European Journal of Phycology*, 50(1):100–111.

McDermid, K. J. and Stuercke, B. (2003). Nutritional composition of edible Hawaiian seaweeds. *Journal of Applied Phycology*, 15(6):513–524.

Nardelli, A. E., Chiozzini, V. G., Braga, E. S., and Chow, F. (2018). Integrated multi-trophic farming system between the green seaweed *Ulva lactuca*, mussel, and fish: a production and bioremediation solution. *Journal of Applied Phycology*, 31(2):847–856.

Pedersen, M., Borum, J., and Fotel, F. L. (2010). Phosphorus dynamics and limitation of fast- and slow-growing temperate seaweeds in Oslofjord, Norway. *Marine Ecology Progress Series*, 399:103–115.

Prabhu, M. S., Israel, A., Palatnik, R. R., Zilberman, D., and Golberg, A. (2020). Integrated biorefinery process for sustainable fractionation of *Ulva ohnoi* (Chlorophyta): process optimization and revenue analysis. *Journal of Applied Phycology*.

Qiu, X., Neori, A., Kim, J. K., Yarish, C., Shpigel, M., Guttman, L., Ezra, D. B., Odintsov, V., and Davis, D. A. (2017). Evaluation of green seaweed *Ulva sp.* as a replacement of fish meal in plant-based practical diets for pacific white shrimp, *Litopenaeus vannamei*. *Journal of Applied Phycology*, 30(2):1305–1316.

Ramesh, R., Chen, Z., Cummins, V., Day, J., D'Elia, C., Dennison, B., Forbes, D., Glaeser, B., Glaser, M., Glavovic, B., Kremer, H., Lange, M., Larsen, J., Tissier, M. L., Newton, A., Pelling, M., Purvaja, R., and Wolanski, E. (2015). Land–ocean interactions in the coastal zone: Past, present & future. *Anthropocene*, 12:85–98.

Raven, J. A. (2013). RNA function and phosphorus use by photosynthetic organisms. *Frontiers in Plant Science*, 4.

Reidenbach, L. B., Fernandez, P. A., Leal, P. P., Noisette, F., McGraw, C. M., Revill, A. T., Hurd, C. L., and Kübler, J. E. (2017). Growth, ammonium metabolism, and photosynthetic properties of *Ulva australis* (Chlorophyta) under decreasing pH and ammonium enrichment. *PLOS ONE*, 12(11):e0188389.

Ribeiro, A. L. N. L., Chiozzini, V. G., Braga, E. S., and Yokoya, N. S. (2016). Physiological responses and biofilter potential of *Hypnea aspera* (Rhodophyta, Gigartinales) cultivated in different availabilities of nitrate, ammonium, and phosphate. *Journal of Applied Phycology*, 29(2):683–694.

Rjiba-Ktita, S., Chermiti, A., Bodas, R., France, J., and López, S. (2016). Aquatic plants and macroalgae as potential feed ingredients in ruminant diets. *Journal of Applied Phycology*, 29(1):449–458.

Robic, A., Rondeau-Mouro, C., Sassi, J.-F., Lerat, Y., and Lahaye, M. (2009). Structure and interactions of ulvan in the cell wall of the marine green algae *Ulva rotundata* (Ulvales, Chlorophyceae). *Carbohydrate Polymers*, 77(2):206–216.

Roleda, M. Y. and Hurd, C. L. (2019). Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. *Phycologia*, 58(5):552–562.

Runcie, J. W., Ritchie, R. J., and Larkum, A. W. (2004). Uptake kinetics and assimilation of phosphorus by *Catenella nipae* and *Ulva lactuca* can be used to indicate ambient phosphate availability. *Journal of Applied Phycology*, 16(3):181–194.

Seghetta, M., Hou, X., Bastianoni, S., Bjerre, A.-B., and Thomsen, M. (2016). Life cycle assessment of macroalgal biorefinery for the production of ethanol, proteins and fertilizers – a step towards a regenerative bioeconomy. *Journal of Cleaner Production*, 137:1158–1169.

Shao, P., Qin, M., Han, L., and Sun, P. (2014). Rheology and characteristics of sulfated polysaccharides from chlorophytan seaweeds *Ulva fasciata*. *Carbohydrate Polymers*, 113:365–372.

Shuuluka, D., Bolton, J. J., and Anderson, R. J. (2012). Protein content, amino acid composition and nitrogen-to-protein conversion factors of *Ulva rigida* and *Ulva capensis* from natural populations and *Ulva lactuca* from an aquaculture system, in South Africa. *Journal of Applied Phycology*, 25(2):677–685.

Smit, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology*, 16(4):245–262.

Sode, S., Bruhn, A., Balsby, T. J., Larsen, M. M., Gotfredsen, A., and Rasmussen, M. B. (2013). Bioremediation of reject water from anaerobically digested waste water sludge with macroalgae (*Ulva lactuca* , Chlorophyta). *Bioresource Technology*, 146:426–435.

Stiger-Pouvreau, V. and Zubia, M. (2020). Macroalgal diversity for sustainable biotechnological development in french tropical overseas territories. *Botanica Marina*, 63(1):17–41.

Teichberg, M., Fox, S. E., Olsen, Y. S., Valiela, I., Martinetto, P., Iribarne, O., Muto, E. Y., Petti, M. A., Corbisier, T. N., Soto-Jiménez, M., et al. (2009). Eutrophication and macroalgal blooms in temperate and tropical coastal waters: nutrient enrichment experiments with *Ulva spp.* *Global Change Biology*, pages 2624–2637.

Thanh, T. T. T., Quach, T. M. T., Nguyen, T. N., Luong, D. V., Bui, M. L., and Tran, T. T. V. (2016). Structure and cytotoxic activity of ulvan extracted from green seaweed *Ulva lactuca*. *International Journal of Biological Macromolecules*, 93:695–702.

Torres, M. D., Kraan, S., and Domínguez, H. (2019). Seaweed biorefinery. *Reviews in Environmental Science and BioTechnology*, 18(2):335–388.

Tran, T. T. V., Truong, H. B., Tran, N. H. V., Quach, T. M. T., Nguyen, T. N., Bui, M. L., Yuguchi, Y., and Thanh, T. T. T. (2017). Structure, conformation in aqueous solution and antimicrobial activity of ulvan extracted from green seaweed *Ulva reticulata*. *Natural Product Research*, 32(19):2291–2296.

Tsagkamilis, P., Danielidis, D., Dring, M. J., and Katsaros, C. (2009). Removal of phosphate by the green seaweed *Ulva lactuca* in a small-scale sewage treatment plant (Ios Island, Aegean Sea, Greece). *Journal of Applied Phycology*, 22(3):331–339.

Tziveleka, L.-A., Ioannou, E., and Roussis, V. (2019). Ulvan, a bioactive marine sulphated polysaccharide as a key constituent of hybrid biomaterials: A review. *Carbohydrate Polymers*, 218:355–370.

Wang, H.-M. D., Li, X.-C., Lee, D.-J., and Chang, J.-S. (2017). Potential biomedical applications of marine algae. *Bioresource Technology*, 244:1407–1415.

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., Smith, A. G., Camire, M. E., and Brawley, S. H. (2016). Algae as nutritional and functional food sources: revisiting our understanding. *Journal of Applied Phycology*, 29(2):949–982.

Wichard, T., Charrier, B., Mineur, F., Bothwell, J. H., Clerck, O. D., and Coates, J. C. (2015). The green seaweed *Ulva*: a model system to study morphogenesis. *Frontiers in Plant Science*, 6.

Wu, H., Huo, Y., Han, F., Liu, Y., and He, P. (2015). Bioremediation using *Gracilaria chouae* co-cultured with *Sparus macrocephalus* to manage the nitrogen and phosphorous balance in an IMTA system in Xiangshan Bay, China. *Marine Pollution Bulletin*, 91(1):272–279.

Xiao, X., Agusti, S., Lin, F., Li, K., Pan, Y., Yu, Y., Zheng, Y., Wu, J., and Duarte, C. M. (2017). Nutrient removal from chinese coastal waters by large-scale seaweed aquaculture. *Scientific Reports*, 7(1).

Yaich, H., Amira, A. B., Abbes, F., Bouaziz, M., Besbes, S., Richel, A., Blecker, C., Attia, H., and Garna, H. (2017). Effect of extraction procedures on structural, thermal and antioxidant properties of ulvan from *Ulva lactuca* collected in monastir coast. *International Journal of Biological Macromolecules*, 105:1430–1439.

Zhou, Y., Tan, L., Pang, Q., Li, F., and Wang, J. (2015). Influence of nutrients pollution on the growth and organic matter output of *Ulva prolifera* in the southern Yellow Sea, China. *Marine Pollution Bulletin*, 95(1):107–114.

Material e métodos gerais

Desenho experimental

Este trabalho é composto de três etapas experimentais interconectadas, cujos desenhos experimentais são apresentados na figura abaixo (Figura 3). Em uma primeira fase, indivíduos de *Ulva fasciata* de duas regiões distintas (Arraial do Cabo e Niterói) foram selecionados para avaliar suas respostas fisiológicas e produção de carboidrato quando cultivados em diferentes concentrações de fosfato. Sequencialmente, indivíduos da região que apresentou a maior produção de carboidratos foram cultivados em água enriquecida com nitrogênio e fósforo em concentrações similares as reportadas para Baía da Guanabara. Nesse desenho experimental foram avaliados três tratamentos distintos: *U. fasciata* cultivada em água enriquecida; *U. fasciata* cultivada em água sem adição de nutrientes (controle fisiológico) e água enriquecida sem a presença de alga (controle da água). Por fim, a biomassa gerada após a experiência de cultivo foi utilizada para obtenção de ulvana que foi caracterizada e comparada com ulvana apresentada na literatura e extraída da biomassa de *U. fasciata* coletada diretamente do ambiente natural.

Análises

As análises que compõem essa tese foram realizadas com base nos protocolos da Unidade Multiusuário de Análises Ambientais (UMAA/CCS), Laboratório de Análise Fitoquímica (IPPN) e Laboratório Multiusuário de Análises por RMN (LAMAR/CCS). Todas as análises apresentadas foram realizadas em triplicata analítica.

Todos os reagentes utilizados possuíam de grau analítico de pureza. Os reagentes utilizados para análise de água, clorofila-*a*, carboidrato no tecido, fósforo, carbono e nitrogênio tecidual, extração de ulvana e infravermelho com transformada de Fourier (FTIR) foram obtidos na Merck®. O óxido de deutério utilizado na Ressonância Magnética Nuclear (RMN) foi obtido na Sigma-Aldrich.

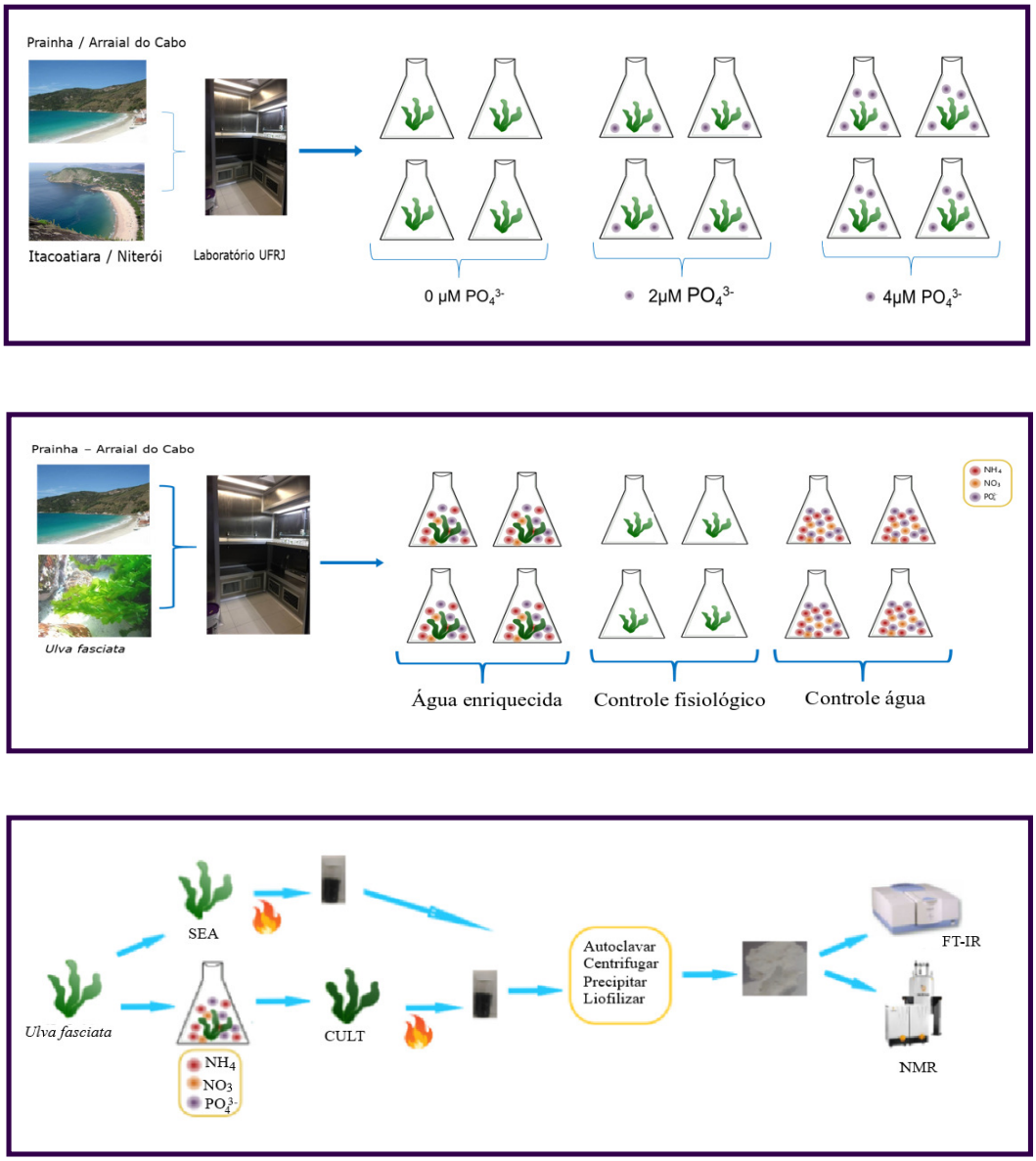

Figura 3: Desenho experimental dos capítulos.

Análise de água

Todas as amostras de água foram imediatamente filtradas em filtro GF/F 0.45 μM no momento da coleta. As amostras foram congeladas a – 20°C quando não analisadas imediatamente. Antes de cada análise de concentrações de fosfato (PO_4^-), amônio (NH_4^+) e nitrato (NO_3^-) foram realizadas curvas padrão com as seguintes concentrações: Branco; 100 ppm; 200 ppm; 500 ppm; 1000 ppm. Todas as análises de água foram realizadas na Unidade Multiusuário de Análises Ambientais/UFRJ.

Fosfato dissolvido

No capítulo 1 a concentração de fosfato no meio foi analisada de acordo método de injeção de fluxo seguindo a ISSO 15681 em equipamento autoanalyser (FIAstar

TM5000 Analyser da FOSS).

No capítulo 2 utilizou-se metodologia adaptada de Murphy and Riley (1962).

Inicialmente foram preparadas três soluções:

Solução 1 - 2, 50 g de ácido ascórbico foram avolumados com água destilada em balão de 50 mL;

Solução 2 – 5 g de Molibdato de amônio + 100 mL de água destilada + 0, 056 g de tartarato + 39 mL de ácido sulfúrico foi avolumado em balão de 250 mL com água destilada;

Solução 3 – 1, 0 mL da solução **1** + 4, 0 mL da solução **2**. 0, 2 mL da solução **3** foi adicionado em 5, 0 mL de amostra, homogeinizado e deixado para reagir por 5 minutos. As amostras foram lidas em espectrofotômetro (Hatch DR 5000) em 885 nm.

Amônio dissolvido

Metodologia adaptada de Koroleff (1970). Foram utilizadas quatro soluções:

Solução 1 – 4 g de hidróxido de sódio foram solubilizadas em 250 mL de água destilada;

Solução 2 – 24 g de citrato foram solubilizadas em 20 mL de água destilada e 2,0 mL de solução **1** foram adicionados. A mistura foi agitada a 80°C até diluição total e avolumada em balão de 50 ml com água destilada;

Solução 3 – 0,50 g de trione foi avolumado em um balão de 50 mL com a solução **1**;

Solução 4 – 0,04 g de nitroprussitato foi diluído em 1,0 mL de água destilada. Separadamente, 3,8 g de fenol foram diluídas em álcool etílico. As duas soluções foram misturadas em balão de 100 mL.

Para cada 4,0 mL de amostra foram adicionados 0,2 mL da solução **4**. Em seguida 0,2 mL da solução **2** e da solução **3** foram adicionados. Entre cada adição de solução a amostra foi homogeneizada. Após 3 horas de reação as amostras foram lidas em espectrofotômetro (Hatch DR 5000) em comprimento de onda de 643 nm.

Nitrato

Metodologia adaptada de Grasshoff and Johannsen (1972). Foram preparadas cinco soluções:

Solução 1 – NH_4Cl: 25 g em 100 mL de água destilada;

Solução 2 – NH_4Cl diluído: 25 mL em 1000 mL (1:40) de água destilada;

Solução 3 – 0,20 g de N-(1-Naphthyl)ethylenediamine foi adicionado em balão volumétrico de 200 mL e avolumado com água destilada;

Solução 4 - Em um balão volumétrico de 1000 mL foi adicionado 25 mL da solução **1** e avolumado com água destilada;

Solução 5 - Em proveta foram adicionados 20 mL de água destilada + 1,0 mL do padrão + 0,4 mL da Solução **1**.

Para essa análise foi montada uma coluna de cádmio. A ativação da coluna foi feita com passagem da solução **5**. Entre cada leitura a coluna foi lavada com 25 mL da solução **2**.

Em cada amostra original (20 mL) foram adicionados 0,4 mL da solução **1**. Essa mistura foi utilizada nas etapas seguintes. Em seguida, uma alíquota de 4,0 mL foi retirada e passada na coluna para retirada de qualquer resíduo da solução anterior. Uma nova alíquota de 2,5 mL foi retirada e 100 μL da solução **3** foi adicionado e a mistura reagiu por 1 minuto. Imediatamente após esse tempo 100 μL da solução **4** foi adicionada e essa mistura reagiu por 20 minutos. A amostra final foi lida em espectrofotômetro (Hatch DR 5000) em comprimento de onda de 543 nm.

Carboidrato no tecido

Utilizou-se o método fenol-ácido sulfúrico proposto por DuBois et al. (1956). Após coleta do tecido as amostras foram lavadas com água destilada e secas em estufa a 50°C até peso constante, identificadas e armazenadas em desumidificador até a análise.

Para a análise, 2,0 mL de ácido sulfúrico 90% foram adicionados em tubo de vidro contendo 0,1 mg de tecido seco macerado e mantido por 20 horas a 4°C. Em seguida foi adicionado 6 mL de água destilada. A uma alíquota de 2,0 ml dessa solução foram acrescentados 50 μL de solução fenólica 80% e 5,0 mL de ácido sulfúrico 90%. Após 15 minutos de reação a amostra foi lida em espectrofotômetro (Hatch DR 5000) em comprimento de onda de 490 nm.

Performance fotossintética

Para análise do rendimento quântico máximo foram medidos os níveis de fluorescência inicial do talo adaptado no escuro (Fv) e fluorescência máxima após pulso de luz saturante (Fm). Para tal, indivíduos de *Ulva* foram aclimatados ao escuro por 15 minutos. Após aclimatação, parte do talo da alga foi posicionado no suporte magnético do fluorímetro Diving PAM (modelo Diving-F) e um pulso de luz de intensidade 8 foi disparado por 0,8 segundos.

Clorofila-*a* no tecido

A amostra de tecido fresco coletadas ao longo dos experimentos foram armazenada congelada (-20°C) e em recipiente escuro ou completamente vedado para que não houvesse degradação da amostra. Inicialmente amostra de tecido fresco pesando 20 mg foram maceradas com nitrogênio líquido. O material macerado é posto em tubo de vidro e 6,0 mL de acetona 90 % foram adicionados. Os tubos são mantidos no escuro a 4°C por 18 horas. Por fim, o material é centrifugado a 300 rpm por cinco minutos. Alíquotas de 1,0 mL do sobrenadante foram coletadas e lidas em espectrofotômetro com comprimento de onda de 665 e 750 nm conforme proposto por Lorenzen (1967). Todo processo de análise foi realizado no escuro.

Fósforo tecidual

Método utilizado foi adaptado de Murphy and Riley (1962). Amostras de tecido secas a 50°C pesando 2,5 mg foram calcinadas em estufa a 550°C por 1 hora. O material remanescente foi pesado e adicionado em tubo contendo 5,0 mL de água destilada e posto em biodigestor por 2 horas a 105°C. Em seguida, 20 ml de água destilada e 1,5 mL de $K_2S_2O_8$ foram adicionados. Para leitura da amostra, uma alíquota de 5,0 mL de sobrenadante foi retirada e o método de análise de fosfato descrito no item foi utilizado.

Carbono e nitrogênio tecidual

As análises de carbono e nitrogênio tecidual foram realizadas pela Unidade Multiusuário de Análises Ambientais/UFRJ em CHNS Elemental Analyser (Flash 2000 – Organic Elemental Analyser with Delta V Advantage – Thermo Scientific) calibrado com padrão de acetanilida. Amostras de tecido seco a 50°C e pesando aproximadamente 3,0 mg foram utilizadas.

Extração de ulvana

Para extração de ulvana utilizou-se metodologia proposta por Reis et al. (2018).

A biomassa imediatamente após a coleta e oriunda do experimento de cultivo é seca em estufa a 50°C, macerada e pesada sendo acrescida água destilada na proporção: 100 mL de água destilada para cada 10 g de alga. Em seguida o material úmido é autoclavado por 40 min a 120°C. A amostra fria é então centrifugada a 10000g por 10 minutos a 4°C. O sobrenadante é coletado e etanol ultrapuro adicionado (3 vezes o volume do sobrenadante) para precipitar o polissacarídeo. Esse material é então mantido em freezer a - 20°C por 48h. O polissacarídeo é

recuperado por centrifugação, 3500 g por 5 min. A ulvana obtida é liofilizada e armazenada em dissecador.

Espectrometria no Infravermelho com transformada de Fourier (FT-IR)

A análise de FT-IR foi realizada no Laboratório de Análise Fitoquímica no Instituto de Pesquisas de Produtos Naturais/UFRJ. Os espectros infravermelhos com transformada de Fourier (FT-IR) foram registrados em espectrofotômetro IR Prestige_21, Shimadzu em temperatura ambiente. Os espectros FT-IR foram obtidos no modo de transmissão em 400-4000 cm^{-1}. Para essa análise foram utilizadas 2,5 mg de ulvana.

Ressonância Magnética Nuclear (RMN)

A análise de RMN foi realizada no Laboratório Multiusuário de Análises por RMN (LAMAR) pertencente ao Instituto de Pesquisas de Produtos Naturais/UFRJ em espectrômetro Varian VNMRSYS 500 MHz Varian Inc., Palo Alto, CA, USA) at 37°C. As frequências de operação de próton e carbono foram 499,77 e 125,68 MHz, respectivamente. Os espectros de ^{1}H NMR foram registrados com uma amplitude de pulso observada de 90 graus (pw = 90 μs), um tempo de aquisição de 2,04 s e um atraso de relaxamento de 1 s. Um total de 32 scans foram realizados para cada amostra. Para ^{13}C NMR, um pulso de 90 graus foi usado (pw = 90 μs), tempo de aquisição de 1,04 s, atraso de relaxamento de 2 s e um total de 114.624 scans foram coletados. Foram utilizadas 20 mg de ulvana solubilizadas em 7,0 ml de óxido de deutério (D_2O) 99,99%.

Referências Bibliográficas

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3):350–356.

Grasshoff, K. and Johannsen, H. (1972). A new sensitive and direct method for the automatic determination of ammonia in sea water. *ICES Journal of Marine Science*, 34(3):516–521.

Koroleff, F. (1970). Direct determination of ammonia in natural waters as indophenol blue. *Information on techniques and methods for seawater analysis*, pages 19–22.

Lorenzen, C. J. (1967). Determination of chlorophyll and pheo-pigments: Spectrophotometric equations. *Limnology and Oceanography*, 12(2):343–346.

Murphy, J. and Riley, J. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27:31–36.

Reis, R. P., de Carvalho Junior, A. A., Facchinei, A. P., dos Santos Calheiros, A. C., and Castelar, B. (2018). Direct effects of ulvan and a flour produced from the green alga ulva fasciata delile on the fungus stemphylium solani weber. *Algal Research*, 30:23–27.

Capítulo 1

The effects of phosphate on physiological responses and carbohydrate production in *Ulva fasciata* (Chlorophyta) from upwelling and non-upwelling site

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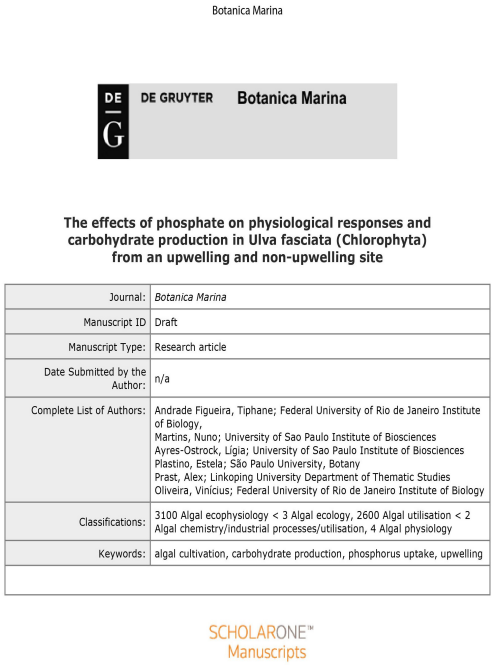

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Figura 1.1: Submission proof to Botanica Marina

Abstract

Phosphorus is an essential element in macroalgae metabolism and carbohydrate anabolism. To identify the influence of phosphorus on different physiological parameters in *Ulva fasciata*, individuals from distinct sites (an upwelling and a non-upwelling site) were cultivated under different phosphate concentrations (0, 2 and 4 μM PO_4^{3-}). Fifteen days into the experiment, the specific growth rates were similar (around 12 % d^{-1}) and carbohydrate contents increased in individuals from both Upwelling and Non-upwelling sites. In individuals from the Upwelling site, the carbohydrate content was high in all treatments (71 % DM), while Non-upwelling individuals cultivated under the highest phosphate concentration presented the lowest carbohydrate content (46 % DM). The most significant phosphorus uptake rates ($p < 0.05$) were observed in individuals from the Non-upwelling site cultivated under the highest phosphate concentration (4 μM PO_4^{3-}), suggesting a stress reaction to elevated nutrient concentrations. Individuals cultivated in the absence of phosphate (control) presented the lowest tissue phosphorus contents and a progressive decline in the maximum quantum yield (Fv/Fm) over the experimental period ($p < 0.05$). Altogether, our results indicate that phosphate influenced the carbohydrate content in *U. fasciata* and that individuals from sites that experiment broad environmental variations can present higher productivity.

Keywords: Carbohydrate production. Macroalgae cultivation. Phosphorus uptake. Upwelling.

1.1 Introduction

The macroalgae market moves approximately 5 billion dollars per year, with the largest share attributed to carbohydrate trade (FAO, 2018; Korzen et al., 2015b; Magnusson et al., 2016). Algae carbohydrates have many different industrial applications, such as cosmetics, pharmaceuticals and foods (Fleurence, 2016; Wang et al., 2015). Thus, the knowledge of physiological factors regarding their production remains as the major hindrance to industrial production (Hafting et al., 2015; Torres et al., 2019). One of the main challenges to the development of macroalgae bioproducts is to maximize the content of carbohydrates, using a low input of nutrients while maintaining high metabolic performance (Martínez et al., 2012).

Phosphorus is a key macronutrient in macroalgae physiology, from the synthesis of nucleic acids to energy transfer, including the composition of the chemical acceptor NADPH, responsible for CO_2 reduction in the Calvin cycle in photosynthesis, where the construction of carbohydrates occurs (Lapointe, 1986; Raven, 2013). However, excessive amounts of phosphorus are among the primary causes of the eutrophication

process (Carpenter and Bennett, 2011), a phenomenon that has become a worldwide issue with increasing severity in developing countries (Cui et al., 2019; Wu et al., 2015). Due to their physiological features, which favor rapid and efficient uptake of this nutrient, macroalgae cultivation has been considered a viable solution to improve water quality (de Oliveira et al., 2016; Lawton et al., 2013; Xiao et al., 2017).

Macroalgae cultivation in eutrophic environments not only benefits coastal ecosystems (Wu et al., 2015; Yu et al., 2014) but represents a valuable source of biomass for carbohydrate production. However, for year-round production, it is crucial that the target species present high productivity and are able to acclimate to dynamic environmental conditions (Lawton et al., 2013; Sfriso and Sfriso, 2017). In this sense, *Ulva fasciata* Delile (Ulvales, Chlorophyta) is a potential marine macroalgae for cultivation in eutrophic waters occurring in the upper mesolittoral zone, with economic potential, year-round presence and worldwide distribution (Chen and Zou, 2015; Wang et al., 2012).

Ulva species presents high internal concentration of carbohydrates, reaching values up to 45% of the dry weight (Khairy and El-Shafay, 2013; Korzen et al., 2015a; Yoza and Masutani, 2013). The use of carbohydrates from *Ulva* species has been investigated in several scientific fields, such as medicine (Ryu et al., 2013; Thanh et al., 2016), food (Khairy and El-Shafay, 2013; McDermid and Stuercke, 2003) and biofuel (Akila et al., 2019; Margareta et al., 2020). The total carbohydrates produced by macroalgae is highly influenced by nutrient availability in the medium, and understanding these interactions is essential to increase the productivity and revenue (Torres et al., 2019). Although phosphorus is an essential element, very few studies have examined its role in the macroalgae metabolism and carbohydrates production (Chopin and Wagey, 1999; Sousa-Pinto et al., 1996). Considering this background, there are no comparative study on the influence of this element in the same species from different populations, considering the variability intrinsic to each species of distinct region.

In this work we examined the relationship between phosphate concentrations and carbohydrate production by *Ulva fasciata* from two distinct regions. For this we assessed the growth rates, photosynthetic efficiency, phosphate uptake and tissue phosphorus content. The general hypothesis was that species from different regions would have different responses to different phosphate concentrations. Moreover, we expect to provide useful information for cultivation of *Ulva* in phosphate enriched water.

1.2 Material and Methods

1.2.1 Biological material

Ulva fasciata was collected in the mesolittoral zone at two distinct sites (Figure 1.2): a Non-upwelling site - Itacoatiara Beach, Niterói / RJ Brazil (22°58' 29"S / 43°02'22"W) - which has suffered low anthropogenic impact, with a water nutrient concentration of approximately 7.5 ± 1.21 μM total inorganic nitrogen and 0.76 ± 0.34 μM of phosphate, as indicated by a three-year survey (Nascimento et al., 2014), and an annual sea surface temperature averaging 24 °C (Catanzaro et al., 2004), and an Upwelling site - Prainha Beach, Arraial do Cabo / RJ Brazil (22°57'40"S / 42°01'13"W) - with high nutrient concentrations due to the seasonal upwelling phenomenon of cold nutrient-rich deep water (Coelho-Souza et al., 2017).

When the upwelling phenomenon is at its peak (January-March), sea surface temperatures reach values as low as 15 °C. The annual sea surface average temperature in this site is 20 °C (de Guimaraens et al., 2005). During upwelling events, the water nutrient concentration can reach values of approximately 15 μM of total inorganic nitrogen and 1 μM of phosphate (Cury et al., 2011). The collection in the Upwelling (UWS) and Non-upwelling sites (NUWS) occurred on February 2016, during the austral summer when the upwelling is strongest (de Guimaraens et al., 2005).

A total of 20 specimens were randomly collected from each site. Immediately after the collection, macroscopic epibionts were removed and the thalli were transported to the laboratory inside a thermal box within 6 hours. The species-level identification as *U.fasciata* was determined by molecular studies (bar-coding using tu$f$A markers)(Martins, 2016). Voucher specimens from Upwelling (UWS) and Non-upwelling (NUWS) sites were deposited at the Institute of Bioscience Herbarium at the University of São Paulo, Brazil (SPF-57878 and SPF-57877, respectively). Permission was obtained from local authorities where appropriate to collect *Ulva* in both sites (SISBIO licence number 17321-2).

1.2.2 Acclimation conditions

To ensure that the observed differences were inherent to the individuals, specimens of both sites were kept in similar culture conditions for six months. For that, unialgal cultures from each site were established, and the individuals maintained in sterile seawater (32 salinity, 0.5 μM total inorganic nitrogen and 0.09 μM of phosphate) enriched with modified von Stosch medium (Ursi and Plastino, 2001) in a controlled temperature room at 24.0 ± 1.0°C, photoperiod of 14:10 (L:D), with photosynthetically active radiation (PAR) kept at 70 μmol photons m^{-2} s^{-1}, provided by Osram 40W daylight fluorescent tubes. The cultivation medium was

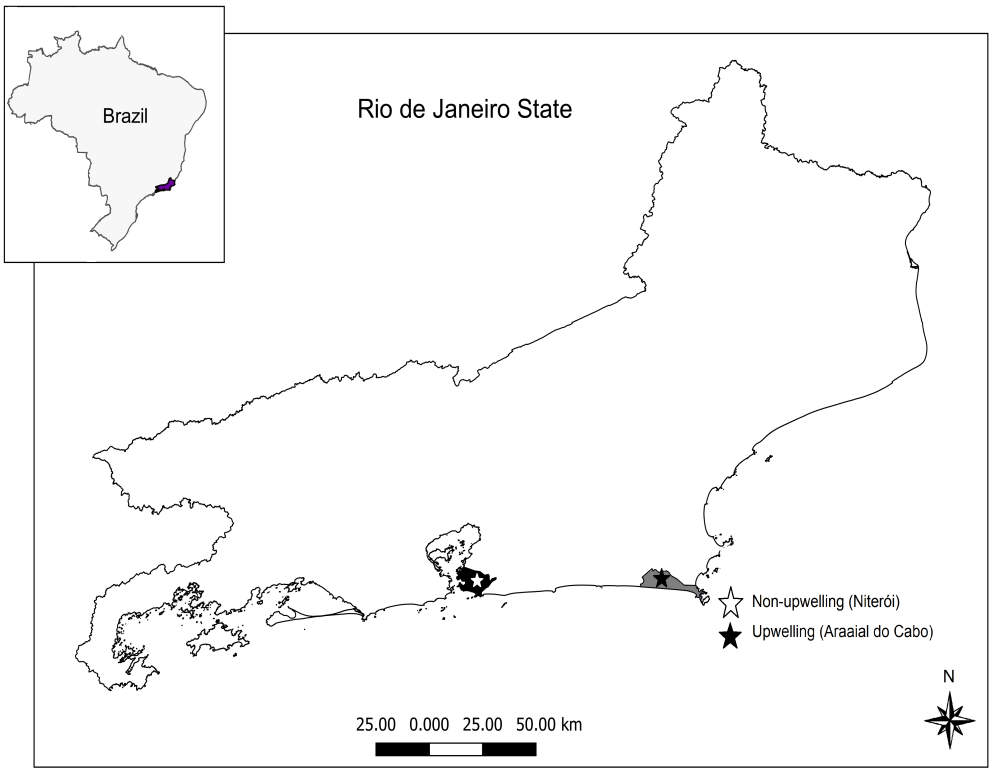

Figura 1.2: Map of sampling sites of *Ulva fasciata*: Upwelling site (Arraial do Cabo) and Non-upwelling site (Niterói).

renewed weekly.

1.2.3 Experimental design

After six months in acclimation conditions, four individuals of *U.fasciata* from each sampled site (UWS and NUWS) were used in the experiment. Thallus fragments from each individual (100 ± 9 mg), were transferred and individually placed in 1 L erlenmeyer flasks (100 mg/L) filled with 1 liter of seawater enriched with modified von Stosch culture medium prepared without phosphorus, with 35 μM of ammonium (single Nitrogen source), vitamins, salts of iron, manganese and ethylenediaminetetraacetic acid (EDTA) as the absence of these compounds could inhibit algal growth. All chemicals used were of analytical reagent grade and purchased from Merck®.

Three different phosphate treatments (PO_4^{3-}) with three replicates each (n = 3) were independently evaluated: 0 (control), 2 and 4 μM of PO_4^{3-}. These concentrations were chosen because they are commonly found in coastal aquaculture systems (Abreu et al., 2011; da Silva Copertino et al., 2008) and in urban estuaries (Ribeiro and Kjerfve, 2002; Soares-Gomes et al., 2016). Light, photoperiod and temperature conditions were the same as those described for the acclimation period.

The experiment lasted fifteen days, and the cultivation medium was renewed

every three days to prevent nutrient depletion. In every medium renewal, a 20 mL aliquot was collected for phosphate assessment and the mass of all individuals was evaluated for growth rate calculations. Photosynthetic performance (Fv/Fm) measurements were performed at the beginning, 7 and 15 days into the experiment. Tissue samples obtained prior to and at the end of the cultivation experiment were used for carbohydrate and phosphorus contents analyses. All samples collected (tissue and water) were kept frozen (-20°C) until analyses.

1.2.4 Specific growth rate (SGR)

All individuals were blotted on paper towel to remove water excess and the fresh mass was assessed every three days, during the medium renewal. Mass assessed prior to and at the end of the experiment were used for SGR calculations, according to the following formula (Yong et al., 2013):

$$SGR = \left[\left(\frac{M_t}{M_0} \right)^{1/t} - 1 \right] * 100 \tag{1.1}$$

Where M_o = wet mass at the beginning of experiment, M_t = wet mass at the time t and t = time interval (days). The SGR is reported as a percentage per day (% d^{-1}).

1.2.5 Photosynthetic performance

The maximum quantum yield (Fv/Fm) measurements were performed at the beginning, 7 and 15 days into the experiment, with a Walz Diving PAM underwater fluorometer, using an optical fiber model Diving-F attached to a magnetic sample holder. The fluorescence levels measured were Fv (initial fluorescence in dark-adapted thalli) and Fm (maximum fluorescence following the application of a single saturating light pulse). Photosynthetic measurements were performed in thalli acclimated for 15 minutes in the dark inside falcon tubes wrapped in aluminum foil (Schreiber 2004). The light exposure time was 0.8s.

1.2.6 Phosphate uptake efficiency

Water aliquots (20 mL) from each treatment were collected every three days, before and after culture medium renewal; immediately filtered through a GF/F glass fiber filter; and stored frozen (-20°C) for subsequent analyses. Phosphate concentrations were analysed by the flux injection method (FIAstar TM5000 Analyser da FOSS) following the ISO 15681. Phosphate uptake by *U.fasciata* was calculated using the following formula proposed by (Pedersen and Borum, 1997):

$$PU = \frac{(C_0 * L_0) - (C_t * L_t)}{(t * B)} \tag{1.2}$$

Where C_o is the initial concentration of phosphorus in the water, L_o is the initial volume of water (liters), L_t is the final concentration of phosphorus in the water, Lt is the final volume of water, M is the algal dry mass and t is the cultivation period.Phosphate uptake is given in mg P g DM^{-1} day^{-1}

1.2.7 Tissue analyses

Tissue samples obtained at the beginning and at the end of the experiment were used for phosphorus content analyses. Tissue P was determined spectrophotometrically using a method adapted from Menzel and Corwin (1965) and Murphy and Riley (1962). Samples with 2 – 3 mg of dried mass were combusted at 550°C for 1 hour in a muffle furnace. The ashes were then digested overnight in 40 ml of hydrochloric acid. Then, 0.2 ml of a mixture of ascorbic acid and molybdate at a proportion of 1:4 was added to 5 ml of the supernatant and measured with a spectrophotometer (Hatch DR 5000) at 885 nm absorbance. Analysis was performed in analytical triplicate for all individuals.

1.2.8 Total carbohydrate content

Individual tissue samples collected at the beginning and at the end of the experimental period were rinsed with distilled water and oven dried at 60°C for 24 hours or until they reached constant mass. The total carbohydrate contents were determined by the colorimetric method with phenol-sulphuric acid (DuBois et al., 1956) and measured with a spectrophotometer (Hatch DR 5000) at 490 nm absorbance. Soluble carbohydrates were compared with a glucose standard solution (Merck).

1.2.9 Statistical analyses

The normality and homogeneity of the variance assumptions were tested by the Kolmogorov-Smirnov and Levene tests, respectively. Growth rate, phosphate uptake, tissue phosphorus and total carbohydrate contents were analysed by the two-way ANOVA (independent variables: site and phosphate concentration). A three-way ANOVA was performed to assess photosynthetic performance (independent variables: time, site and phosphate concentration). In all cases, a posteriori Student-Newman-Keuls test (SNK) was used to establish statistical differences. Statistical analyses were performed using Statistica 10 software, considering $p < 0.05$ as significance level.

1.3 Results

1.3.1 Specific growth rate (SGR)

The average specific growth rates of *Ulva fasciata* from UWS were 13.40 ± 1.77 (0 μM PO_4^{3-}), 15.64 ± 0.37 (2 μM PO_4^{3-}) and 15.39 ± 2.53% d^{-1} (4 μM PO_4^{3-}). For macroalgae from NUWS, SGR were 15.47 ± 2.08 (0 μM PO_4^{3-}), 15.11 ± 2.12 (2 μM PO_4^{3-}) and 13.87 ± 0.58% d^{-1} (4 μM PO_4^{3-}). No differences in SGR were observed among the macroalgae under different phosphate concentrations ($p = 0.62$), nor between sites ($p = 0.23$; Figure A.2).

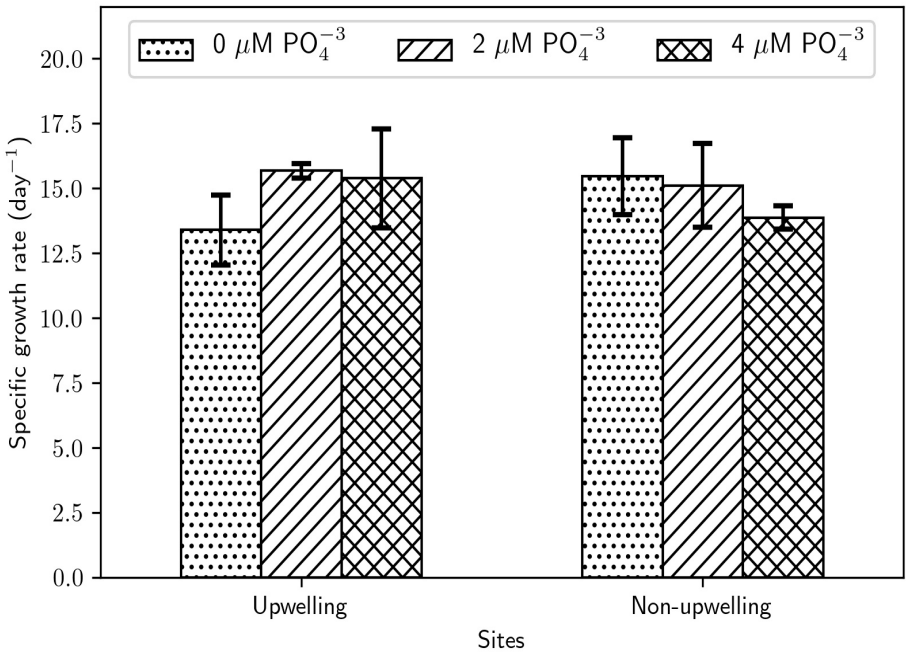

Figura 1.3: Growth rates calculated from the average fresh mass (mg) of *Ulva fasciata* from two distinct sites (Upwelling and Non-upwelling) in three different phosphate concentrations (0, 2, and 4 μM PO_4^{3-}) fifteen days into the experiment. Data presented as mean and standard deviation (n = 3). Different letters indicate significant differences.

1.3.2 Photosynthetic performance

Analyses of photosynthetic performance showed no interaction between sites and phosphate concentrations ($p = 0.99$), between time and sites ($p = 0.27$) or among time, phosphate concentration and sites ($p = 0.39$). However, the interaction between phosphate concentration and time influenced the photosynthetic responses ($p < 0.05$). Therefore, data from the two sites were merged (Figure 1.3). Treatments with phosphate addition kept their algal photosynthetic system healthy throughout

the experimental period (Fv/Fm = 0.788 ± 0.01, Figure 1.3). However, the absence of phosphorus negatively influenced the maximum quantum yield ($p < 0.05$). At the beginning of the experiment (t = 0), the average maximum quantum yield was 0.771 ± 0.01. After 7 days, the photosynthetic performance decreased (Fv/Fm = 0.738 ± 0.36) and continued to decay until the end of the experiment (t = 15, Fv/Fm = 0.693 ± 0.04, Figure 1.3).

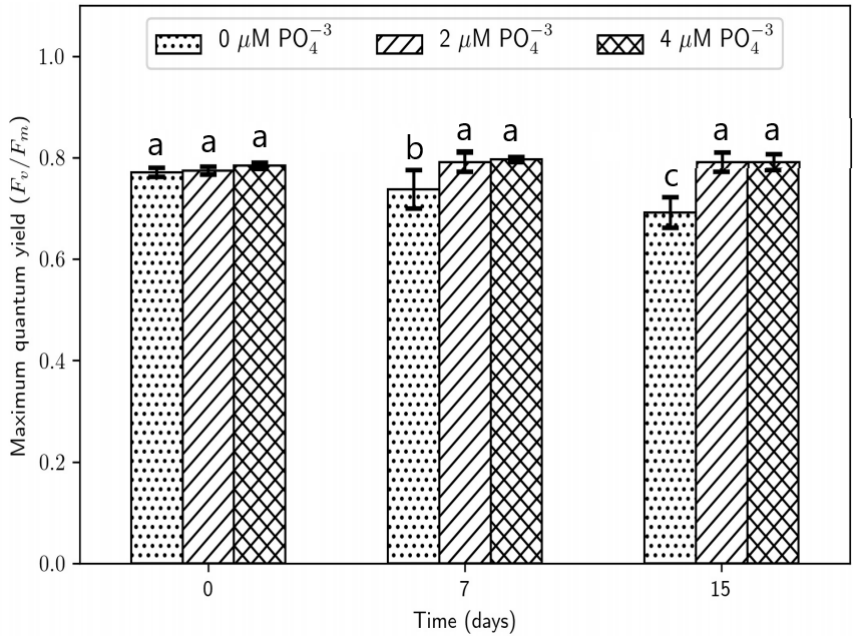

Figura 1.4: Maximum quantum yield (Fv/Fm) of *Ulva fasciata* in three different phosphate concentrations (0, 2, and 4 μM P_4^{3-}) over the experimental period (fifteen days). Data presented as mean and standard deviation (n = 3). Treatments with different letters indicate significant differences.

1.3.3 Phosphate uptake efficiency

The average phosphate uptake was 1.80 ± 0.32 mg P gDM^{-1} day^{-1} in UWS individuals cultivated in 2 and 4 μM PO_4^{3-} treatments and NUWS individuals in the 2 μM PO_4^{3-} treatment ($p > 0.05$). Individuals from NUWS cultivated in the 4 μM PO_4^{3-} treatment presented the highest phosphate uptake, 3.42 ± 0.87 mg P gDM^{-1} day^{-1} ($p < 0.01$) (Figure 1.4).

1.3.4 Tissue phosphorus analyses

At the beginning of the experiment, tissue phosphorus contents in macroalgae from NUWS were twice the value obtained in the UWS ($p < 0.01$), and these values were higher than those found after the experimental period for individuals of both sites in all treatments ($p < 0.01$; Figure 1.5). After the experimental period, the average tissue P value was 0.29 ± 0.04% for *U.fasciata* from UWS and 0.20 ± 0.01% for NUWS (Figure 1.5). Tissue P in UWS site was higher than in the NUWS site in

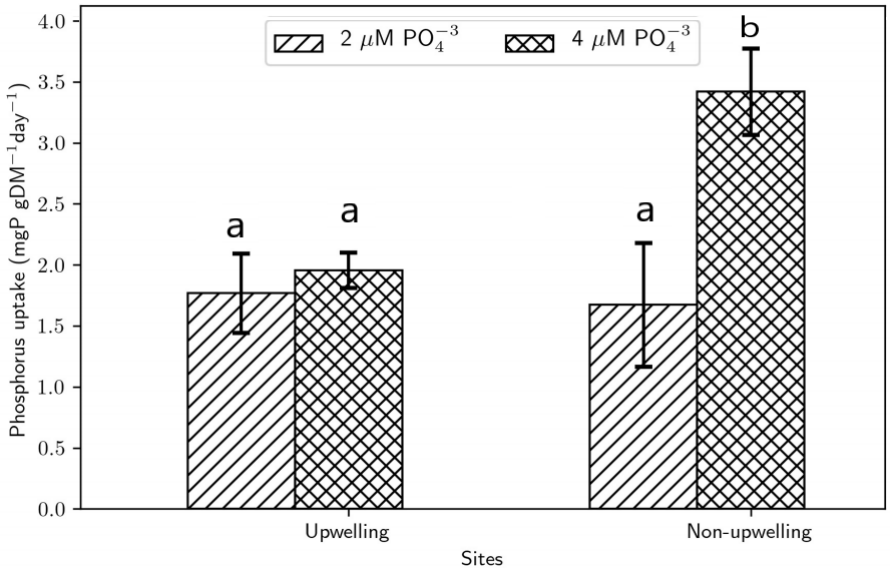

Figura 1.5: Phosphate uptake by *Ulva fasciata* cultivated in three different phosphate concentrations (0, 2, and 4 μM PO_4^{3-}), fifteen days into the experiment. The phosphate concentration in the 0 μM PO_4^{3-} treatment was bellow the detection limits of the equipment (< 0.1 μM). Data presented as mean and standard deviation (n = 3). Different letters indicate significant differences.

the treatment with 2 μM PO_4^{3-} ($p < 0.01$). There was no difference between sites in the 4 μM PO_4^{3-} treatment ($p > 0.05$). The tissue P in the treatment without phosphate addition (control) presented the lowest value for both sites ($p < 0.01$).

1.3.5 Carbohydrate content

The carbohydrate content observed before the experiment were 33.54 ± 9.35 and 21.34 ± 3.83% DM in the UWS and NUWS individuals, respectively. After the experimental period, the carbohydrate content increased in all treatments ($p < 0.01$) and in both sites ($p < 0.01$) approximately 136 and 264% for UWS and NUWS, respectively. Considering the UWS individuals, the carbohydrate content in the treatment with 4 μM PO_4^{3-} was higher than in the treatment without phosphate addition ($p < 0.05$), but there was no difference between the treatments without phosphate addition and with 2 µM PO43- (p = 0.80), or between 2 and 4 µM PO_4^{3-} ($p > 0.05$). In the NUWS individuals, no differences were found between treatments with 0 and 2 μM PO_4^{3-} ($p = 0.87$). The carbohydrate content in the treatment with 4 μM PO_4^{3-} was the lowest recorded (46.42 ± 0.05% DM) in NUWS ($p < 0.01$) and when compared with all treatments in the UWS site ($p < 0.01$) (Figure 1.6).

1.4 Discussion

Ulva fasciata from Upwelling (UWS) and Non-upwelling sites (NUWS) presented similarly high growth rates in all treatments (around 12% day^{-1}), showing the

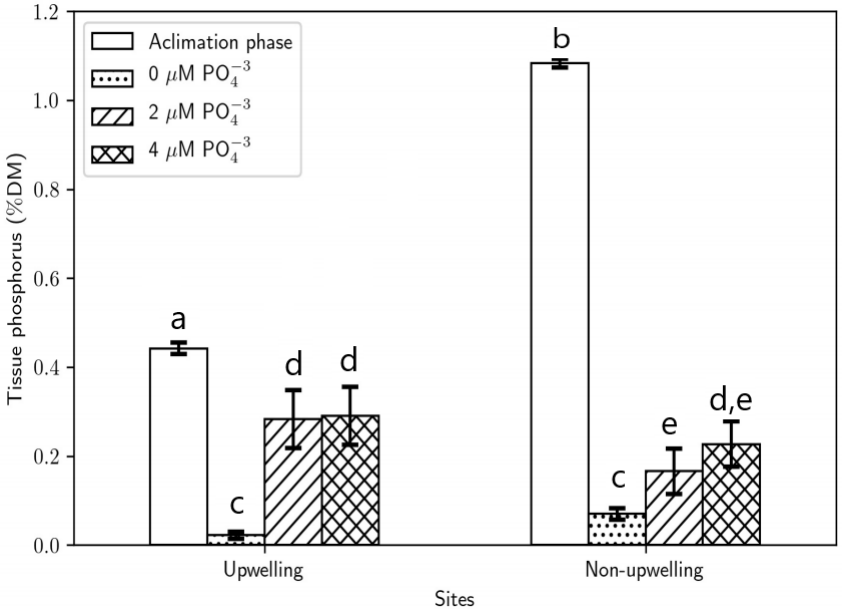

Figura 1.6: Tissue phosphorus of *Ulva fasciata* in three different phosphate concentrations (0, 2 and 4 μM PO_4^{3-}) over the experimental period (fifteen days). Data presented as means and standard deviations (n = 3). Treatments with different letters indicate significant differences ($p < 0.05$).

physiological plasticity and fast response of this species when submitted to different phosphate availabilities, as phosphorus is essential for growth, being a major constituent of RNA and protein synthesis (Chávez-Sánchez et al., 2017; Douglas et al., 2014). These SGR were similar to those found on previous studies conducted with other *Ulva* species in laboratory conditions (Ale et al., 2010; Li et al., 2016) and in outdoor experiments (Ben-Ari et al., 2014; Msuya and Neori, 2008). High growth rates are an important factor to consider when evaluating a species' potential for biotechnological purposes (Juneja et al., 2013). These results show that *U.fasciata* is a potential candidate for commercial biomass production. Individuals cultivated in the absence of phosphate presented positive growth, attributed to the use of internal nutrient reserves, as macroalgae store nutrients in their tissue to grow and survive in periods of nutrient limitation (Pedersen et al., 2010).

Phosphate starved individuals presented a progressive decline in the photosynthetic performance (Fv/Fm), once the photosynthetic metabolism responds to environmental changes faster than other physiological indicators (Schreiber, 2004). Phosphorus starvation for longer periods of time would lead to other metabolic disorders, such as decreasing growth and photosynthesis rates (Lapointe, 1986; Lee et al., 2005). Similar patterns were observed for *Ulva* prolifera cultivated in treatments without phosphate, after 8 days of experiment (Li et al., 2016).

The photosynthetic performance of *U.fasciata* in treatments with phosphate addition maintained a constantly high Fv/Fm throughout the experimental period

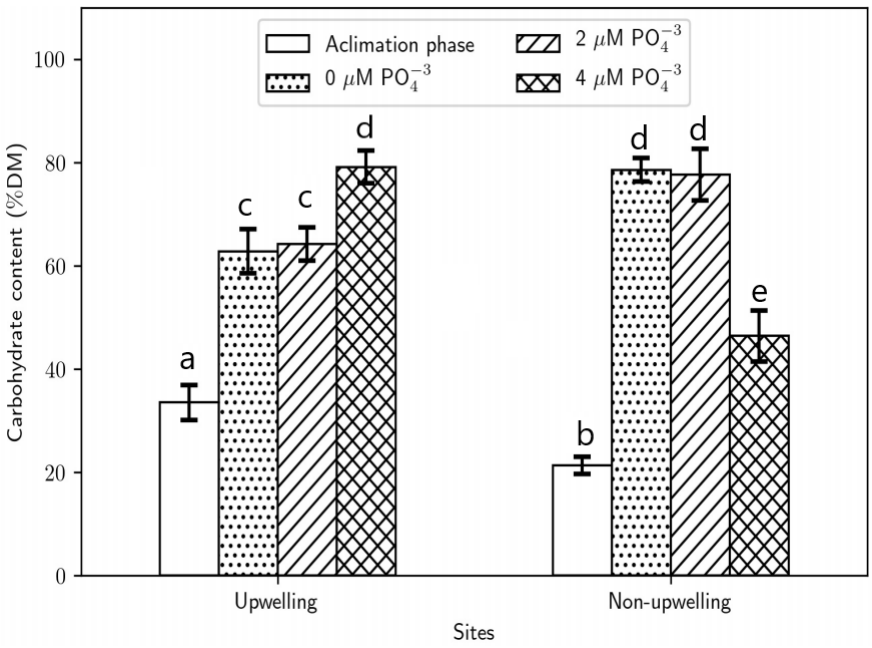

Figura 1.7: Carbohydrate contents of *Ulva fasciata* from two distinct sites (Upwelling and Non-upwelling) in three different phosphate treatments (0, 2, and 4 μM PO_4^{3-}), fifteen days into the experiment. Data presented as means and standard deviations (n = 3). Treatments with different letters indicate significant differences.

(Fv/Fm =0.788 ± 0.020), indicating healthy photosynthetic performance (Li et al., 2016; Scherner et al., 2012), while evaluating the potential impact of urban water on the photosynthetic response of *U. lactuca*, found that in eutrophic waters the photosynthetic efficiency was enhanced, suggesting that the physiological acclimation of this species improves in nutrient-rich sites.

Individuals from NUWS cultivated in 4 μM PO_4^{3-} presented an uptake two-fold higher than in the 2 μM PO_4^{3-} treatment and in individuals from UWS in all treatments (Figure 4, $p < 0.01$). Differently from nitrogen, phosphorus uptake is less studied with few investigations into its mechanism in macroalgae metabolism (see Lee et al., 2005 and Runcie et al., 2004). The phosphorus uptake observed here reflects differences between UWS and NUWS individuals, with uptake in UWS being demand driven (controlled by the algae maximum P uptake), while in NUWS uptake was supply driven (controlled by phosphate concentration in the water), as reported by Douglas et al. (2014) in a study on phosphorus uptake by six different macroalgae species. NUWS individuals were originally from a warmer site with low nutrient availability and differences in uptake suggest a stress reaction to high external nutrient concentrations, as life history traits are an important factor when determining nutrient uptake rate and affinity (Douglas et al., 2014).

Prior to the experiment, *U.fasciata* from NUWS presented higher tissue P than the individuals from the UWS. Our results suggest that the high tissue P found in NUWS individuals was due to an adaptive mechanism similar to that reported by

Gao et al. (2012), where individuals of *Undaria pinnatifida* from a warmer site had a greater capacity to accumulate nitrogen reserves, increasing its heat tolerance. *Ulva* species tend to accumulate phosphorus as polyphosphates and TCA-soluble P (lipids, sugar and nucleotides), fast mobile phosphorus forms (Runcie et al., 2004). After the experiment, tissue P contents decreased in all individuals and treatments; however, values were within the range observed in other studies (Bjornsater and Wheeler, 1990; Tsagkamilis et al., 2009). The decrease in tissue P can be attributed to the use of these reserves for reproductive maturation, demanding phosphorus to create reproductive tissue, and to the constant efflux of phosphorus from the macroalgae, which is independent of the phosphate concentration in the medium (Douglas et al., 2014; Runcie et al., 2004).

The carbohydrate contents increased at rates of 92 and 136% in individuals from UWS and 264 and 117% in individuals from NUWS in the treatments with 2 and 4 μM PO_4^{3-}, respectively. This result shows that phosphate concentration influenced the carbohydrate anabolism. The carbohydrate contents in individuals from both sites were higher than the year-round variations (42 and 45% DM) observed for *U. lactuca* collected in Abu Quir Bay, Egypt (Khairy and El-Shafay 2013), and also higher than in those cultivated in an Integrated Multi Trophic Aquaculture System (44 % DM) (Ben-Ari et al., 2014). Individuals from UWS presented high values of carbohydrate in all treatments, while those from NUWS presented a sharp decrease in the carbohydrate content at 4 μM PO_4^{3-}, although their growth rates were constant and phosphate uptake was around 3.48 mg P gDM^{-1} day^{-1}. This indicates that the phosphate removed from the medium was used in biomass production.

High values of carbohydrates were found in treatments with no phosphate addition. In conditions where phosphorus levels are limited, internal nutrient reserves can be used to produce carbohydrates, providing the energy necessary to maintain physiological and biochemical processes (Lee et al., 2005; Pedersen et al., 2010; Rocha et al., 2018). This result, along with the lower value of tissue phosphorus observed, indicated that *U.fasciata* individuals cultivated in the absence of phosphate resorted to their reserves, showing a high carbohydrate content. This pattern was also observed in an experiment conducted with *U. rigida*, where the carbohydrate content increased 131% in individuals cultivated in a low nutrient site for 48 hours after 14 days in a nutrient-rich site (Korzen et al., 2015a).

Different responses of *U.fasciata* from the two sites were observed throughout the experiment. NUWS individuals had higher tissue P and UWS had the highest carbohydrate content. After cultivation in different phosphate concentrations, UWS presented constant tissue P and high carbohydrate content, while NUWS macroalgae presented a decrease in carbohydrate in the treatment with 4 μM PO_4^{3-}. It should be noted that all individuals were cultivated for six months in the same laboratory

conditions to prevent any influence from the collection site.

Niterói (NUWS) is not affected by the upwelling phenomenon, being an oceanic beach located in an environmentally protected area, thus presenting a narrower temperature variation and low nutrient availability (Nascimento et al., 2014). The lowest carbohydrate content found in NUWS individuals could be due to the lack of environmental stimuli (Padilla-Gamiño and Carpenter, 2007). Arraial do Cabo (UWS) suffers the upwelling phenomenon of cold and nutrient-rich deep water and therefore experiences broad year-round temperature and nutrient availability variations (Coelho-Souza et al., 2017). These oscillations in abiotic factors can induce individuals to develop adaptive mechanisms, such as higher energetic reserves, to better cope with periods of suboptimal conditions (Boyer, 1982).

In this study, individuals of *U.fasciata* from both sites stored high tissue P during the acclimation phase and resorted to these reserves to sustain growth when deprived of phosphate. However, these individuals had the photosystem impaired by phosphate deprivation over time. Future studies with previous acclimation of *U.fasciata* individuals in seawater with no phosphate addition could help determine the influence of phosphate deprivation on other physiological aspects of this macroalgae.

In summary, the phosphate concentration influenced the total carbohydrate content and physiological responses in *U.fasciata*. The cultivation of *U.fasciata* in nutrient-rich environments, such as urban estuaries or eutrophic regions, could present a valuable service to the ecosystem, reducing costs with nutrient inputs and increasing productivity, which is particularly important in developing countries. The results suggest differences between individuals from the two collection sites, with UWS individuals presenting higher productivity, thus being recommended for future biotechnological studies.

1.5 Acknowledgment

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Referências Bibliográficas

Abreu, M. H., Pereira, R., Yarish, C., Buschmann, A. H., and Sousa-Pinto, I. (2011). IMTA with *Gracilaria vermiculophylla*: Productivity and nutrient removal performance of the seaweed in a land-based pilot scale system. *Aquaculture*, 312(1-4):77–87.

Akila, V., Manikandan, A., Sukeetha, D. S., Balakrishnan, S., Ayyasamy, P. M., and Rajakumar, S. (2019). Biogas and biofertilizer production of marine macroalgae: An effective anaerobic digestion of *Ulva* sp. *Biocatalysis and Agricultural Biotechnology*, 18:101035.

Ale, M. T., Mikkelsen, J. D., and Meyer, A. S. (2010). Differential growth response of *Ulva lactuca* to ammonium and nitrate assimilation. *Journal of Applied Phycology*, 23(3):345–351.

Ben-Ari, T., Neori, A., Ben-Ezra, D., Shauli, L., Odintsov, V., and Shpigel, M. (2014). Management of *Ulva lactuca* as a biofilter of mariculture effluents in IMTA system. *Aquaculture*, 434:493–498.

Bjornsater, B. R. and Wheeler, P. A. (1990). Effect of nitrogen and phosphorus supply on growth and tissue composition of *Ulva fenestrata* and *Enteromorpha intestinalis* (Ulvales, Chlorophyta) 1. *Journal of Phycology*, 26(4):603–611.

Boyer, J. S. (1982). Plant productivity and environment. *Science*, 218(4571):443–448.

Carpenter, S. R. and Bennett, E. M. (2011). Reconsideration of the planetary boundary for phosphorus. *Environmental Research Letters*, 6(1):014009.

Catanzaro, L. F., Neto, J. A. B., Guimarães, M. S. D., and Silva, C. G. (2004). Distinctive sedimentary processes in Guanabara Bay - SE/Brazil, based on the analysis of echo-character (7.0 kHz). *Revista Brasileira de Geofísica*, 22(1):69–83.

Chávez-Sánchez, T., Piñón-Gimate, A., Serviere-Zaragoza, E., Sánchez-González, A., Hernández-Carmona, G., and Casas-Valdez, M. (2017). Recruitment in *Ulva* blooms in relation to temperature, salinity and nutrients in a subtropical bay of the Gulf of California. *Botanica Marina*, 60(3).

Chen, B. and Zou, D. (2015). Altered seawater salinity levels affected growth and photosynthesis of *Ulva fasciata* (Ulvales, Chlorophyta) germlings. *Acta Oceanologica Sinica*, 34(8):108–113.

Chopin, T. and Wagey, B. T. (1999). Factorial study of the effects of phosphorus and nitrogen enrichments on nutrient and carrageenan content in *Chondrus crispus* (Rhodophyceae) and on residual nutrient concentration in seawater. *Botanica Marina*, 42(1).

Coelho-Souza, S. A., Pereira, G. C., Lopez, M. S., Guimaraes, J. R., and Coutinho, R. (2017). Seasonal sources of carbon to the Brazilian upwelling system. *Estuarine, Coastal and Shelf Science*, 194:162–171.

Cui, J., Zhang, J., Monotilla, A. P., Huo, Y., Shi, J., Zhao, X., Kang, X., and He, P. (2019). Assessment of blooming *Ulva* macroalgae production potential in the Yellow Sea, China. *Phycologia*, 58(5):535–541.

Cury, J. C., Araujo, F. V., Coelho-Souza, S. A., Peixoto, R. S., Oliveira, J. A. L., Santos, H. F., Dávila, A. M. R., and Rosado, A. S. (2011). Microbial diversity of a Brazilian coastal region influenced by an upwelling system and anthropogenic activity. *PLoS ONE*, 6(1):e16553.

da Silva Copertino, M., Tormena, T., and Seeliger, U. (2008). Biofiltering efficiency, uptake and assimilation rates of *Ulva clathrata* (Roth) J. Agardh (Clorophyceae) cultivated in shrimp aquaculture waste water. *Journal of Applied Phycology*, 21(1):31–45.

de Guimaraens, M. A., de Moraes Paiva, A., and Coutinho, R. (2005). Modeling *Ulva spp.* dynamics in a tropical upwelling region. *Ecological Modelling*, 188(2-4):448–460.

de Oliveira, V. P., Martins, N. T., de Souza Guedes, P., Pollery, R. C. G., and Enrich-Prast, A. (2016). Bioremediation of nitrogenous compounds from oilfield wastewater by *Ulva* lactuca (Chlorophyta). *Bioremediation Journal*, 20(1):1–9.

Douglas, E., Haggitt, T., and Rees, T. (2014). Supply- and demand-driven phosphate uptake and tissue phosphorus in temperate seaweeds. *Aquatic Biology*, 23(1):49–60.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3):350–356.

FAO (2018). *The state of world fisheries and aquaculture 2018 : meeting the sustainable development goals*. Food and Agriculture Organization of the United Nations, Rome.

Fleurence, J. (2016). *Seaweed in health and disease prevention*. Academic Press is an imprint of Elsevier, London, UK.

Gao, X., Endo, H., Taniguchi, K., and Agatsuma, Y. (2012). Genetic differentiation of high-temperature tolerance in the kelp *Undaria pinnatifida* sporophytes from geographically separated populations along the Pacific coast of Japan. *Journal of Applied Phycology*, 25(2):567–574.

Hafting, J. T., Craigie, J. S., Stengel, D. B., Loureiro, R. R., Buschmann, A. H., Yarish, C., Edwards, M. D., and Critchley, A. T. (2015). Prospects and challenges for industrial production of seaweed bioactives. *Journal of Phycology*, 51(5):821–837.

Juneja, A., Ceballos, R., and Murthy, G. (2013). Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A review. *Energies*, 6(9):4607–4638.

Khairy, H. M. and El-Shafay, S. M. (2013). Seasonal variations in the biochemical composition of some common seaweed species from the coast of Abu Qir Bay, Alexandria, Egypt. *Oceanologia*, 55(2):435–452.

Korzen, L., Abelson, A., and Israel, A. (2015a). Growth, protein and carbohydrate contents in *Ulva rigida* and *Gracilaria bursa-pastoris* integrated with an offshore fish farm. *Journal of Applied Phycology*, 28(3):1835–1845.

Korzen, L., Pulidindi, I. N., Israel, A., Abelson, A., and Gedanken, A. (2015b). Marine integrated culture of carbohydrate rich *Ulva rigida* for enhanced production of bioethanol. *RSC Advances*, 5(73):59251–59256.

Lapointe, B. E. (1986). Phosphorus-limited photosynthesis and growth of *Sargassum natans* and *Sargassum fluitans* (Phaeophyceae) in the western North Atlantic. *Deep Sea Research Part A. Oceanographic Research Papers*, 33(3):391–399.

Lawton, R. J., Mata, L., de Nys, R., and Paul, N. A. (2013). Algal bioremediation of waste waters from land-based aquaculture using *Ulva*: Selecting target species and strains. *PLoS ONE*, 8(10):e77344.

Lee, T.-M., Tsai, P.-F., Shyu, Y.-T., and Sheu, F. (2005). The effects of phosphite on phosphate starvation responses of *Ulva Lactuca* (Ulvales, Chlorophyta) 1. *Journal of Phycology*, 41(5):975–982.

Li, H., Zhang, Y., Han, X., Shi, X., Rivkin, R. B., and Legendre, L. (2016). Growth responses of *Ulva prolifera* to inorganic and organic nutrients: Implications for macroalgal blooms in the southern Yellow Sea, China. *Scientific Reports*, 6(1).

Magnusson, M., Carl, C., Mata, L., de Nys, R., and Paul, N. A. (2016). Seaweed salt from *Ulva*: A novel first step in a cascading biorefinery model. *Algal Research*, 16:308–316.

Margareta, W., Nagarajan, D., Chang, J.-S., and Lee, D.-J. (2020). Dark fermentative hydrogen production using macroalgae (*Ulva sp.*) as the renewable feedstock. *Applied Energy*, 262:114574.

Martínez, B., Pato, L. S., and Rico, J. M. (2012). Nutrient uptake and growth responses of three intertidal macroalgae with perennial, opportunistic and summer-annual strategies. *Aquatic Botany*, 96(1):14–22.

Martins, N. T. (2016). *Physiological responses of Ulva fasciata Delile Ulvales, Chlorophyta comparison of two populations from thermally distinct sites from Brazilian coast*. PhD thesis, Universidade de São Paulo.

McDermid, K. J. and Stuercke, B. (2003). Nutritional composition of edible Hawaiian seaweeds. *Journal of Applied Phycology*, 15(6):513–524.

Menzel, D. W. and Corwin, N. (1965). The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation 1. *Limnology and oceanography*, 10(2):280–282.

Msuya, F. E. and Neori, A. (2008). Effect of water aeration and nutrient load level on biomass yield, n uptake and protein content of the seaweed *Ulva lactuca* cultured in seawater tanks. *Journal of Applied Phycology*, 20(6):1021–1031.

Murphy, J. and Riley, J. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27:31–36.

Nascimento, A., Coelho-Gomes, C., Barbarino, E., and Lourenço, S. O. (2014). Temporal variations of the chemical composition of three seaweeds in two tropical coastal environments. *Open Journal of Marine Science*, 04(02):118–139.

Padilla-Gamiño, J. L. and Carpenter, R. C. (2007). Seasonal acclimatization of *Asparagopsis taxiformis* (Rhodophyta) from different biogeographic regions. *Limnology and Oceanography*, 52(2):833–842.

Pedersen, M. and Borum, J. (1997). Nutrient control of estuarine macroalgae:growth strategy and the balance between nitrogen requirements and uptake. *Marine Ecology Progress Series*, 161:155–163.

Pedersen, M., Borum, J., and Fotel, F. L. (2010). Phosphorus dynamics and limitation of fast- and slow-growing temperate seaweeds in Oslofjord, Norway. *Marine Ecology Progress Series*, 399:103–115.

Raven, J. A. (2013). RNA function and phosphorus use by photosynthetic organisms. *Frontiers in Plant Science*, 4.

Ribeiro, C. and Kjerfve, B. (2002). Anthropogenic influence on the water quality in Guanabara Bay, Rio de Janeiro, Brazil. *Regional Environmental Change*, 3(1-3):13–19.

Rocha, G. S., Parrish, C. C., Lombardi, A. T., and da Graça Gama Melão, M. (2018). Biochemical and physiological responses of *Selenastrum gracile* (Chlorophyceae) acclimated to different phosphorus concentrations. *Journal of Applied Phycology*, 30(4):2167–2177.

Runcie, J. W., Ritchie, R. J., and Larkum, A. W. (2004). Uptake kinetics and assimilation of phosphorus by *Catenella nipae* and *Ulva lactuca* can be used to indicate ambient phosphate availability. *Journal of Applied Phycology*, 16(3):181–194.

Ryu, M. J., Kim, A. D., Kang, K. A., Chung, H. S., Kim, H. S., Suh, I. S., Chang, W. Y., and Hyun, J. W. (2013). The green algae *Ulva fasciata* Delile extract induces apoptotic cell death in human colon cancer cells. *In Vitro Cellular & Developmental Biology - Animal*, 49(1):74–81.

Scherner, F., Barufi, J. B., and Horta, P. (2012). Photosynthetic response of two seaweed species along an urban pollution gradient: evidence of selection of pollution-tolerant species. *Marine pollution bulletin*, 64(11):2380–2390.

Schreiber, U. (2004). Pulse-amplitude-modulation (pam) fluorometry and saturation pulse method: an overview. In *Chlorophyll a fluorescence*, pages 279–319. Springer.

Sfriso, A. A. and Sfriso, A. (2017). In situ biomass production of *Gracilariaceae* and *Ulva rigida*: the Venice Lagoon as a study case. *Botanica Marina*, 60(3).

Soares-Gomes, A., da Gama, B., Neto, J. B., Freire, D., Cordeiro, R., Machado, W., Bernardes, M., Coutinho, R., Thompson, F., and Pereira, R. (2016). An environmental overview of Guanabara Bay, Rio de Janeiro. *Regional Studies in Marine Science*, 8:319–330.

Sousa-Pinto, I., Lewis, R., and Polne-Füller, M. (1996). The effect of phosphate concentration on growth and agar content of *Gelidium robustum* (Gelidiaceae,

Rhodophyta) in culture. In *Fifteenth International Seaweed Symposium*, pages 437–443. Springer Netherlands.

Thanh, T. T. T., Quach, T. M. T., Nguyen, T. N., Luong, D. V., Bui, M. L., and Tran, T. T. V. (2016). Structure and cytotoxic activity of ulvan extracted from green seaweed *Ulva lactuca*. *International Journal of Biological Macromolecules*, 93:695–702.

Torres, M. D., Kraan, S., and Domínguez, H. (2019). Seaweed biorefinery. *Reviews in Environmental Science and BioTechnology*, 18(2):335–388.

Tsagkamilis, P., Danielidis, D., Dring, M. J., and Katsaros, C. (2009). Removal of phosphate by the green seaweed *Ulva lactuca* in a small-scale sewage treatment plant (Ios Island, Aegean Sea, Greece). *Journal of Applied Phycology*, 22(3):331–339.

Ursi, S. and Plastino, E. M. (2001). Crescimento *in vitro* de linhagens de coloração vermelha e verde clara de *Gracilaria birdiae* (Gracilariales, Rhodophyta) em dois meios de cultura: análise de diferentes estádios reprodutivos. *Revista Brasileira de Botânica*, 24(4).

Wang, H.-M. D., Chen, C.-C., Huynh, P., and Chang, J.-S. (2015). Exploring the potential of using algae in cosmetics. *Bioresource Technology*, 184:355–362.

Wang, Y., Wang, Y., Zhu, L., Zhou, B., and Tang, X. (2012). Comparative studies on the ecophysiological differences of two green tide macroalgae under controlled laboratory conditions. *PLoS ONE*, 7(8):e38245.

Wu, H., Huo, Y., Zhang, J., Liu, Y., Zhao, Y., and He, P. (2015). Bioremediation efficiency of the largest scale artificial *Porphyra yezoensis* cultivation in the open sea in China. *Marine Pollution Bulletin*, 95(1):289–296.

Xiao, X., Agusti, S., Lin, F., Li, K., Pan, Y., Yu, Y., Zheng, Y., Wu, J., and Duarte, C. M. (2017). Nutrient removal from chinese coastal waters by large-scale seaweed aquaculture. *Scientific Reports*, 7(1).

Yong, Y. S., Yong, W. T. L., and Anton, A. (2013). Analysis of formulae for determination of seaweed growth rate. *Journal of Applied Phycology*, 25(6):1831–1834.

Yoza, B. A. and Masutani, E. M. (2013). The analysis of macroalgae biomass found around Hawaii for bioethanol production. *Environmental Technology*, 34(13-14):1859–1867.

Yu, Z., Zhu, X., Jiang, Y., Luo, P., and Hu, C. (2014). Bioremediation and fodder potentials of two *Sargassum spp.* in coastal waters of Shenzhen, South China. *Marine Pollution Bulletin*, 85(2):797–802.

Capítulo 2

Nitrogen and phosphorus uptake and effects in *Ulva fasciata* physiology and extracted ulvan

2.1 Introduction

Global macroalgae production increased from 13.5 million tonnes in 1995 to 30 million tonnes in 2016 (FAO, 2018) due to its versatility and potential as feedstock for food, chemicals and fuel industries (Gupta et al., 2018; Magnusson et al., 2016; Margareta et al., 2020). However uncertainties in the biomass supply associated with cultivation management and economic viability for its production are among the main hindrances to this market development (Akila et al., 2019; Torres et al., 2019).

The high input of nitrogen and phosphorus in marine water caused by anthropogenic activities is considered the principal cause of water quality impairment in coastal zones (Nascimento et al., 2014; Reidenbach et al., 2017), and the use of macroalgae to control marine eutrophication is growing worldwide due to its capacity to efficiently uptake nitrogen and phosphorus in the water (Xiao et al., 2017), cost effectiveness and to promote sustainable development (Tremblay-Gratton et al., 208;Nardelli et al., 2019).

Cultivation of macroalgae in sites with elevated concentrations of nitrogen and phosphorus with the subsequent extraction of valuable-add products is an alternative for more reliable biomass production, returning these nutrients to the technological cycle, promoting the valorization of the biomass while improving water quality (Mata et al., 2015; Seghetta et al., 2016; Torres et al., 2019). Suggested uses of this biomass include animal feed (Bikker et al., 2016), biogas (Akila et al., 2019)and pigment (Angell et al., 2014).

Macroalgae belonging to the genus *Ulva* (Chlorophyta) are opportunist species dominating environments with toxic nutrient conditions for other species, presenting high growth rate, cosmopolitan distribution and a cell wall thickness of two-cell layers that allows higher nutrient absorption and biomass production (Li et al.,

2016; Lubsch and Timmermans, 2018). The advantages of *Ulva* species can be further enhanced as this biomass can provide products of economic interest such as ulvan, a bioactive polysaccharide with biotechnological interest (Li et al., 2018; Tziveleka et al., 2019).

Ulvan has drawn attention with studies showing its potential as antioxidant (Li et al., 2018; Qi et al., 2005), antiviral (Hardouin et al., 2016; Lopes et al., 2017), anticancer (Shao et al., 2014; Thanh et al., 2016) among others (Adrien et al., 2017; del Rocío Quezada-Rodríguez and Fajer-Ávila, 2016). This sulfated heteropolysaccharide is built on sequences of two major repeating disaccharides unities designated as ulvanobiuronic acid 3-sulfate type A (A3s) and type B (B3s), composed of variable amounts of rhamnose, glucuronic acid, iduronic acid, xylose and sulfate (Tziveleka et al., 2019).The presence of the rare sugars rhamnose – used in anti-aging cosmetics and iduronic acid - required in the synthesis of heparin analogs - single out ulvan from other algal polysaccharides (Bindschädler et al., 2010; Hallak et al., 2000).

Although the biotechnological applications of ulvan are promising, structural variations may occur due to ecophysiological factors acting on *Ulva* (Robic et al., 2009b). The commercial use of polysaccharides requires ulvan with predictive structure and functional properties that could be obtained by the controlled cultivation of *Ulva*.

In this sense, the present work evaluated nitrogen and phosphorus uptake potential and its effects in the physiology and structure of ulvan extracted from the macroalgae *Ulva fasciata*. To enhance potential structural differences, ulvan from the cultivated biomass was compared against ulvan from biomass collected from the natural environment. Furthermore, an equation estimating the nutrient recovery from the produced biomass was proposed.

2.2 Material and Methods

2.2.1 Biological material

Individuals of *Ulva fasciata* were collected in the mesolittoral zone at Prainha Beach, Arraial do Cabo / RJ Brazil (22°57'40"S / 42°01'13"W) (Figure 2.1), rinsed with local seawater to remove sediments and encrusting material and immediately transported to the laboratory inside coolers filled with seawater.

In the laboratory individuals were further cleaned with distilled water. Part of the fresh biomass (SEA) was oven dried at 50°C until constant mass and stored in desiccator until ulvan extraction. The remaining material was used in the cultivation experiment (CULT). Voucher specimens were deposited in the Institute of Bioscience

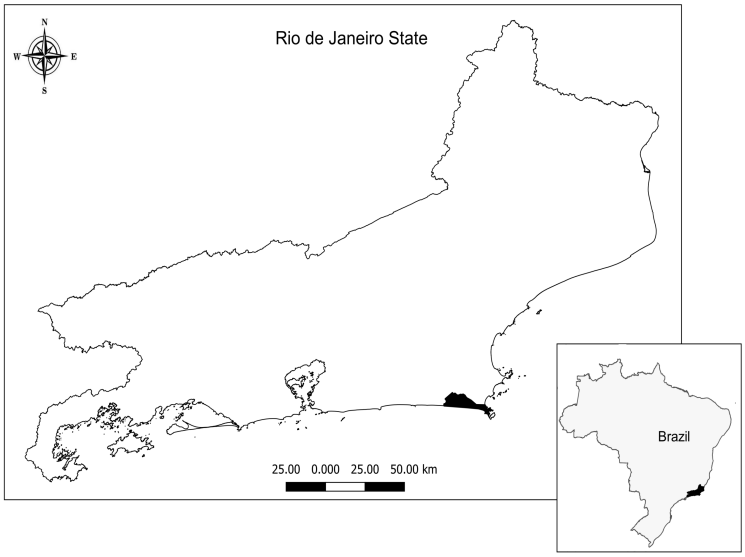

Figura 2.1: Map of sampling site of *Ulva fasciata*

Herbarium at the University of São Paulo, Brazil (SPF-57877).

Individuals were acclimated to the laboratory conditions for seven days prior to the experiment. Healthy thalli were maintained in sterilized seawater enriched with von Stosch culture medium (Ursi and Plastino, 2001) in a temperature-controlled room at 24.0 ± 1.0°C and photoperiod of 12 hours light. Photosynthetically active radiation (PAR) was kept at 70 μmol photons m^{-2} s^{-1} and was provided by 40 W daylight fluorescent tubes.

2.2.2 Experiment design

After acclimatization to laboratory conditions, health individuals weighing approximately 3.0 g (fresh mass) were selected and placed in erlenmeyers flasks filled with 2 L of sterilized seawater (0.05 μM NH_4, 0.03 μM NO_2, 0.41 μM NO_3 and 0.09 μM PO_4^{3-}) for a three days starvation period, ensuring that all individuals presented comparable initial physiological conditions (Lubsch and Timmermans, 2019). For the experiment individuals were cultivated in 3 L erlenmeyer flasks (1g/L), with four replicates for each treatment (enriched water and control). The enriched water treatment consisted of sterilized seawater enriched with 200 μM ammonium (NH_4Cl), 8 μM nitrate ($NaNO_3$) and 12 μM phosphate ($Na_2.HPO_4.12H_2O$). These concentrations were chosen with reference to the mean maximum nutrient concentration after a five years monitoring of one of the points of a eutrophic bay (Valentin et al., 2018) with potential for *Ulva* cultivation. For the control treatment (n = 4), *U. fasciata* individuals were cultivated in unenriched sterilized seawater (0.05 μM NH_4, 0.41 μM NO_3 and 0.09 μM PO_4^{3-}). Erlenmeyer flasks (n = 4) filled with enriched water without macroalgae were used as blanks. All flasks

were randomly distributed and manually shaken twice a day to minimize possible differences in light availability and reduce boundary layer effects. Conditions of light, photoperiod and temperature were the same as those described for the acclimatization period. The experiment lasted five days and was repeated twice.

Daily, a 60 ml aliquot was collected for dissolved nitrogen and phosphate uptake assessment. Tissue samples obtained prior to and at the end of the cultivation experiment were used for chlorophyll-*a*, tissue nitrogen, phosphorus and carbon contents analyses. All samples collected (tissue and water) were kept frozen (−20 °C,) until analyses. All analyses were performed with analytical triplicates. After the cultivation experiment, biomass from natural environment and cultivation (SEA and CULT, respectively) were used for ulvan extraction (Figure 2.2).

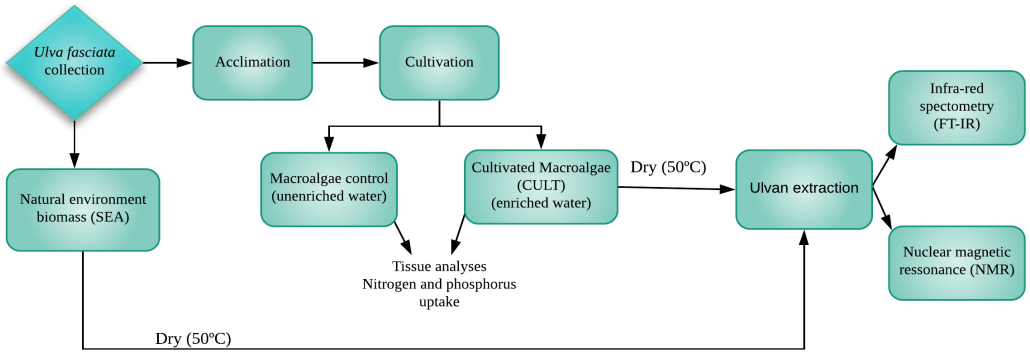

Figura 2.2: Schematic representation of *Ulva fasciata* cultivation and ulvan extraction

2.2.3 Specific growth rates

Specific growth rates were assessed by measuring the fresh weight using the following formula proposed by Schmidt et al. (2010):

$$\mathrm{SGR}(\%\ \mathrm{d}^{-1}) = [(\mathrm{W}_t/\mathrm{W}_0)\text{-}1]*(100/\mathrm{t})$$

Where, W_0 is the initial wet weight, W_t is the final wet weight, and t is the time between weighing (in days). The SGR is reported as a percentage per day (% d^{-1}).

2.2.4 Nutrient uptake

Medium aliquots (60ml) were collected daily, filtered through GF/F glass filter and analysed. Ammonium concentrations were measured by the indophenol blue method adapted from Koroleff (1970), nitrate was analysed according with Grasshoff and Johannsen (1972) and phosphate was analysed by the phosphomolybdenum blue method as described by Murphy and Riley (1962). All analyses were performed

in triplicate. Nutrient uptake was calculated according to the following formula (Pedersen and Borum, 1997)

$$\text{Nutrient uptake(mg nutrient g } DM^{-1} \text{ day}^{-1}\text{)}=[(C_0 * L_0)\text{-}(C_t * L_t)/\ (t*B)]$$

Where C_0 is the initial nutrient concentration in the water (mg/L), L_0 is the initial volume (liter), C_t is the final nutrient concentration in the water, L_t is the final volume of water, B is the algal biomass dry mass (gram) and t is the cultivation period (days).

Nutrient uptake efficiency (%) was calculated as follows (Tremblay-Gratton et al., 2017)

$$\text{Nutrient uptake efficiency}(\%) = [(C_0\text{-}C_t)/C_0]*100$$

Where C_0 is the initial nutrient concentration and C_t is the final nutrient concentration.

2.2.5 Fresh mass and surface area correlation

The methodology to construct an equation describing the correlation between fresh mass (FM, in g) and surface area (SA, in cm^2) was adapted from Lubsch and Timmermans (2018). Health fragments (n = 34) of *U. fasciata* ranging from 0.002 to 0.085 g (0.153 to 6.391 cm^2) were placed on a white surface and covered with a petri dish to avoid folding of the fragments. A ruler placed by the side of the fragments was used as reference. Photographs (Cannon PowerShot G16) were taken and the SA was calculated using the open source software ImageJ (ImageJ, U. S. National Institutes of Health, Bethesda, MD, USA). The colored photograph was first converted into grayscale (type 8-bit) and then in pixels using the function "binary", to highlight the pigmented (dark) area and exclude any holes. The threshold routine was set to manual and adjusted for refined analysis when necessary.

2.2.6 Tissue chlorophyll-*a*

Chlorophyll-*a* contents were calculated according with LORENZEN (1967). Fresh tissue samples were collected at the beginning and at the end of the experiment, washed with distilled water and frozen for posterior analysis. Acetone 90% (Merck®) was added to flask tubes with 20 mg of fresh tissue macerated with liquid nitrogen. The tubes were kept at 4°C for 18 hours, and then centrifuged at 3000 rpm for 5 minutes. The supernatant was analysed with a Spectrophotometer (Hatch DR 5000) at 665 and 750 nm absorbance. The entire procedure was performed in the dark.

2.2.7 Tissue phosphorus, nitrogen and carbon content

Tissue samples collected at the beginning and at the end of the experiment were dried at 50°C until they reached constant mass, grinded into a powder and used in tissue analyses.

The tissue phosphorus contents were determined spectrophotometrically using method adapted from Murphy and Riley (1962). For total nitrogen and carbon contents' analyses, a CHNS Elemental Analyzer (Flash 2000 – Organic Elemental Analyzer with Delta V Advantage – Thermo Scientific) previously calibrated with acetanilide as a reference standard was used.

2.2.8 Ulvan extraction

After the cultivation experiment, individuals cultivated in enriched water (CULT) were washed with distilled water to remove salts and oven dried at 50°C until constant mass.

The polysaccharide was extracted according to method described by Reis et al. (2018). Dried algal biomass (SEA and CULT) were grinded into a powder, suspended in ultrapure water (Milli-q®) (100 ml/10 g) and autoclaved at 120°C for 40 min. The supernatant was centrifuged at 10000 g and 4°C for 10 min (Eppendorf centrifuge 5810 r). Ulvan was precipitated with three volumes of ultrapure ethanol (Merck®), cooled at $-20\,^{\circ}$C, for 48 hours and further centrifuged at 3500 g for 5 min. The recovered pellet (ulvan) was freeze-dried. The ulvan yield for both samples was 16.29 ± 0.93 % as calculated using the formula proposed by Yaich et al. (2017).

2.2.9 Fourier-transform infrared spectroscopy (FT-IR) analysis

Ulvan infrared spectra with a Fourier transform (FT-IR), was recorded on a spectrophotometer (IR Prestige−21, Shimadzu) at room temperature. The FT-IR spectra were obtained in the transmission mode at 400-4000 cm^{-1}. The transmission spectra data were recorded using potassium bromide (Merck®) pellets containing 2.5 mg of ulvan powder.

2.2.10 Nuclear magnetic resonance spectroscopy

NMR analyses were performed using a Varian VNMRSYS 500 MHz spectrometer (Varian Inc., Palo Alto, CA, USA) at 37^0C. Proton and carbon operating frequencies were 499.77 and 125.68 MHz, respectively. ^{1}H NMR spectra were recorded with a 90 degree observe pulse width (pw = 90 μs), acquisition time of 2.04 s and a 1 s

relaxation delay.A total of 32 scans were performed for each sample. For ^{13}C NMR, a 90 degree pulse was used (pw = 90 μs), 1.04 s acquisition time, 2 s relaxation delay and a total of 114.624 scans were collected. Ulvan samples (2% w/v) were dissolved in D_2O 99.99% (Sigma-Aldrich). ^{1}H and ^{13}C NMR chemical shifts were expressed in parts per million (ppm).

2.2.11 Statistics

Growth rates were analysed by one-way ANOVA (independent variable: treatment). Dissolved nutrient concentrations, chlorophyll a and tissue nutrient contents were analysed by the two-way ANOVA (independent variables: treatment and time). The normality and homogeneity of the variance assumptions were tested by the Shapiro-Wilks and Cochram tests, respectively. When the data failed to meet these assumptions, log transformation was used (Baptista-Neto et al., 2013). In all cases, a posteriori Student-Newman-Keuls test (SNK) was used to establish statistical differences. Statistical analyses were performed using the Statistica 10 software, and considering $p < 0.05$.

2.3 Results

2.3.1 Specific growth rates (SGR)

The growth of *U. fasciata* was significantly affected by the nitrogen and phosphorus concentration in the water ($p < 0.01$). Macroalgae in the water enriched treatment presented the highest growth rate (5.69 ± 0.48 % d^{-1}), while in the control treatment was 2.76 ± 0.91 % d^{-1}.

2.3.2 Nutrient uptake

The uptake efficiency was 22.48 ± 0.9 for phosphate, 99.95 ± 0.05 for ammonium and 100% for nitrate. The nutrient uptake varied throughout the experiment with higher uptake rates in the second day for both nitrogen ($NH_4 - NO_3$) (Figure A.3)and phosphorus (PO_4^{3-}) (Figure A.4). The average uptake rates were 4.20 ± 0.43 mg N g DW^{-1} day^{-1} and 0.25 ± 0.07 mg P g DW^{-1} day^{-1} for nitrogen and phosphorus, respectively. In the macroalgae control treatment the nutrient concentration was bellow detection limits ($< 0.01\mu$M) by the first day of experiment. Water blank treatment (enriched seawater without macroalgae) showed no difference in nitrogen ($p = 0.30$) or in phosphate concentrations ($p = 0.63$) throughout the experimental period.

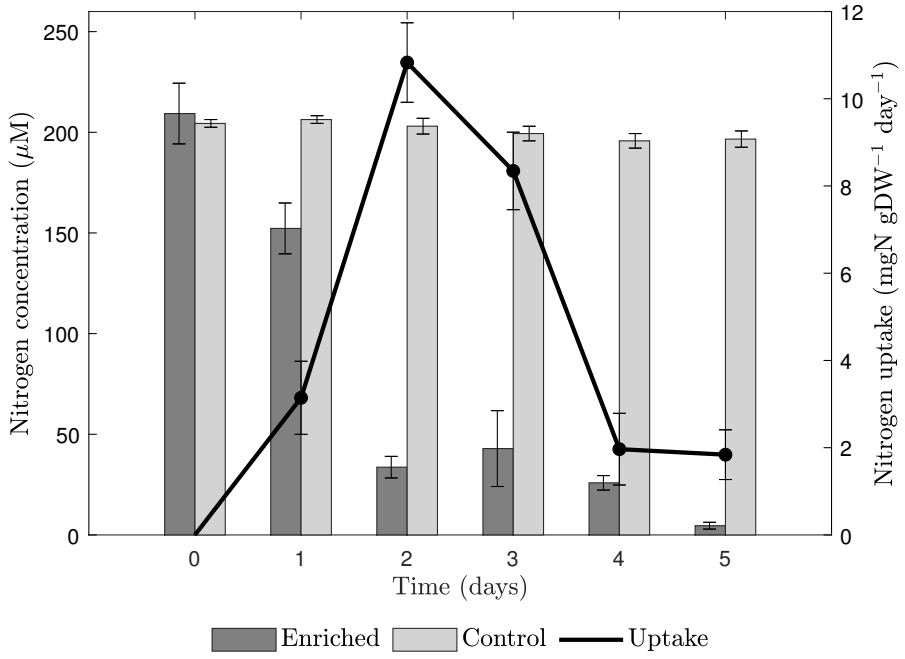

Figura 2.3: Nitrogen (NH_4 – NO_3) uptake by *Ulva fasciata* over the experimental period (five days). There was no difference in nutrient concentrations in the blank treatment throughout the experiment ($p > 0.05$). Data presented as mean and standard deviations (n = 4).

2.3.3 Tissue analysis

Chlorophyll *a* increased 244% in individuals cultivated in the enriched water treatment, being higher than in individuals from the control ($p < 0.01$). Comparisons between the beginning and the end of the experiment showed no difference in the chlorophyll-*a* contents in macroalgae from the control treatment ($p > 0.05$) (Table 2.1).

Tabela 2.1: Elemental composition (% dry mass) and chlorophyll-*a* contents (mg g fresh mass^{-1}) for *Ulva fasciata* cultivated in two different treatments (enriched water and control). Data presented as mean and standard deviations (n = 3). Bold values indicate significant differences according to two-way ANOVA and Newman-Keuls test ($p < 0.05$).

Treatment	**Chl-*a*** (mg gFM^{-1})	**P** (% DM)	**N** (% DM)	**C** (% DM)
Enriched				
T = 0 day	0.34 ± 0.07	0.05 ± 0.01	1.89 ± 0.16	27.07 ± 0.60
T = 5 days	**1.17 ± 0.27**	**0.06 ± 0.00**	**3.27 ± 0.07**	29.27 ± 0.71
Control				
T = 0 day	0.34 ± 0.08	0.05 ± 0.01	2.00 ± 0.21	28.20 ± 1.23
T = 5 days	0.34 ± 0.03	**00.03 ± 0.01**	2.03 ± 0.35	29.73 ± 1.50

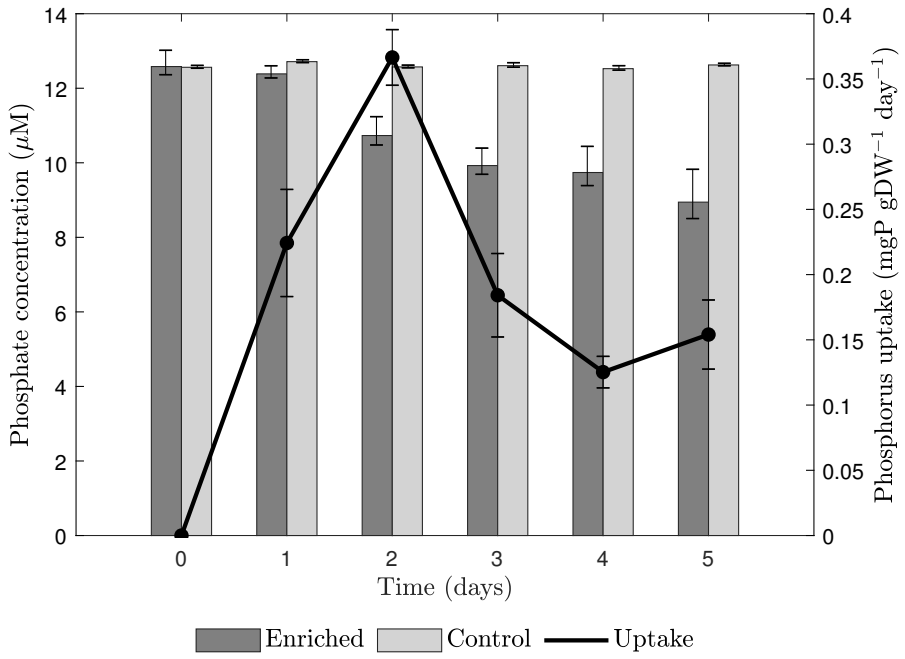

Figura 2.4: Phosphorus uptake by *Ulva fasciata* over the experimental period (five days). There was no difference in nutrient concentrations in the blank treatment throughout the experiment ($p > 0.05$). Data presented as mean and standard deviations (n = 4).

2.3.4 Fresh mass and surface area correlation

Surface Area (SA) was highly correlated with Fresh Mass (FM) ($R^2 = 0.973$), and the trend line used to calculate the equivalent area versus biomass is: y = 0.0119x + 0.0003 (Figure 2.5).

Based on this correlation, for example, an individual of *U. fasciata* weighting 1g would have an equivalent SA of 82.7 cm^2. This correlation allowed us to construct a model for the estimation of the nutrient uptake potential over a chosen period of time and area.

$$NR = \left[1 + \frac{(SGR * t)}{100}\right] * \left[\frac{(area - 0.252)}{84.03}\right] * \frac{DM}{FM} * \frac{TNC}{100} \tag{2.1}$$

where, NR is nutrient recovery (mass nutrient $area^{-1}$ $time^{-1}$), SGR is the specific growth rate of the macroalgae, t is time, DM is the dry mass of the macroalgae and FM is the fresh mass of the macroalgae and TNC is the tissue nutrient content in percentage.

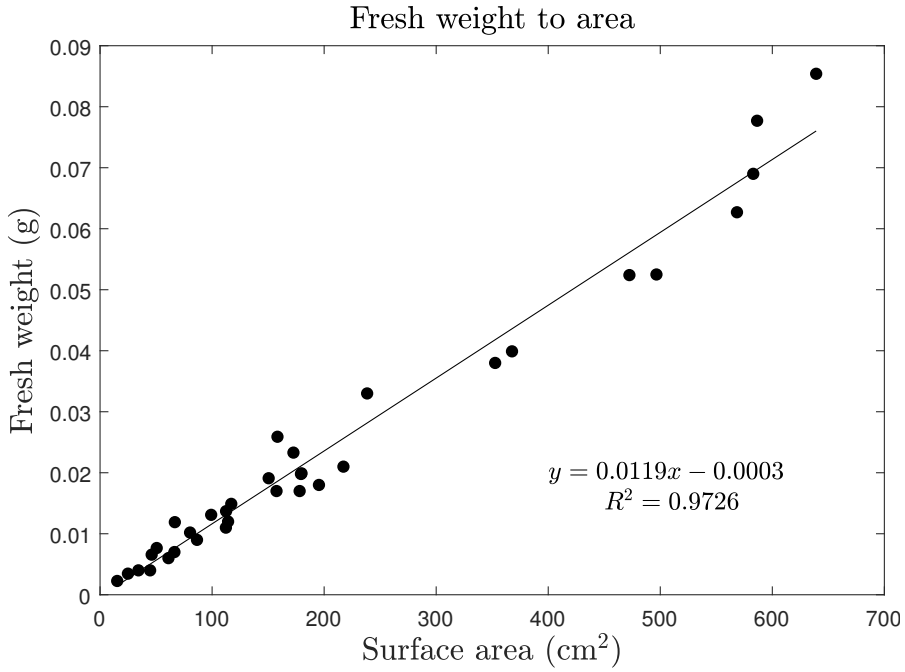

Figura 2.5: Correlation between fresh mass (g) and surface area (cm^2) of *U. fasciata*. Trend line: y = 0.0119x + 0.0003; R^2 = 0.973

2.3.5 Ulvan characterization

2.3.6 Fourier-transform infrared spectroscopy (FT-IR)

IR Spectra of SEA (biomass from the natural environment) and CULT (biomass from the enriched water treatment) ulvan are presented in Figure 2.6 with the signals assignment provided by comparison with published data (Alves et al., 2010; Qi et al., 2005; Tako et al., 2015; Yaich et al., 2017). The absorption band at around 3300 cm^{-1} was attributed to a stretching of hydroxyl groups (O - H). The signal observed at approximately 2937 cm^{-1} is due to C - H stretching vibration and is a characteristic of polysaccharides (Li et al., 2018). Bands of carboxylate groups of uronic acid with similar intensities are present in both spectra at around 1651 and 1435 cm^{-1}. The absorptions between 1147 and 848 cm^{-1} are known as the fingerprint region for ulvan. Signal at 983 cm^{-1} is characteristic of the vibration of glycosidic bonds, and the one at 848 cm^{-1} corresponds to the bending vibration of C – O – S from sulfate in axial position. Peaks bellow 900 cm^{-1} in both ulvan samples are characteristic of the presence of sulfate.

2.3.7 Nuclear Magnetic Resonance (NMR)

The ^{13}C NMR spectra are shown in Figure 2.7, with typical signals of ulvan structure attributed by comparison with published data (Costa et al., 2012; Lahaye, 1998; Lahaye et al., 1999; Lahaye and Ray, 1996). Signals in the resonance region corresponding to carbon rings (70.06 – 80.28 ppm and 69.60 – 79.88 ppm in CULT

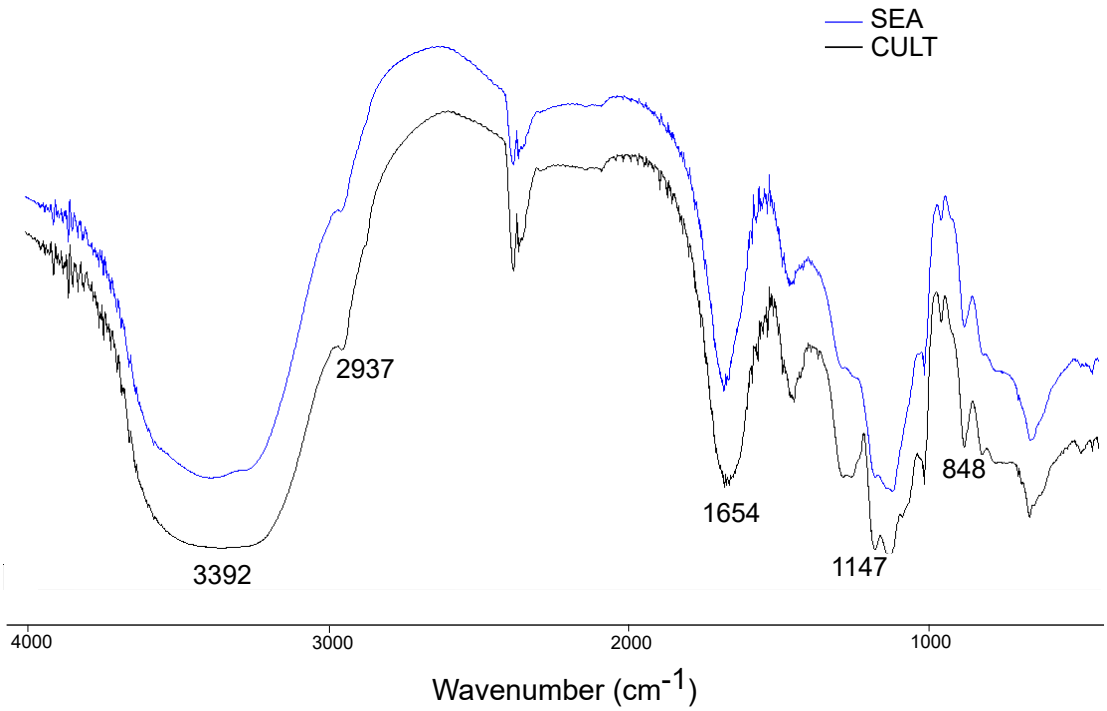

Figura 2.6: Infrared spectra of ulvan from *Ulva fasciata* from natural environment (SEA) and cultivated in enriched water (CULT) between 4000 and 400 cm^{-1}.

and SEA, respectively), C-6 methyl group of rhamnose at around 19 ppm and carboxyl signal of uronic acid at approximately 177 ppm are present. Further assigned signals of anomeric carbons (99.28 - 104.62 ppm) were observed in SEA.

^{1}H NMR spectra are presented in Figure 4.6 and the signals associated to each hydrogen atom are assigned according with reference data (Alves et al., 2012; Lahaye, 1998; Thanh et al., 2016; Yaich et al., 2017). Anomeric protons of rhamnose and iduronic acid are present between 4.28 - 5.13 ppm. Proton chemical shifts of rhamnose are shown at 1.30 ppm. Glucuronic acid chemical shifts are between 3.36 and 3.79 ppm. Signals of xylose are indicated at 3.20 and 5.27 ppm at SEA ulvan.

2.4 Discussion

This study showed that *Ulva fasciata* can efficiently absorb the nitrogen and phosphorus dissolved in the water. The global structure of ulvan from biomass cultivated in enriched water and natural environment were similar, with cultivated Ulva presenting more sulfate. The production of ulvan from biomass cultivated in enriched water can be a viable pathway to the development of this emerging market.

The growth rate of *U. fasciata* cultivated in enriched water was higher than in studies with *U. lactuca* cultivated in oilfield wastewater (0.51 % day^{-1}) and integrated with abalone (3.2 % day^{-1}) or shrimp production (4.7 % day^{-1}) (de Oliveira et al., 2016; Lavania-Baloo et al., 2014; Robertson-Andersson et al.,

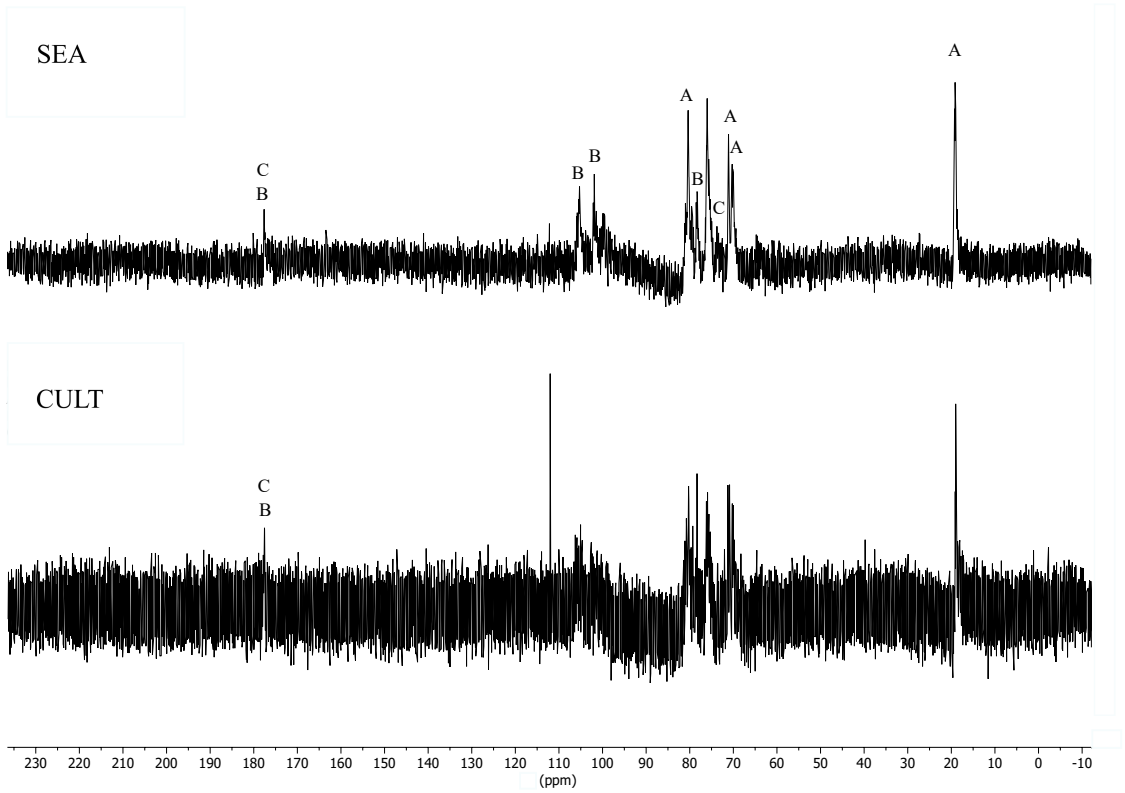

Figura 2.7: ^{13}C NMR spectra of ulvan from *Ulva fasciata* collected in natural environment (SEA) and cultivated in enriched water (CULT). Residue A = rhamnose, B = glucuronic acid, C = iduronic acid and D = xylose.

2008). High growth rates are an important factor to consider when evaluating a specie potential to biotechnological purposes (Juneja et al., 2013). In macroalgae control (cultivated in unenriched water) the growth rate was fifty percent lower than in enriched treatment. The metabolism of opportunistic algae concentrates primarily on the absorption of large amount of nutrient to sustain high growth and reproductive maturation making these species more susceptible to nutrient limitation (Martínez et al., 2012; Pedersen et al., 2010).

The nitrogen concentration used here was greater than that reported in most studies, but *U. fasciata* efficiently removed it from the enriched water. These uptake efficiency confirm that nutrient concentration in the reference area is suitable for *Ulva* cultivation.The uptake efficiency of *U. fasciata* was higher than reported for *U. lactuca* grown in Integrated multi-trophic sistem (IMTA)(49.7%, 142μM - N) and *U. lactuca* cultivated in rejected water from anaerobically digested sludge (64%, 115μM - N) (Shpigel et al., 2019; Sode et al., 2013). Phosphate uptake was similar to those reported by *U. lactuca* cultivated in a two level IMTA system (27%), co-cultured with the Pacific white shrimp (24.6%) or for *U. lactuca* cultivated in the effluent discharged from an abalone tank (25%) (Brito et al., 2013; Nardelli et al., 2018; Neori et al., 1998).

Uptake was highest in the second day of the experiment for both nitrogen and

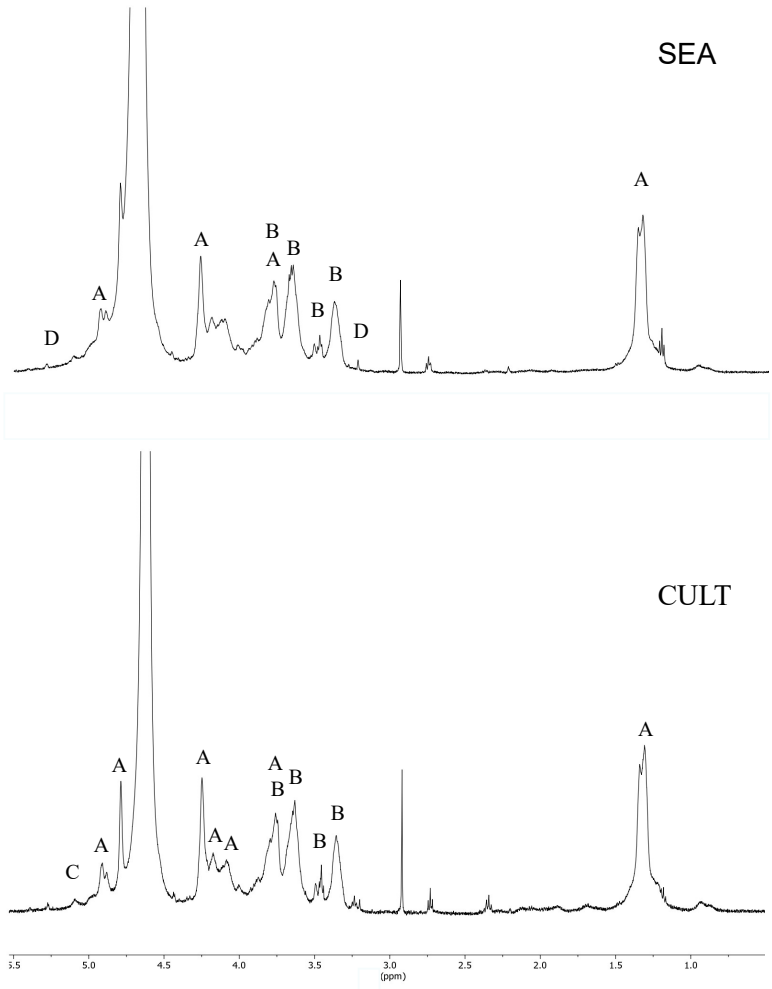

Figura 2.8: ^{1}H NMR of ulvan from *Ulva fasciata* collected in natural environment (SEA) and cultivated in enriched water (CULT). Residue A = rhamnose, B = glucuronic acid, C = iduronic acid and D = xylose

phosphorus (10 mg N g DW^{-1} day^{-1} and 0.53 mg P g DM^{-1} day^{-1}, respectively) followed by a sharp decreased (Figure 11). Nitrogen uptake continued to decay while phosphorus uptake increased in the fourth day, suggesting that nitrogen was the limiting nutrient. When nitrogen pools were filled the phosphate uptake resumed.

The hypothesis of nitrogen being the limiting nutrient is supported by the C/N ratio (14.32) and the tissue N close to minimum content allowing for maximum growth recorded at the onset of the experiment. After individuals' cultivation in enriched water, tissue N increased to a concentration similar to found in literature (da Silva Copertino et al., 2008; Hernández et al., 2002). Nitrogen assimilation promotes the synthesis of metabolic, structural and storage compounds such as pigments, lipids and proteins (Angell et al., 2014).

Chlorophyll-*a* content can give information on the specie light harvesting capacity and response to environmental conditions (da Silva et al., 2015). Individuals cultivated in enriched water presented higher chlorophyll-*a* and darker thalli than

control individuals, indicating high photosynthetic activity (Lin et al., 2011) . This chlorophyll-a content is similar to concentrations found in *Ulva* collected in nutrient rich sites (da Silva et al., 2015; Lin et al., 2011).

In macroalgae from control treatment, tissue P content decreased 40% but no difference in tissue N, C and chlorophyll-*a* was observed (Table 1). This decoupling between tissue phosphorus and other physiological parameters may represent a tradeoff between phosphorus reserves to sustain growth and photosynthetic capacity, as stored polyphosphates can be hydrolyzed to meet requirements of starved macroalgae (Lee et al., 2005; Reidenbach et al., 2017).

Phosphorus is present in lipids, nucleotides and Calvin cycle (Hernández et al., 2002; Lee et al., 2005) and is the limiting nutrient in tropical regions, but only a few in depth studies have examined the phosphorus metabolism in macroalgae (Lee et al., 2005; Lubsch and Timmermans, 2018; Pedersen et al., 2010). Understanding nutrient demand and role in macroalgae metabolism is of central importance to forecast ecological impacts and commercial application (Roleda and Hurd, 2019).

In our study we used nutrients concentrations found at an important Brazilian coastal area under heavy eutrophic conditions. Given the results gathered and the equation constructed, considering a year-round period and a cultivated area of one square kilometer, the nutrient recovery potential would be around 0.17 and 11.35 kilograms km^{-2} $year^{-1}$ of phosphorus and nitrogen, respectively. Furthermore, 347 kilograms km^{-2} $year^{-1}$ of dry biomass could be produced, given around 56 kilograms km^{-2} $year^{-1}$ of extracted ulvan. The production of this polysaccharide from *Ulva* biomass grown during the bioremediation process can amortize costs of this important ecosystem service, representing an economic incentive for macroalgae cultivation by coastal communities.

FTIR and NMR spectroscopy are rapid and non-destructive analysis that provide fundamental information on ulvan polysaccharide structure (Robic et al., 2009a). In this work, such techniques showed that polysaccharides from natural environment (SEA) and cultivated *U. fasciata* (CULT) presented all the characteristics peaks described in literature (Qi et al., 2005; Robic et al., 2009b; Tabarsa et al., 2018), confirming that the extracted polysaccharides are ulvan. According with FTIR spectra, there was no visible difference between the two ulvan extracts and those reported in the literature (Li et al., 2018; Thanh et al., 2016; Yaich et al., 2017).

IR spectra of CULT ulvan presented stronger signals of sulfate groups than SEA between 1159 and 625 cm^{-1} (Figure 4.4). The presence, degree and distribution of the sulfate groups are important in determining the biological activity of ulvan, as previous studies have found that the antioxidant activity and regulation of physiological stress by ulvan is related to it is sulfate content (Leiro et al., 2007; Qi and Sun, 2015). This result suggests that CULT ulvan could have a higher

antioxidant potential.

^{13}C NMR spectra showed signals characteristics of type A (A3s) and type B ulvanobiuronic acid (B3s), confirming that the extracted polysaccharides are mainly composed of repeated sequences of these two disaccharides. The noise observed in these NMR spectra was related to sample dilution increased by the high molecular weight of the polymer and solution viscosity. Ulvan molecular weight can vary from 1.8×10^5 to 2×10^6 depending of extraction methods, specie and polydispersity of the samples (Thanh et al., 2016; Yaich et al., 2017). According to Lahaye and Robic (2007) ulvan extracted with temperatures between 80 – 90 °C, close to the used in this study, tend to present higher molecular weight. Future studies with purification and sugar quantification procedures could help elucidated the fine structure of both ulvan samples.

In ^{13}C and ^{1}H NMR spectra of SEA ulvan (Figure 4.6 and 4.5) we could observe signals of xylose, suggesting the presence of ulvanobiose (U3s) in this ulvan structure. In CULT we could not detect U3s, but peak characteristic to C-1 of rhamnose in A3s disaccharide (4.82 ppm) was present (Lahaye and Robic, 2007). According to Robic et al. (2009a) during the active growth of *Ulva* the macroalgae tends to synthesize more ulvanobiuronic acid type A, with the production of ulvanobiose being developmentally regulated. In this study, *U. fasciata* presented a growth rate of 5.7 % day^{-1} and active nutrient uptake throughout the cultivation experiment, an indication that individuals had not reach their growth plateau when collected.

Cultivation in laboratory system is important to the understanding of macroalgae ecophysiology and demand regarding nutrient availability prior to field scale-up (Zhou et al., 2015). In open-water culture, other variables should be further considered for a more accurate estimative of the macroalga production such as cultivation cycle, light intensity, temperature variation and herbivory (Hiraoka and Oka, 2008; Wei et al., 2017). *U. fasciata* is a fast growing macroalga with worldwide distribution, being potential specie for biotechnological purposes (Chen and Zou, 2015; Wang et al., 2012). Therefore future studies assessing the cultivation *U. fasciata* in larger scales with these nutrient conditions and characterization of the extracted ulvan is needed to better understand and evaluate its full potential and challenges.

In summary, this study showed that *U. fasciata* efficiently removed the dissolved nitrogen and phosphorus from the enriched water, increasing the content of tissue nutrient, pigment and protein. The ulvan extracted from the cultivated biomass is similar to those reported in the literature, suggesting the feasibility of obtaining this polysaccharide from cultivated biomass.

Referências Bibliográficas

Adrien, A., Bonnet, A., Dufour, D., Baudouin, S., Maugard, T., and Bridiau, N. (2017). Pilot production of ulvans from *Ulva sp.* and their effects on hyaluronan and collagen production in cultured dermal fibroblasts. *Carbohydrate Polymers*, 157:1306–1314.

Akila, V., Manikandan, A., Sukeetha, D. S., Balakrishnan, S., Ayyasamy, P. M., and Rajakumar, S. (2019). Biogas and biofertilizer production of marine macroalgae: An effective anaerobic digestion of *Ulva* sp. *Biocatalysis and Agricultural Biotechnology*, 18:101035.

Alves, A., Caridade, S. G., Mano, J. F., Sousa, R. A., and Reis, R. L. (2010). Extraction and physico-chemical characterization of a versatile biodegradable polysaccharide obtained from green algae. *Carbohydrate Research*, 345(15):2194–2200.

Alves, A., Sousa, R. A., and Reis, R. L. (2012). *In Vitro* cytotoxicity assessment of ulvan, a polysaccharide extracted from green algae. *Phytotherapy Research*, 27(8):1143–1148.

Angell, A. R., Mata, L., de Nys, R., and Paul, N. A. (2014). Variation in amino acid content and its relationship to nitrogen content and growth rate in *Ulva ohnoi* (Chlorophyta). *Journal of Phycology*, 50(1):216–226.

Baptista-Neto, J., Gingele, F., and Leipe, T. (2013). *Biostatistical analysis*. Environmental Pollution, Nova Jersey.

Bikker, P., van Krimpen, M. M., van Wikselaar, P., Houweling-Tan, B., Scaccia, N., van Hal, J. W., Huijgen, W. J. J., Cone, J. W., and López-Contreras, A. M. (2016). Biorefinery of the green seaweed *Ulva lactuca* to produce animal feed, chemicals and biofuels. *Journal of Applied Phycology*, 28(6):3511–3525.

Bindschädler, P., Adibekian, A., Grünstein, D., and Seeberger, P. H. (2010). De novo synthesis of differentially protected l-iduronic acid glycosylating agents. *Carbohydrate Research*, 345(7):948–955.

Brito, L. O., Arantes, R., Magnotti, C., Derner, R., Pchara, F., Olivera, A., and Vinatea, L. (2013). Water quality and growth of pacific white shrimp *Litopenaeus vannamei* (Boone) in co-culture with green seaweed *Ulva lactuca* (linaeus) in intensive system. *Aquaculture International*, 22(2):497–508.

Chen, B. and Zou, D. (2015). Altered seawater salinity levels affected growth and photosynthesis of *Ulva fasciata* (Ulvales, Chlorophyta) germlings. *Acta Oceanologica Sinica*, 34(8):108–113.

Costa, C., Alves, A., Pinto, P. R., Sousa, R. A., da Silva, E. A. B., Reis, R. L., and Rodrigues, A. E. (2012). Characterization of ulvan extracts to assess the effect of different steps in the extraction procedure. *Carbohydrate Polymers*, 88(2):537–546.

da Silva, M. L., de Araújo, F. V., Castro, R. O., and Sales, A. S. (2015). Spatial–temporal analysis of marine debris on beaches of niterói, RJ, brazil: Itaipu and itacoatiara. *Marine Pollution Bulletin*, 92(1-2):233–236.

da Silva Copertino, M., Tormena, T., and Seeliger, U. (2008). Biofiltering efficiency, uptake and assimilation rates of *Ulva clathrata* (Roth) J. Agardh (Clorophyceae) cultivated in shrimp aquaculture waste water. *Journal of Applied Phycology*, 21(1):31–45.

de Oliveira, V. P., Martins, N. T., de Souza Guedes, P., Pollery, R. C. G., and Enrich-Prast, A. (2016). Bioremediation of nitrogenous compounds from oilfield wastewater by *Ulva* lactuca (Chlorophyta). *Bioremediation Journal*, 20(1):1–9.

del Rocío Quezada-Rodríguez, P. and Fajer-Ávila, E. J. (2016). The dietary effect of ulvan from *Ulva clathrata* on hematological-immunological parameters and growth of tilapia (*Oreochromis niloticus*). *Journal of Applied Phycology*, 29(1):423–431.

FAO (2018). *The state of world fisheries and aquaculture 2018 : meeting the sustainable development goals*. Food and Agriculture Organization of the United Nations, Rome.

Grasshoff, K. and Johannsen, H. (1972). A new sensitive and direct method for the automatic determination of ammonia in sea water. *ICES Journal of Marine Science*, 34(3):516–521.

Gupta, V., Trivedi, N., Simoni, S., and Reddy, C. (2018). Marine macroalgal nursery: A model for sustainable production of seedlings for large scale farming. *Algal Research*, 31:463–468.

Hallak, L. K., Collins, P. L., Knudson, W., and Peeples, M. E. (2000). Iduronic acid-containing glycosaminoglycans on target cells are required for efficient respiratory syncytial virus infection. *Virology*, 271(2):264–275.

Hardouin, K., Bedoux, G., Burlot, A.-S., Donnay-Moreno, C., Bergé, J.-P., Nyvall-Collén, P., and Bourgougnon, N. (2016). Enzyme-assisted extraction (eae) for the production of antiviral and antioxidant extracts from the green seaweed ulva armoricana (ulvales, ulvophyceae). *Algal Research*, 16:233–239.

Hernández, I., Martínez-Aragón, J., Tovar, A., Pérez-Lloréns, J., and Vergara, J. (2002). Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 2. ammonium. *Journal of Applied Phycology*, 14(5):375–384.

Hiraoka, M. and Oka, N. (2008). Tank cultivation of ulva prolifera in deep seawater using a new "germling cluster" method. *Journal of applied phycology*, 20(1):97–102.

Juneja, A., Ceballos, R., and Murthy, G. (2013). Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A review. *Energies*, 6(9):4607–4638.

Koroleff, F. (1970). Direct determination of ammonia in natural waters as indophenol blue. *Information on techniques and methods for seawater analysis*, pages 19–22.

Lahaye, M. (1998). NMR spectroscopic characterisation of oligosaccharides from two *Ulva rigida* ulvan samples (Ulvales, Chlorophyta) degraded by a lyase. *Carbohydrate Research*, 314(1-2):1–12.

Lahaye, M., Cimadevilla, E. A.-C., Kuhlenkamp, R., Quemener, B., Lognoné, V., and Dion, P. (1999). Chemical composition and 13C NMR spectroscopic characterisation of ulvans from *Ulva* (Ulvales, Chlorophyta). In *Sixteenth International Seaweed Symposium*, pages 515–521. Springer Netherlands.

Lahaye, M. and Ray, B. (1996). Cell-wall polysaccharides from the marine green alga *Ulva* rigida (Ulvales, Chlorophyta) — NMR analysis of ulvan oligosaccharides. *Carbohydrate Research*, 283:161–173.

Lahaye, M. and Robic, A. (2007). Structure and functional properties of ulvan, a polysaccharide from green seaweeds. *Biomacromolecules*, 8(6):1765–1774.

Lavania-Baloo, Azman, S., Said, M. I. M., Ahmad, F., and Mohamad, M. (2014). Biofiltration potential of macroalgae for ammonium removal in outdoor tank shrimp wastewater recirculation system. *Biomass and Bioenergy*, 66:103–109.

Lee, T.-M., Tsai, P.-F., Shyu, Y.-T., and Sheu, F. (2005). The effects of phosphite on phosphate starvation responses of ulva lactuca (ulvales, chlorophyta) 1. *Journal of Phycology*, 41(5):975–982.

Leiro, J. M., Castro, R., Arranz, J. A., and Lamas, J. (2007). Immunomodulating activities of acidic sulphated polysaccharides obtained from the seaweed *Ulva rigida* C. Agardh. *International Immunopharmacology*, 7(7):879–888.

Li, H., Zhang, Y., Han, X., Shi, X., Rivkin, R. B., and Legendre, L. (2016). Growth responses of *Ulva prolifera* to inorganic and organic nutrients: Implications for macroalgal blooms in the southern Yellow Sea, China. *Scientific Reports*, 6(1).

Li, W., Jiang, N., Li, B., Wan, M., Chang, X., Liu, H., Zhang, L., Yin, S., Qi, H., and Liu, S. (2018). Antioxidant activity of purified ulvan in hyperlipidemic mice. *International Journal of Biological Macromolecules*, 113:971–975.

Lin, A., Shen, S., Wang, G., Yi, Q., Qiao, H., Niu, J., and Pan, G. (2011). Comparison of chlorophyll and photosynthesis parameters of floating and attached ulva prolifera. *Journal of integrative plant biology*, 53(1):25–34.

Lopes, N., Ray, S., Espada, S. F., Bomfim, W. A., Ray, B., Faccin-Galhardi, L. C., Linhares, R. E. C., and Nozawa, C. (2017). Green seaweed *Enteromorpha compressa* (Chlorophyta, Ulvaceae) derived sulphated polysaccharides inhibit herpes simplex virus. *International Journal of Biological Macromolecules*, 102:605–612.

LORENZEN, C. J. (1967). Determination of chlorophyll and pheo-pigments: Spectrophotometric equations. *Limnology and Oceanography*, 12(2):343–346.

Lubsch, A. and Timmermans, K. (2018). Uptake kinetics and storage capacity of dissolved inorganic phosphorus and corresponding N:P dynamics in *Ulva lactuca* (Chlorophyta). *Journal of Phycology*, 54(2):215–223.

Lubsch, A. and Timmermans, K. R. (2019). Uptake kinetics and storage capacity of dissolved inorganic phosphorus and corresponding dissolved inorganic nitrate uptake in *Saccharina latissima* and *Laminaria digitata* (Phaeophyceae). *Journal of Phycology*, 55(3):637–650.

Magnusson, M., Carl, C., Mata, L., de Nys, R., and Paul, N. A. (2016). Seaweed salt from *Ulva*: A novel first step in a cascading biorefinery model. *Algal Research*, 16:308–316.

Margareta, W., Nagarajan, D., Chang, J.-S., and Lee, D.-J. (2020). Dark fermentative hydrogen production using macroalgae (*Ulva sp.*) as the renewable feedstock. *Applied Energy*, 262:114574.

Martínez, B., Pato, L. S., and Rico, J. M. (2012). Nutrient uptake and growth responses of three intertidal macroalgae with perennial, opportunistic and summer-annual strategies. *Aquatic Botany*, 96(1):14–22.

Mata, L., Magnusson, M., Paul, N. A., and de Nys, R. (2015). The intensive land-based production of the green seaweeds *Derbesia tenuissima* and *Ulva ohnoi: biomass and bioproducts*. *Journal of Applied Phycology*, 28(1):365–375.

Murphy, J. and Riley, J. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27:31–36.

Nardelli, A. E., Chiozzini, V. G., Braga, E. S., and Chow, F. (2018). Integrated multi-trophic farming system between the green seaweed *Ulva lactuca*, mussel, and fish: a production and bioremediation solution. *Journal of Applied Phycology*, 31(2):847–856.

Nascimento, A., Coelho-Gomes, C., Barbarino, E., and Lourenço, S. O. (2014). Temporal variations of the chemical composition of three seaweeds in two tropical coastal environments. *Open Journal of Marine Science*, 04(02):118–139.

Neori, A., Ragg, N. L., and Shpigel, M. (1998). The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: II. performance and nitrogen partitioning within an abalone (*Haliotis tuberculata*) and macroalgae culture system. *Aquacultural Engineering*, 17(4):215–239.

Pedersen, M. and Borum, J. (1997). Nutrient control of estuarine macroalgae:growth strategy and the balance between nitrogen requirements and uptake. *Marine Ecology Progress Series*, 161:155–163.

Pedersen, M., Borum, J., and Fotel, F. L. (2010). Phosphorus dynamics and limitation of fast- and slow-growing temperate seaweeds in Oslofjord, Norway. *Marine Ecology Progress Series*, 399:103–115.

Qi, H. and Sun, Y. (2015). Antioxidant activity of high sulfate content derivative of ulvan in hyperlipidemic rats. *International Journal of Biological Macromolecules*, 76:326–329.

Qi, H., Zhao, T., Zhang, Q., Li, Z., Zhao, Z., and Xing, R. (2005). Antioxidant activity of different molecular weight sulfated polysaccharides from *Ulva pertusa* Kjellm (Chlorophyta). *Journal of Applied Phycology*, 17(6):527–534.

Reidenbach, L. B., Fernandez, P. A., Leal, P. P., Noisette, F., McGraw, C. M., Revill, A. T., Hurd, C. L., and Kübler, J. E. (2017). Growth, ammonium metabolism, and photosynthetic properties of *Ulva australis* (Chlorophyta) under decreasing pH and ammonium enrichment. *PLOS ONE*, 12(11):e0188389.

Reis, R. P., de Carvalho Junior, A. A., Facchinei, A. P., dos Santos Calheiros, A. C., and Castelar, B. (2018). Direct effects of ulvan and a flour produced from the green alga ulva fasciata delile on the fungus stemphylium solani weber. *Algal Research*, 30:23–27.

Robertson-Andersson, D. V., Potgieter, M., Hansen, J., Bolton, J. J., Troell, M., Anderson, R. J., Halling, C., and Probyn, T. (2008). Integrated seaweed cultivation on an abalone farm in South Africa. *Journal of Applied Phycology*, 20(5):579–595.

Robic, A., Rondeau-Mouro, C., Sassi, J.-F., Lerat, Y., and Lahaye, M. (2009a). Structure and interactions of ulvan in the cell wall of the marine green algae *Ulva rotundata* (Ulvales, Chlorophyceae). *Carbohydrate Polymers*, 77(2):206–216.

Robic, A., Sassi, J.-F., Dion, P., Lerat, Y., and Lahaye, M. (2009b). Seasonal variability of physicochemical and rheological properties of ulvan in two ulva species (chlorophyta) from the brittany coast 1. *Journal of Phycology*, 45(4):962–973.

Roleda, M. Y. and Hurd, C. L. (2019). Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. *Phycologia*, 58(5):552–562.

Schmidt, E. C., Nunes, B. G., Maraschin, M., and Bouzon, Z. L. (2010). Effect of ultraviolet-b radiation on growth, photosynthetic pigments, and cell biology of kappaphycus alvarezii (rhodophyta, gigartinales) macroalgae brown strain. *Photosynthetica*, 48(2):161–172.

Seghetta, M., Hou, X., Bastianoni, S., Bjerre, A.-B., and Thomsen, M. (2016). Life cycle assessment of macroalgal biorefinery for the production of ethanol, proteins and fertilizers – a step towards a regenerative bioeconomy. *Journal of Cleaner Production*, 137:1158–1169.

Shao, P., Qin, M., Han, L., and Sun, P. (2014). Rheology and characteristics of sulfated polysaccharides from chlorophytan seaweeds *Ulva fasciata*. *Carbohydrate Polymers*, 113:365–372.

Shpigel, M., Guttman, L., Ben-Ezra, D., Yu, J., and Chen, S. (2019). Is ulva sp. able to be an efficient biofilter for mariculture effluents? *Journal of Applied Phycology*, 31(4):2449–2459.

Sode, S., Bruhn, A., Balsby, T. J., Larsen, M. M., Gotfredsen, A., and Rasmussen, M. B. (2013). Bioremediation of reject water from anaerobically digested waste water sludge with macroalgae (*Ulva lactuca* , Chlorophyta). *Bioresource Technology*, 146:426–435.

Tabarsa, M., You, S., Dabaghian, E. H., and Surayot, U. (2018). Water-soluble polysaccharides from *Ulva intestinalis*: Molecular properties, structural elucidation and immunomodulatory activities. *Journal of Food and Drug Analysis*, 26(2):599–608.

Tako, M., Tamanaha, M., Tamashiro, Y., and Uechi, S. (2015). Structure of ulvan isolated from the edible green seaweed, *Ulva pertusa*. *Advances in Bioscience and Biotechnology*, 06(10):645–655.

Thanh, T. T. T., Quach, T. M. T., Nguyen, T. N., Luong, D. V., Bui, M. L., and Tran, T. T. V. (2016). Structure and cytotoxic activity of ulvan extracted from green seaweed *Ulva lactuca*. *International Journal of Biological Macromolecules*, 93:695–702.

Torres, M. D., Kraan, S., and Domínguez, H. (2019). Seaweed biorefinery. *Reviews in Environmental Science and BioTechnology*, 18(2):335–388.

Tremblay-Gratton, A., Boussin, J.-C., Tamigneaux, É., Vandenberg, G. W., and François, N. R. L. (2017). Bioremediation efficiency of *Palmaria palmata* and *Ulva lactuca* for use in a fully recirculated cold-seawater naturalistic exhibit: effect of high NO3 and PO4 concentrations and temperature on growth and nutrient uptake. *Journal of Applied Phycology*, 30(2):1295–1304.

Tziveleka, L.-A., Ioannou, E., and Roussis, V. (2019). Ulvan, a bioactive marine sulphated polysaccharide as a key constituent of hybrid biomaterials: A review. *Carbohydrate Polymers*, 218:355–370.

Ursi, S. and Plastino, E. M. (2001). Crescimento *in vitro* de linhagens de coloração vermelha e verde clara de *Gracilaria birdiae* (Gracilariales, Rhodophyta) em dois meios de cultura: análise de diferentes estádios reprodutivos. *Revista Brasileira de Botânica*, 24(4).

Valentin, J. L., Paiva, P., and Salomon, P. (2018). *A Baía de Guanabara: passado, presente e futuro de um ecossistema ameaçado*. Editora CRV.

Wang, Y., Wang, Y., Zhu, L., Zhou, B., and Tang, X. (2012). Comparative studies on the ecophysiological differences of two green tide macroalgae under controlled laboratory conditions. *PLoS ONE*, 7(8):e38245.

Wei, Z., You, J., Wu, H., Yang, F., Long, L., Liu, Q., Huo, Y., and He, P. (2017). Bioremediation using gracilaria lemaneiformis to manage the nitrogen and phosphorous balance in an integrated multi-trophic aquaculture system in yantian bay, china. *Marine Pollution Bulletin*, 121(1-2):313–319.

Xiao, X., Agusti, S., Lin, F., Li, K., Pan, Y., Yu, Y., Zheng, Y., Wu, J., and Duarte, C. M. (2017). Nutrient removal from chinese coastal waters by large-scale seaweed aquaculture. *Scientific Reports*, 7(1).

Yaich, H., Amira, A. B., Abbes, F., Bouaziz, M., Besbes, S., Richel, A., Blecker, C., Attia, H., and Garna, H. (2017). Effect of extraction procedures on structural, thermal and antioxidant properties of ulvan from *Ulva lactuca* collected in monastir coast. *International Journal of Biological Macromolecules*, 105:1430–1439.

Zhou, Y., Tan, L., Pang, Q., Li, F., and Wang, J. (2015). Influence of nutrients pollution on the growth and organic matter output of *Ulva prolifera* in the southern Yellow Sea, China. *Marine Pollution Bulletin*, 95(1):107–114.

Capítulo 3

Conclusão geral

Nesse estudo nós mostramos a importância que cada etapa da geração de biomassa de *Ulva* tem no produto final. A hipótese "*Indivíduos de uma mesma espécie originários de diferentes regiões de coleta respondem de forma distinta as diferentes concentrações de fosfato*" foi **aceita**. Baseado nos resultados apresentados no Capítulo 1 (fósforo tecidual, eficiência de absorção de fósforo, teor de carboidrato) pode-se observar que a origem dos indivíduos de *Ulva fasciata* influencia nas respostas fisiológicas e produção de carboidratos quando cultivados em diferentes concentrações de fosfato. Indivíduos oriundos da região afetada pelo fenômeno da ressurgência (Arraial do Cabo) apresentaram maior produção de carboidratos (aproximadamente 71% de massa seca) e, portanto, foram selecionados para as etapas seguintes. Esses resultados indicam a existência de um mecanismo de adaptação para sobrevivência a oscilações nas condições ambientais e períodos de condições subótimas.

A hipótese "*A Ulva fasciata é um eficiente agente na remoção de nitrogênio e fósforo dissolvidos na água*" foi **aceita**, com eficiência de remoção de 99% para amônio, 100% para nitrato e 22% para fosfato pelas macroalgas cultivadas em meio enriquecido (Capítulo 2). Espécies de *Ulva* são r-estrategistas prosperando em condições ambientais potencialmente tóxicas para outras macroalgas. O conhecimento da demanda e papel dos nutrientes no metabolismo algáceo é fundamental, com a absorção de nitrogênio promovendo a síntese de compostos metabólicos e pigmentos, como observado pelo aumento de 244% do teor de clorofila-*a* nos indivíduos cultivados em meio enriquecido. Nos indivíduos cultivados em meio não-enriquecido a taxa de crescimento foi metade da registrada para indivíduos cultivados em meio enriquecido e análise tecidual (nitrogênio, fósforo, carbono e clorofila-*a*) mostrando queda apenas no teor de fósforo no tecidual (40%), o que indica que a *Ulva fasciata* recorre às reservas de fósforo como mecanismo de compensação quando em condições subótimas de cultivo.

Por fim, a hipótese "*cultivo em meio enriquecido não altera a estrutura do*

polissacarídeo ulvana" foi aceita. A ulvana extraída da biomassa oriunda do cultivo é similar à reportada na literatura e à ulvana extraída de biomassa do ambiente natural. O Espectro de FT-IR da ulvana extraída da biomassa cultivada apresentou sinais de sulfato mais fortes do que a ulvana extraída de *Ulva fasciata* do ambiente natural, sugerindo que este polissacarídeo pode apresentar maior potencial antioxidante.

Em um momento onde a conscientização dos consumidores em relação às matrizes produtivas e os impactos que o consumo tem no meio ambiente crescem, indústrias se vêem forçadas a buscar produtos e matrizes mais sustentáveis. A *Ulva* é uma macroalga oportunista, capaz de produzir grande quantidade de biomassa prestando um importante serviço ambiental e com potencial biotecnológico nos mais diversos setores produtivos. Os resultados apresentados neste estudo ampliam o conhecimento da ecofisiologia, produção e potencial tecnológico da macroalga *Ulva fasciata*, contribuindo para transposição da fronteira entre a pesquisa e indústria. Estudos futuros avaliando o cultivo *U. fasciata* em maior escala com essas condições nutricionais e a caracterização da ulvana extraída são necessários para melhor compreendermos e avaliar todo o seu potencial e desafios.

Apêndice A

Artigo publicado: Structural Characterization of ulvan polysaccharide from cultivated and collected *Ulva fasciata* (Chlorofita)

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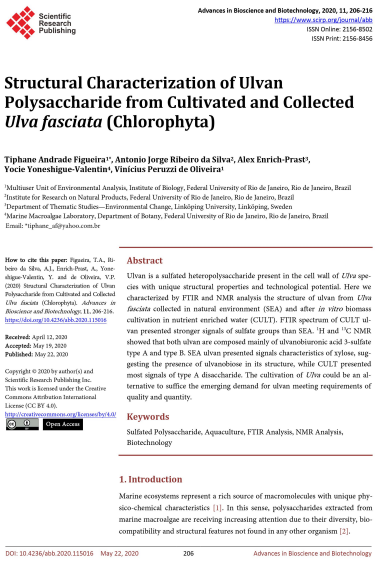

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Structural Characterization of Ulvan Polysaccharide from Cultivated and Collected *Ulva fasciata* (Chlorophyta)

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Abstract

Ulvan is a sulfated heteropolysaccharide present in the cell wall of *Ulva* species with unique structural properties and technological potential. Here we characterized by FTIR and NMR analysis the structure of ulvan from *Ulva fasciata* collected in natural environment (SEA) and after *in vitro* biomass cultivation in nutrient enriched water (CULT). FTIR spectrum of CULT ulvan presented stronger signals of sulfate groups than SEA. ^{1}H and ^{13}C NMR showed that both ulvan are composed mainly of ulvanobiuronic acid 3-sulfate type A and type B. SEA ulvan presented signals characteristics of xylose, suggesting the presence of ulvanobiose in its structure, while CULT presented most signals of type A disaccharide. The cultivation of *Ulva* could be an alternative to suffice the emerging demand for ulvan meeting requirements of quality and quantity.

Keywords

Sulfated Polysaccharide, Aquaculture, FTIR Analysis, NMR Analysis, Biotechnology

1. Introduction

Marine ecosystems represent a rich source of macromolecules with unique physico-chemical characteristics [1]. In this sense, polysaccharides extracted from marine macroalgae are receiving increasing attention due to their diversity, biocompatibility and structural features not found in any other organism [2].

Abstract

Ulvan is a sulfated heteropolysaccharide present in the cell wall of *Ulva* species with unique structural properties and technological potential. Here we characterized by FTIR and NMR analysis the structure of ulvan from *Ulva fasciata* collected in natural environment (SEA) and after *in vitro* biomass cultivation in nutrient enriched water (CULT). FTIR spectrum of CULT ulvan presented stronger signals of sulfate groups than SEA. ^{1}H and ^{13}C NMR showed that both ulvan are composed mainly of ulvanobiuronic acid 3-sulfate type A and type B. SEA ulvan presented signals characteristics of xylose, suggesting the presence of ulvanobiose in its structure, while CULT presented most signals of type A disaccharide. The cultivation of *Ulva* could be an alternative to suffice the emerging demand for ulvan meeting requirements of quality and quantity.

Keywords:Sulfated polysaccharide; aquaculture; FTIR analysis; NMR analysis; biotechnology

Introduction

Marine ecosystems represent a rich source of macromolecules with unique physico-chemical characteristics (Wang et al., 2015). In this sense, polysaccharides extracted from marine macroalgae are receiving increasing attention due to their diversity, biocompatibility and structural features not found in any other organism (Cunha and Grenha, 2016).

Species of the genus *Ulva* are the most abundant and cosmopolitan macroalgae in the Chlorophyta Division being able to adapt across diverse geo-climatic conditions

with high productivity and opportunistic growth. *Ulva* cultivation is increasing worldwide due to their potential as functional foods, feed and biofuel (Holdt and Kraan, 2011; Wells et al., 2016). Although polysaccharides from the red (carrageenan and agar) and brown (alginate) macroalgae have been used in the food industry, polysaccharides from green macroalgae remains largely unexploited. The main polysaccharide of *Ulva* species is ulvan, corresponding to 29% of dry weight with a promising technological application.

Ulvan is homogeneously distributed within the intercellular space and in the fibrillar wall (Chiellini and Morelli, 2011; Lahaye and Robic, 2007) being responsible for maintaining the osmolar stability and protecting the thallus from marine bacterial attack (Alves et al., 2012a; Cunha and Grenha, 2016). Ulvan is composed of variable amounts of rhamnose, glucuronic acid, iduronic acid, xylose and sulfate (Kidgell et al., 2019; Tziveleka et al., 2019). This sulfated heteropolysaccharide is built on sequences of two major repeating disaccharides unities designated as ulvanobiuronic acid 3-sulfate type A (→4)-β-D-GlcA-(1→4)-α-L-Rha 3S-(1→ and type B →4)-α-L-IdoA-(1→4)-α-L-Rha 3s(1→. Minor sulfated residues with xylose (O-2 sulfated or not) denominated ulvanobiose (U3s) [(→4)-β-D-GlcA-(1-2)-α-D-Xyl-(1→] can also occur in place of uronic acids (Costa et al., 2012).

With the growing interest in novel and renewable polymers, ulvan has drawing attention with studies showing its potential as antioxidant (Li et al., 2018; Qi et al., 2005), antiviral (Hardouin et al., 2016; Lopes et al., 2017), anticancer (Shao et al., 2014; Thanh et al., 2016) among others (Adrien et al., 2017; Araújo et al., 2014; del Rocío Quezada-Rodríguez and Fajer-Ávila, 2016). The presence of the remarkable rare sugars, rhamnose and iduronic acid, similar to mammalian glycosaminoglycans (Holdt and Kraan, 2011; Kidgell et al., 2019) single out ulvan from other algal polysaccharides. L-rhamnose used in a variety of anti-aging cosmetics (Adrien et al., 2017; Pageon et al., 2019) is specifically recognized by a number of mammalians lectins (Lahaye and Robic, 2007). Iduronic acid, which has never been identified in algal polysaccharides (Alban et al., 2002; Chiellini and Morelli, 2011) is required in the synthesis of heparin analogs being used against respiratory syncytial virus infection and antithrombotic activities (Bindschädler et al., 2010; Hallak et al., 2000). Currently, this substance is obtained through several steps that could be avoided using ulvan (Bindschädler et al., 2010).

Although the biotechnological applications of ulvan are promising, structural variations may occur due to ecophysiological factors acting on *Ulva* (Robic et al., 2009a,b). The commercial use of polysaccharides requires ulvan with predictive structure and functional properties, that could be obtained by the controlled cultivation of *Ulva*. To determine the potential of cultivated *Ulva* for ulvan

production in this work we characterized (FTIR and NMR) the ulvan extracts from *Ulva fasciata* Delile (Chlorophyta) after in vitro biomass cultivation in nutrient enriched water and compared it against ulvan from biomass collected in an oligotrophic natural environment, to enhance potential structural differences.

Material and methods

Algal material

Healthy thalli of *Ulva fasciata* were collected in the intertidal zone at Prainha Beach, Arraial do Cabo / RJ Brazil (22°57'40"S / 42°01'13"W) rinsed with local seawater and transported to laboratory inside coolers. Individuals were cleaned with distilled water for further removal of sediment and macroscopic epibionts. Voucher specimens were deposited in the Institute of Bioscience Herbarium, at the University of São Paulo, Brazil (SPF-57877). Part of the fresh biomass (SEA) was oven dried at 50°C until constant weight and stored in desiccator until ulvan extraction. The remaining material was used in the cultivation experiment (CULT). In sequence biomass from natural environment and cultivation experiment (SEA and CULT, respectively) were used for ulvan extraction (Figure A.1).

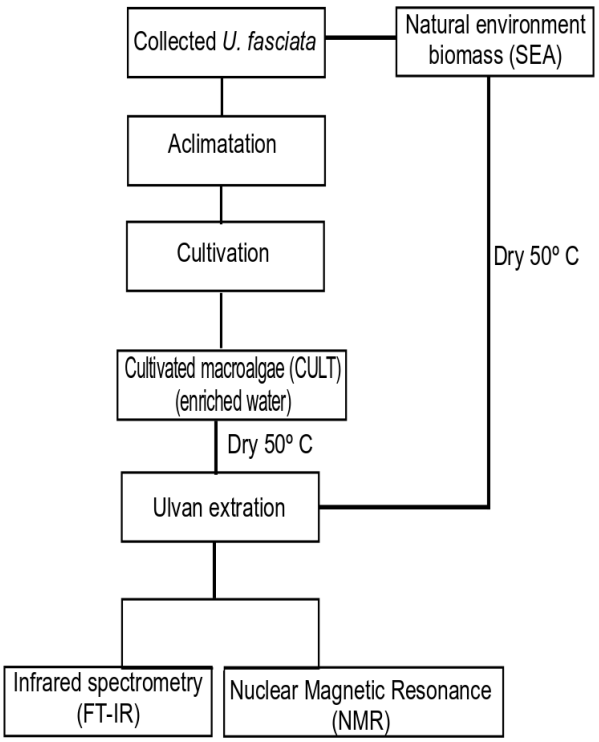

Figura A.1: Schematic representation of steps to ulvan extraction and characterization.

Ulva cultivation

To ensure that all individuals presented comparable initial physiological conditions, *U. fasciata* thalli underwent a seven-day acclimatization period to the laboratory conditions followed by a three-day starvation period prior to the cultivation. For acclimatization, individuals were kept in sterilized seawater enriched with von Stosch culture medium (Ursi and Plastino, 2001) in a temperature-controlled room at 24.0 ± 1.0 °C, 70 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) and 12 hours light photoperiod. For starvation, individuals weighing approximately 3.5 g were placed in 2.8 L Erlenmeyer flasks filled with sterilized seawater (0.5 μM NH_4, 0.03 μM NO_2, 0.41 μM NO_3 and 0.09 μM PO_4^{3-}).

For the cultivation, starved individuals weighting 3.0 ± 0.11 g were cultivated in 3 liters Erlenmeyer flasks (n = 4) filled with seawater enriched with 200 μM of ammonium (NH_4Cl), 8 μM of nitrate ($NaNO_3$), 12μ of phosphate ($Na_2.HPO_4.12H_2O$), salts and vitamins (Ursi and Plastino, 2001). The nutrient concentrations were chosen with reference to the mean maximum nutrient concentration after a five years monitoring of one of the points of an important Brazilian bay (Valentin et al., 2018) with potential for *Ulva* cultivation. The same light, photoperiod and temperature conditions of the acclimatization and starvation period were maintained. The experiment lasted five days.

Ulvan extraction

After the cultivation, individuals were washed with distilled water to remove salts and oven dried at 50°C until constant weight. The polysaccharide was extracted according to method described by Reis et al. (2018). Dried algal biomass (SEA and CULT) were grinded into a powder, suspended in ultrapure water (Milli-q®) (100 ml/10 g) and autoclaved at 120°C for 40 min. The supernatant was centrifuged at 10000 g and 4°C for 10 min (Eppendorf centrifuge 5810 r). Ulvan was precipitated with three volumes of ultrapure ethanol (Merck®), cooled at – 20°C for 48 hours and further centrifuged at 3500 g for 5 min. The recovered pellet (ulvan) was freeze-dried. Ulvan extraction yield calculated using formula proposed by Yaich et al. (2017) was 16.29 ± 0.93%.

Fourier-transform Infrared Spectroscopy (FT-IR) Analysis

Ulvan (SEA and CULT) infrared spectra with Fourier transform (FT-IR) were recorded on a spectrophotometer (IR Prestige_21, Shimadzu) at room temperature. The FT-IR spectra were obtained in the transmission mode at 400-4000 cm^{-1}. The transmission spectra were recorded using KBr (Merck®) pellets containing 2.5 mg

of ulvan powder.

Nuclear Magnetic Resonance Spectroscopy

NMR analyses were performed using a Varian VNMRSYS 500 MHz spectrometer (Varian Inc., Palo Alto, CA, USA) at 37°C. Proton and carbon operating frequencies were 499.77 and 125.68 MHz, respectively. ^{1}H NMR spectra were recorded with a 90 degree observe pulse width (pw = 90 μs), a 2.04 s acquisition time and a 1 s relaxation delay. A total of 32 scans were performed for each sample. For ^{13}C NMR, a 90 degree pulse was used (pw = 90 μs), 1.04 s acquisition time, 2 s relaxation delay and a total of 114.624 scans were collected. Ulvan samples (2% w/v) were dissolved in D2O 99.99% (Sigma-Aldrich®). ^{1}H and ^{13}C NMR chemical shifts were expressed in parts per million (ppm).

Results and discussion

FTIR and NMR spectroscopy are rapid and non-destructive analysis that provide fundamental information on ulvan polysaccharide structure (Robic et al., 2009a). In this work, such techniques showed that polysaccharides from natural environment (SEA) and cultivated *U. fasciata* (CULT) are mainly constituted of rhamnose, iduronic and glucuronic acid, sulfate and, in the case of SEA, xylose.

IR Spectra of SEA and CULT ulvan are presented in Figure A.2 with the signals assignment provided by comparison with published data (Alves et al., 2010; Qi et al., 2005; Tako et al., 2015; Yaich et al., 2017). CULT and SEA spectra presented all the characteristics peaks described in literature (Qi et al., 2005; Robic et al., 2008; Tabarsa et al., 2018), confirming that the extracted polysaccharides are ulvan. According with FTIR spectra, there was no visible difference between the two ulvan extracts and those reported in the literature (Li et al., 2018; Tako et al., 2015; Thanh et al., 2016).

The absorption band at around 3300 cm^{-1} was attributed to a stretching of hydroxyl groups (O - H). Signal observed at approximately 2937 cm^{-1} is due to C - H stretching vibration and is characteristic of polysaccharides (Li et al., 2018). Bands of carboxylate groups of uronic acid with similar intensities are present in both spectra at around 1651 and 1435 cm^{-1}. The absorptions between 1147 and 848 cm^{-1} are known as the fingerprint region for ulvan, being the most important absorptions. At 983 cm^{-1} signal is characteristic of the vibration of glycosidic bonds and at 848 cm^{-1} corresponds to the bending vibration of C – O - S of sulfate in axial position. Peaks bellow 900 cm^{-1} in both ulvan samples are characteristic of the presence of sulfate.

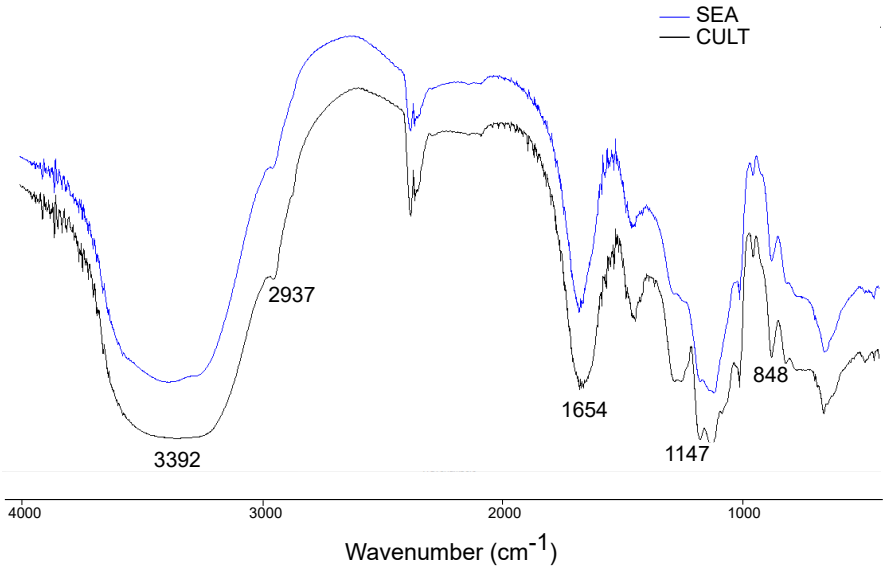

Figura A.2: Infrared spectra of ulvan from *Ulva fasciata* from natural environment (SEA) and cultivated in enriched water (CULT) between 4000 and 400 cm^{-1}.

The presence, degree and distribution of the sulfate groups are important in determining the biological activity of ulvan (Kidgell et al., 2019). CULT ulvan presented stronger signals of sulfate groups than SEA between 1159 and 625 cm^{-1}. Previous studies have found that the antioxidant activity and regulation of physiological stress by ulvan is related to it is sulfate content (Leiro et al., 2007; Qi and Sun, 2015). This result suggests that CULT ulvan could have a higher antioxidant potential.

The ^{13}C NMR spectra are shown in Figure A.3, with typical signals of ulvan structure attributed by comparison with published data (Costa et al., 2012; Lahaye, 1998; Lahaye et al., 1999; Lahaye and Ray, 1996). Carbon of rhamnose and glucuronic acid that constitutes the type A ulvanobiuronic acid (A3s) and the chemical shifts attributed to rhamnose 3-sulfate linked to iduronic acid in type B ulvanobiuronic acid (B3s) were identified, confirming that the extracted polysaccharides are mainly composed of repeated sequences of these two disaccharides. Signals in the resonance region corresponding to carbon rings (70.06 – 80.28 ppm and 69.60 – 79.88 ppm in CULT and SEA, respectively), C-6 methyl group of rhamnose at around 19 ppm and carboxyl signal of uronic acid at approximately 177 ppm are present. Further assigned signals of anomeric carbons (99.28 - 104.62 ppm) were observed in SEA.

^{1}H NMR spectra are presented in Figure A.4 and the signals associated to each hydrogen atom are assigned according with reference data (Alves et al., 2012b; Lahaye, 1998; Thanh et al., 2016; Yaich et al., 2017). Anomeric protons of rhamnose and iduronic acid are present between 4.28 - 5.13 ppm. Proton chemical shifts of rhamnose are shown at 1.30 ppm. Glucuronic acid chemical shifts are between 3.36

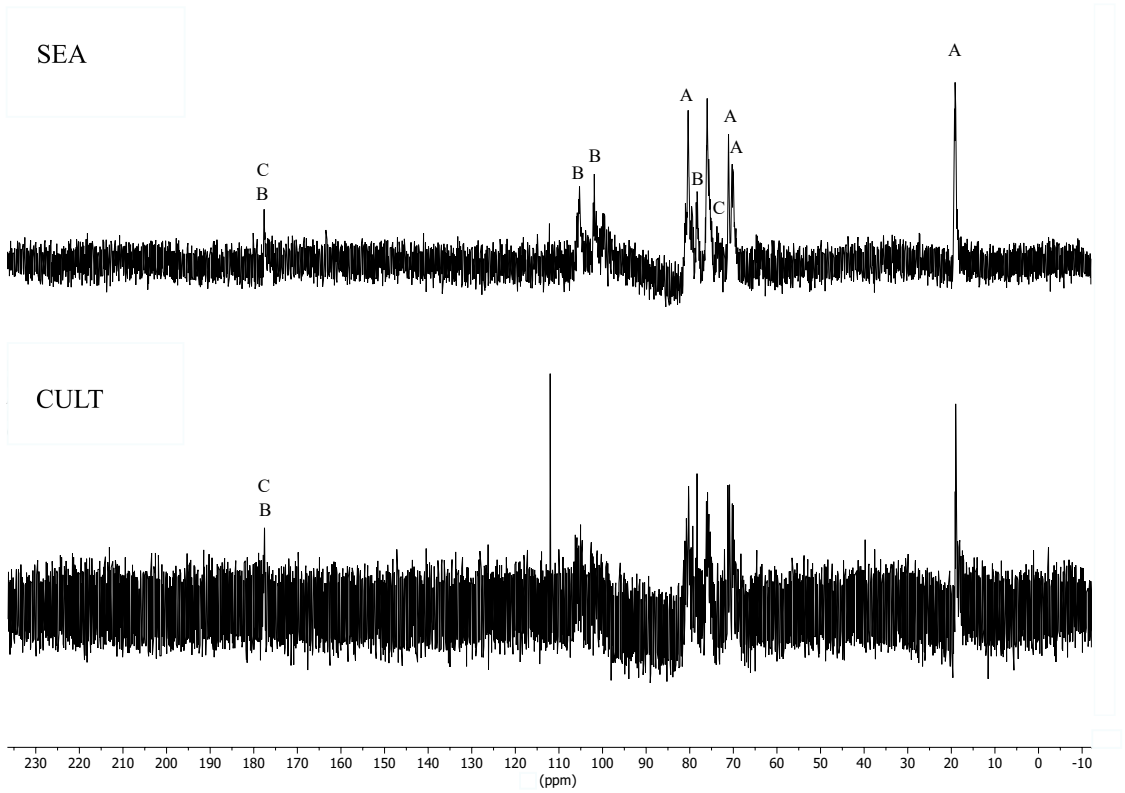

Figura A.3: ^{13}C NMR spectra of ulvan from *Ulva fasciata* collected in natural environment (SEA) and cultivated in enriched water (CULT). Residue A = rhamnose, B = glucuronic acid, C = iduronic acid and D = xylose.

and 3.79 ppm. Signals of xylose are indicated at 3.20 and 5.27 ppm at SEA ulvan.

Noise observed in the ^{13}C NMR spectra is related to sample dilution increased by the high molecular weight of the polymer and solution viscosity. Ulvan molecular weight can vary from 1.8×10^5 to 2×10^6 depending of extraction methods, specie and polydispersity of the samples (Tran et al., 2017; Yaich et al., 2017). According to (Lahaye and Robic, 2007) ulvan extracted with temperatures between 80 – 90°C, close to the used in this study, tend to present higher molecular weight.

In ^{13}C and ^{1}H NMR spectra of SEA ulvan we could observe signals of xylose, suggesting the presence of ulvanobiose (U3s) in this ulvan structure. In CULT we could not detect U3s, but peak characteristic to C-1 of rhamnose in A3s disaccharide (4.82 ppm) was present (Lahaye and Robic, 2007). According to (Robic et al., 2009b) during the active growth of *Ulva* the macroalgae tends to synthesize more ulvanobiuronic acid type A, with the production of ulvanobiose being developmentally regulated. In this study, *U. fasciata* presented an average growth rate of 5.7 % day^{-1} and active nutrient uptake throughout the cultivation experiment (data not shown), an indication that individuals had not reach their growth plateau when collected.

In this study both ulvan presented similar global structure, but ulvan from cultivated *U. fasciata* presented stronger signals of sulfate and ulvan from natural

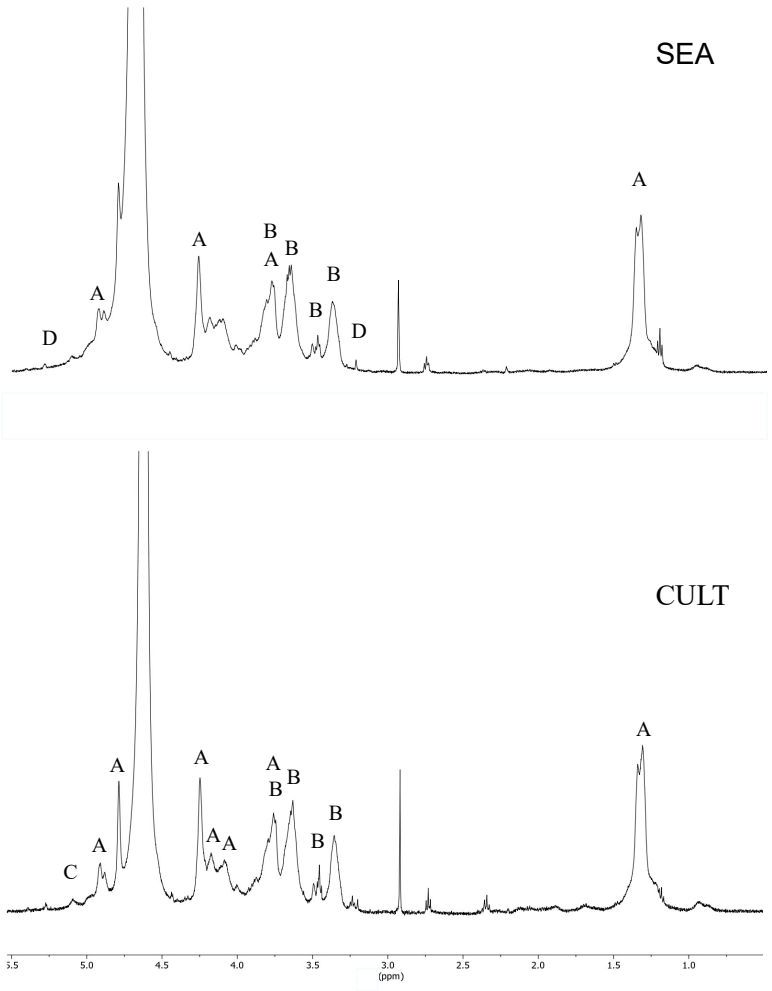

Figura A.4: ^{1}H NMR of ulvan from *Ulva fasciata* collected in natural environment (SEA) and cultivated in enriched water (CULT). Residue A = rhamnose, B = glucuronic acid, C = iduronic acid and D = xylose

environment had signals of xylose. Future studies with purification and sugar quantification procedures could help elucidated the fine structure of both ulvan samples and assess the efficacy of CULT ulvan for different applications such as antioxidant.

Conclusion

The production of ulvan with predictive structure and in necessary amounts is one of the hindrances for the ulvan market development. The results gathered here shows that ulvan from cultivated *U. fasciata* is similar to those reported in literature and could be a source for obtaining this polysaccharide. By controlling abiotic conditions ulvan production could be maximized meeting commercial requirements.

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Referências Bibliográficas

Adrien, A., Bonnet, A., Dufour, D., Baudouin, S., Maugard, T., and Bridiau, N. (2017). Pilot production of ulvans from *Ulva sp.* and their effects on hyaluronan and collagen production in cultured dermal fibroblasts. *Carbohydrate Polymers*, 157:1306–1314.

Alves, A., Caridade, S. G., Mano, J. F., Sousa, R. A., and Reis, R. L. (2010). Extraction and physico-chemical characterization of a versatile biodegradable polysaccharide obtained from green algae. *Carbohydrate Research*, 345(15):2194–2200.

Alves, A., Sousa, R. A., and Reis, R. L. (2012a). A practical perspective on ulvan extracted from green algae. *Journal of Applied Phycology*, 25(2):407–424.

Alves, A., Sousa, R. A., and Reis, R. L. (2012b). *In Vitro* cytotoxicity assessment of ulvan, a polysaccharide extracted from green algae. *Phytotherapy Research*, 27(8):1143–1148.

Araújo, L., Gonçalves, A. E., and Stadnik, M. J. (2014). Ulvan effect on conidial germination and appressoria formation of *Colletotrichum gloeosporioides*. *Phytoparasitica*, 42(5):631–640.

Bindschädler, P., Adibekian, A., Grünstein, D., and Seeberger, P. H. (2010). De novo synthesis of differentially protected l-iduronic acid glycosylating agents. *Carbohydrate Research*, 345(7):948–955.

Chiellini, F. and Morelli, A. (2011). Ulvan: A versatile platform of biomaterials from renewable resources. In *Biomaterials - Physics and Chemistry*. InTech.

Costa, C., Alves, A., Pinto, P. R., Sousa, R. A., da Silva, E. A. B., Reis, R. L., and Rodrigues, A. E. (2012). Characterization of ulvan extracts to assess the effect of different steps in the extraction procedure. *Carbohydrate Polymers*, 88(2):537–546.

Cunha, L. and Grenha, A. (2016). Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. *Marine Drugs*, 14(3):42.

del Rocío Quezada-Rodríguez, P. and Fajer-Ávila, E. J. (2016). The dietary effect of ulvan from *Ulva clathrata* on hematological-immunological parameters and growth of tilapia (*Oreochromis niloticus*). *Journal of Applied Phycology*, 29(1):423–431.

Hallak, L. K., Collins, P. L., Knudson, W., and Peeples, M. E. (2000). Iduronic acid-containing glycosaminoglycans on target cells are required for efficient respiratory syncytial virus infection. *Virology*, 271(2):264–275.

Hardouin, K., Bedoux, G., Burlot, A.-S., Donnay-Moreno, C., Bergé, J.-P., Nyvall-Collén, P., and Bourgougnon, N. (2016). Enzyme-assisted extraction (EAE) for the production of antiviral and antioxidant extracts from the green seaweed *Ulva armoricana* (Ulvales, Ulvophyceae). *Algal Research*, 16:233–239.

Holdt, S. L. and Kraan, S. (2011). Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology*, 23(3):543–597.

Kidgell, J. T., Magnusson, M., de Nys, R., and Glasson, C. R. (2019). Ulvan: A systematic review of extraction, composition and function. *Algal Research*, 39:101422.

Lahaye, M. (1998). NMR spectroscopic characterisation of oligosaccharides from two *Ulva rigida* ulvan samples (Ulvales, Chlorophyta) degraded by a lyase. *Carbohydrate Research*, 314(1-2):1–12.

Lahaye, M., Cimadevilla, E. A.-C., Kuhlenkamp, R., Quemener, B., Lognoné, V., and Dion, P. (1999). Chemical composition and 13C NMR spectroscopic characterisation of ulvans from *Ulva* (Ulvales, Chlorophyta). In *Sixteenth International Seaweed Symposium*, pages 515–521. Springer Netherlands.

Lahaye, M. and Ray, B. (1996). Cell-wall polysaccharides from the marine green alga *Ulva* rigida (Ulvales, Chlorophyta) — NMR analysis of ulvan oligosaccharides. *Carbohydrate Research*, 283:161–173.

Lahaye, M. and Robic, A. (2007). Structure and functional properties of ulvan, a polysaccharide from green seaweeds. *Biomacromolecules*, 8(6):1765–1774.

Leiro, J. M., Castro, R., Arranz, J. A., and Lamas, J. (2007). Immunomodulating activities of acidic sulphated polysaccharides obtained from the seaweed *Ulva rigida* C. Agardh. *International Immunopharmacology*, 7(7):879–888.

Li, W., Jiang, N., Li, B., Wan, M., Chang, X., Liu, H., Zhang, L., Yin, S., Qi, H., and Liu, S. (2018). Antioxidant activity of purified ulvan in hyperlipidemic mice. *International Journal of Biological Macromolecules*, 113:971–975.

Lopes, N., Ray, S., Espada, S. F., Bomfim, W. A., Ray, B., Faccin-Galhardi, L. C., Linhares, R. E. C., and Nozawa, C. (2017). Green seaweed *Enteromorpha compressa* (Chlorophyta, Ulvaceae) derived sulphated polysaccharides inhibit herpes simplex virus. *International Journal of Biological Macromolecules*, 102:605–612.

Pageon, H., Azouaoui, A., Zucchi, H., Ricois, S., Tran, C., and Asselineau, D. (2019). Potentially beneficial effects of rhamnose on skin ageing: an *in vitro* and *in vivo* study. *International Journal of Cosmetic Science*, 41(3):213–220.

Qi, H. and Sun, Y. (2015). Antioxidant activity of high sulfate content derivative of ulvan in hyperlipidemic rats. *International Journal of Biological Macromolecules*, 76:326–329.

Qi, H., Zhao, T., Zhang, Q., Li, Z., Zhao, Z., and Xing, R. (2005). Antioxidant activity of different molecular weight sulfated polysaccharides from *Ulva pertusa* Kjellm (Chlorophyta). *Journal of Applied Phycology*, 17(6):527–534.

Reis, R. P., de Carvalho Junior, A. A., Facchinei, A. P., dos Santos Calheiros, A. C., and Castelar, B. (2018). Direct effects of ulvan and a flour produced from the green alga *Ulva fasciata* Delile on the fungus *Stemphylium solani* Weber. *Algal Research*, 30:23–27.

Robic, A., Bertrand, D., Sassi, J.-F., Lerat, Y., and Lahaye, M. (2008). Determination of the chemical composition of ulvan, a cell wall polysaccharide from *Ulva spp.* (Ulvales, Chlorophyta) by FT-IR and chemometrics. *Journal of Applied Phycology*, 21(4):451–456.

Robic, A., Rondeau-Mouro, C., Sassi, J.-F., Lerat, Y., and Lahaye, M. (2009a). Structure and interactions of ulvan in the cell wall of the marine green algae *Ulva rotundata* (Ulvales, Chlorophyceae). *Carbohydrate Polymers*, 77(2):206–216.

Robic, A., Sassi, J.-F., Dion, P., Lerat, Y., and Lahaye, M. (2009b). Seasonal variability of physicochemical and rheological properties of ulvan in two *Ulva* species (Chlorophyta) from the Brittany coast 1. *Journal of Phycology*, 45(4):962–973.

Shao, P., Qin, M., Han, L., and Sun, P. (2014). Rheology and characteristics of sulfated polysaccharides from chlorophytan seaweeds *Ulva fasciata*. *Carbohydrate Polymers*, 113:365–372.

Tabarsa, M., You, S., Dabaghian, E. H., and Surayot, U. (2018). Water-soluble polysaccharides from *Ulva intestinalis*: Molecular properties, structural elucidation

and immunomodulatory activities. *Journal of Food and Drug Analysis*, 26(2):599–608.

Tako, M., Tamanaha, M., Tamashiro, Y., and Uechi, S. (2015). Structure of ulvan isolated from the edible green seaweed, *Ulva pertusa*. *Advances in Bioscience and Biotechnology*, 06(10):645–655.

Thanh, T. T. T., Quach, T. M. T., Nguyen, T. N., Luong, D. V., Bui, M. L., and Tran, T. T. V. (2016). Structure and cytotoxic activity of ulvan extracted from green seaweed *Ulva lactuca*. *International Journal of Biological Macromolecules*, 93:695–702.

Tran, T. T. V., Truong, H. B., Tran, N. H. V., Quach, T. M. T., Nguyen, T. N., Bui, M. L., Yuguchi, Y., and Thanh, T. T. T. (2017). Structure, conformation in aqueous solution and antimicrobial activity of ulvan extracted from green seaweed *Ulva reticulata*. *Natural Product Research*, 32(19):2291–2296.

Tziveleka, L.-A., Ioannou, E., and Roussis, V. (2019). Ulvan, a bioactive marine sulphated polysaccharide as a key constituent of hybrid biomaterials: A review. *Carbohydrate Polymers*, 218:355–370.

Ursi, S. and Plastino, E. M. (2001). Crescimento *in vitro* de linhagens de coloração vermelha e verde clara de *Gracilaria birdiae* (Gracilariales, Rhodophyta) em dois meios de cultura: análise de diferentes estádios reprodutivos. *Revista Brasileira de Botânica*, 24(4).

Valentin, J. L., Paiva, P., and Salomon, P. (2018). *A Baía de Guanabara: passado, presente e futuro de um ecossistema ameaçado*. Editora CRV.

Wang, H.-M. D., Chen, C.-C., Huynh, P., and Chang, J.-S. (2015). Exploring the potential of using algae in cosmetics. *Bioresource Technology*, 184:355–362.

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., Smith, A. G., Camire, M. E., and Brawley, S. H. (2016). Algae as nutritional and functional food sources: revisiting our understanding. *Journal of Applied Phycology*, 29(2):949–982.

Yaich, H., Amira, A. B., Abbes, F., Bouaziz, M., Besbes, S., Richel, A., Blecker, C., Attia, H., and Garna, H. (2017). Effect of extraction procedures on structural, thermal and antioxidant properties of ulvan from *Ulva lactuca* collected in monastir coast. *International Journal of Biological Macromolecules*, 105:1430–1439.

Apêndice B

Prêmio Yocie Yoneshigue-Valentin

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Rede Nacional em Biotecnologia
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CERTIFICADO

Certificamos que a Dra. Tiphane Andrade Figueira recebeu o *Prêmio Científico Yocie Yoneshigue-Valentin* no 7º WORKSHOP OF REDEALGAS: BIOTECHNOLOGY FOR WELLNESS.

R. Coutinho
Ricardo Coutinho
Presidente do Workshop

Yocie Yoneshigue-Valentin
Yocie Yoneshigue-Valentin
Profa. Emérita da Redealgas

Pio Colepicolo
Pio Colepicolo
Presidente da Redealgas

Apêndice C

Capítulo: Macroalgas e suas aplicações biotecnológicas

Tiphane Andrade Figueira[1], Nuno Tavares Martins[2]

[1] Universidade Federal do Rio de Janeiro; [2] Universidade de São Paulo

Imagem de fundo da capa: Colagem da folha diafanizada de *Tetrapterys mucronata* Cav., uma das espécies de Malpighiaceae estudadas no projeto de doutorado da discente Leyde Nayane Nunes dos Santos Silva, do programa de Ciências Biológicas (Botânica) da Universidade de São Paulo.

VIII Botânica no Inverno 2018 / Org. Aline Possamai Della [et al.]. – São Paulo: Instituto de Biociências da Universidade de São Paulo, Departamento de Botânica, 2018. 275 p. : il.

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Figura C.1: Schematic representation of steps to ulvan extraction and characterization.

Plano de Fundo

Macroalgas marinhas são organismos autotróficos fotossintetizantes, talófitos (ou seja, não são diferenciados em raízes, caule e folhas), que possuem em comum o pigmento clorofila-*a*. Estes organismos são agrupados em três divisões taxonômicas, feofíceas (algas pardas), rodófitas (algas vermelhas) e clorófitas (algas verdes). No sul do Chile foram encontradas algas cozidas e parcialmente consumidas em um sítio arqueológico de 14 mil anos, sugerindo seu uso na alimentação e medicina (Dillehay et al., 2008), e o primeiro registro escrito do uso de macroalgas data de 1700 anos atrás na China.

Historicamente, as macroalgas foram utilizadas pelas populações costeiras na alimentação humana, forragem animal, como fertilizantes e com fins medicinais, principalmente em países do Oriente. Na medicina tradicional chinesa, estes organismos foram utilizados, por exemplo, no tratamento de gota e problemas estomacais (Buschmann et al., 2017). Ainda hoje, países como China, Japão e República da Coréia figuram entre os principais consumidores de algas tanto frescas quanto desidratadas, em sopas, saladas, sobremesas e como condimentos (McHugh, 2003). Inicialmente eram utilizadas apenas domesticamente, mas nas últimas décadas, diversas aplicações industriais foram desenvolvidas.

Durante a Primeira Guerra Mundial (1914-1918), ocorreu embargo ao comércio de potássio mineral pela principal exportadora mundial – a Alemanha. O potássio é amplamente utilizado na produção de armamentos, mas também muito utilizado como fertilizantes. O embargo afetou principalmente os Estados Unidos, maior consumidor de fertilizantes. Visando abastecer estes mercados, empresas americanas começaram a utilizar a alga parda kelp na obtenção de potássio, para produção de fertilizantes (Neushul, 1989; van Hal et al., 2014). A partir de então, diversas outras aplicações tecnológicas para macroalgas surgiram. Segundo dados mais recentes da Organização das Nações Unidas para Alimentação e Agricultura (FAO, na sigla em inglês) em 2015 foram produzidas, aproximadamente, 30 milhões de toneladas de macroalgas (peso fresco), movimentando um mercado de 5 bilhões de dólares (FAO, 2018) (Figura C.2)

Mundialmente, aproximadamente 221 espécies de macroalgas são utilizadas, sendo 66% delas para alimentação (Milledge et al., 2014) illedge et al. 2014). Os sete principais gêneros cultivados com fins comerciais são: *Eucheuma sp.*, *Kappaphycus alvarezii* e *Gracilaria sp.* (para produção de carragenana e ágar), *Saccharina japonica*, *Undaria pinnatifida*, *Porphyra sp.* e *Sargassum fusiforme* (usados na alimentação humana) (Buschmann et al., 2017). As macroalgas utilizadas comercialmente são obtidas de duas formas: coletadas na natureza e através de cultivo. Com a expansão do uso de macroalgas, apenas a coleta de populações

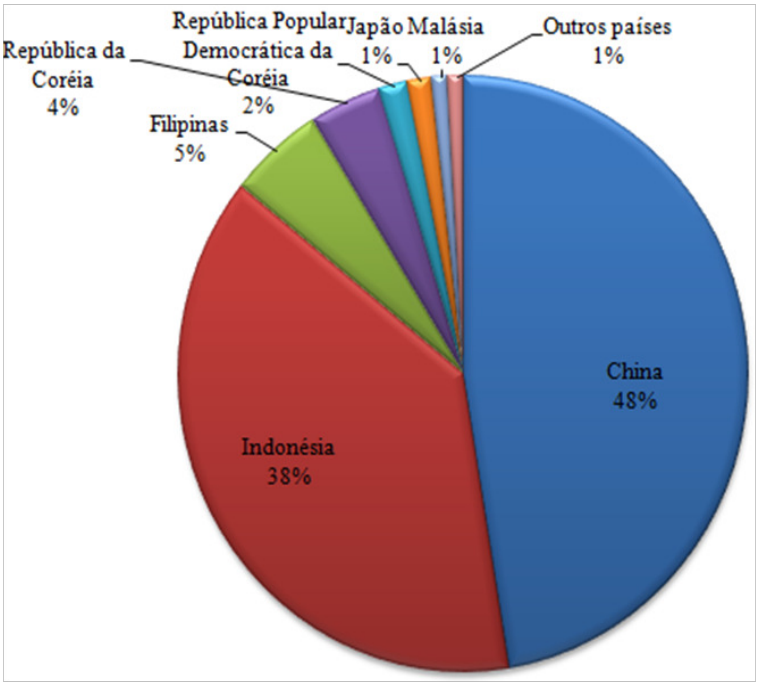

Figura C.2: Principais países produtores de macroalgas em 2015 (Adaptado de FAO, 2018).

nativas deixou de ser suficiente para suprir a demanda comercial. Além disso, perda de biodiversidade e extinção de populações locais são alguns dos impactos ambientais que podem ser associados a essa atividade. Por conta disso, a obtenção de biomassa a partir de depósitos naturais tem se mantido estável, enquanto a produção de macroalgas através de cultivo apresenta um crescimento de aproximadamente 7,5% ao ano (Mazarrasa et al., 2013).

O cultivo de macroalgas é o setor para produção de alimentos que cresce com a maior velocidade, contando com quase 50% dos recursos aquáticos globais (FAO, 2018). Acredita-se que a aquicultura tenha grande potencial para enfrentar os desafios da crescente necessidade de alimentos (Stévant et al., 2017). A maioria das pessoas utiliza diariamente produtos oriundos de macroalgas, na forma de comidas processadas (como iogurtes, carnes e frutas) e produtos domésticos como tintas, pasta de dentes, suplementos alimentares, purificadores de ar, cosméticos e etc (Dhargalkar e Pereira, 2005).

Dentre as múltiplas aplicações biotecnológicas existentes para macroalgas, apresentamos a seguir um breve panorama de seus principais usos e mercados futuros mais proeminentes.

Hidrocolóides

Hidrocolóides são substâncias não cristalinas formadas por estruturas de elevado peso molecular que se dissolvem em água formando uma solução viscosa. Alginato, ágar e carragenana são os principais carboidratos utilizados para formar géis com variadas viscosidades e graus de firmeza. Atualmente, aproximadamente 13% das macroalgas produzidas mundialmente são utilizadas pela indústria de hidrocolóides (Rioux et al., 2017). O primeiro registro escrito do uso de macroalgas como fonte de hidrocolóides (ágar) data de 330 D. C. pelo chinês Chi Han (Craigie, 2010). Extratos de Irish Moss (*Chondrus crispus*) contendo carragenana foi um agente espessante muito usado na Europa do século 19, porém somente a partir de 1930 extratos de algas pardas contendo alginato foram produzidos e comercializados em escala industrial como agentes gelificantes e espessantes (McHugh, 2003). Após a Segunda Guerra Mundial, com a necessidade de alimentar uma crescente população, as aplicações industriais de extratos de macroalgas se expandiram e diversificaram rapidamente, e hoje estão presentes na fabricação de produtos como sorvetes, cosméticos e géis usados em eletroforese (Craigie, 2011; Renn, 1990). A produção de hidrocolóides é o principal mercado de produtos extraídos de macroalgas. Em 2013, foram produzidas mais de 100000 toneladas de biomassa, totalizando aproximadamente 1,2 bilhão de dólares (Yarish, 2016).

Ágar

O ágar é obtido principalmente a partir de dois tipos de algas vermelhas que apresentam uma ampla distribuição geográfica: *Gelidium sp.* e a *Gracilaria sp*, que pode ter uma concentração de ágar de até 31% do seu peso seco (Holdt and Kraan, 2011; Rhein-Knudsen et al., 2015). A produção de ágar gira em torno de 10,600 toneladas/ano, e aproximadamente 90% do ágar produzido é utilizado na indústria alimentícia (McHugh, 2003). Alguns tipos de ágar, especialmente os extraídos de *Gracilaria chilensis*, são usados na produção de produtos com elevados teores de açúcar, como doces de frutas. Este tipo de ágar é conhecido como "açúcar reativo", pois o açúcar (sacarose) aumenta a força do gel (McHugh, 2003). Em panificações, a capacidade desses géis de suportar elevadas temperaturas permite que este hidrocolóide possa ser usado como estabilizante e espessante para tortas e glacês. O ágar também é usado como aditivo em inúmeros produtos como laticínios, carne e peixe enlatados, sopas, molhos e bebidas (Rioux et al., 2017). Na indústria farmacêutica, o ágar é há muito usado como um laxante suave. Já no setor de microbiologia bacteriana este hidrocolóide, com um grau de pureza especial, é utilizado para testar a presença de bactérias. Devido ao tratamento especial necessário, esta forma de ágar pode custar até duas vezes mais do que o

utilizado na indústria alimentícia (McHugh, 2003).

Carragenana

A produção de carragenana originalmente era dependente da coleta de algas que crescem em águas frias. Contudo, a partir dos anos 1970 macroalgas nativas de países tropicais contendo carragenana passaram a ser cultivadas, permitindo assim o escalonamento de sua produção. Atualmente, a maior parte deste hidrocolóide é extraído das macroalgas *Kappaphycus alvarezii* e *Eucheuma sp.*, produzidas em cultivo e, *Sarcothalia sp.* e *Gigartina sp.* (Chile e México) e *Chondrus crispus* (Canadá e França) coletadas na natureza (Valderrama et al., 2015). A principal aplicação da carragenana é na indústria de alimentos, especialmente em produtos lácteos, onde frequentemente apenas pequenas quantidades desse produto são necessárias. Em produtos de baixa caloria, a carragenana pode ser utilizada para melhorar a textura de alimentos salgados e como substituto para pectina em alimentos doces, como geléias (McHugh, 2003). Na produção de bebidas, a carragenana é usada para o clareamento de cervejas, vinhos e mel (Rioux et al., 2017). Na indústria farmacêutica, este hidrocolóide é usado como agentes de suspensão e estabilizantes em medicamentos, loções e pomadas (Holdt and Kraan, 2011). A carragenana pode ser encontrada em muitos outros produtos utilizados no dia-a-dia como em rações animais, pasta de dentes e purificadores de ar em gel (Dhargalkar e Pereira, 2005).

Alginato

O alginato foi descoberto em 1880 pelo farmacêutico britânico E. C. C. Stanford, e sua produção comercial teve início em 1929 na Califórnia (Holdt and Kraan, 2011). O alginato é extraído de algas pardas principalmente coletadas na natureza, pois os custos para o cultivo desse grupo de macroalgas é muito elevado. As espécies mais utilizadas para extração deste hidrocolóide são: *Ascophyllum spp.*, *Durvillaea spp.*, *Ecklonia spp.*, *Laminaria spp.*, *Lessonia spp.*, *Macrocystis spp.*. O alginato é amplamente utilizado em alimentos, cosméticos, medicamentos e também encontra aplicação na indústria têxtil e de papel. Na impressão têxtil os alginatos são usados como espessantes para a pasta contendo o corante (Yarish, 2016)(Yarish et al., 2016). Graças às propriedades gelificantes do alginato este foi muito utilizado no início da produção de cerejas artificiais em 1946 (McHugh, 2003). O alginato também é usado na produção de molhos, ketchup, caldas e cobertura para sorvete dada sua função espessante (Rioux et al., 2017). Na indústria cervejeira, pequenas concentrações de alginato de propileno glicol promovem a formação de uma espuma mais estável e duradoura (McHugh, 2003). Quando em contato com a água, pós

de alginato absorvem e aumentam seu volume, por isso estes pós são utilizados em produtos dietéticos gerando maior sensação de saciedade, em medicamentos para dores estomacais entre outros (Smit, 2004). Anualmente são produzidas 26,500 toneladas de alginato (Holdt and Kraan, 2011).

Alimentação Humana

Segundo dados da FAO, em 2014, 75% das macroalgas produzidas mundialmente foram utilizadas na indústria de alimentos, e a produção de Kombu (*Saccharina japonica*), Wakame (*Undaria pinnatifida*) e Nori (*Porphyra spp.*), utilizadas na comida tradicional japonesa, representou 40% dessa produção (Rioux et al., 2017). Países da Ásia (China, Japão e Coréia) e Pacífico (Filipinas, Nova Zelândia, Indonésia e outros) são os principais consumidores de alga, utilizando-as em alimentos como saladas, sopas, biscoitos, acompanhando alimentos crus (como sushi) e diversos outros pratos (Stévant et al., 2017). De forma mais tímida, países como Gales, França, Irlanda, Chile e Canadá possuem algumas tradições alimentares com pratos baseados em algas (Rioux et al., 2017).

Com a imigração internacional, pessoas originárias de países com tradição no uso de algas levaram consigo costumes e receitas que facilitaram a difusão do consumo desses alimentos. Com isso, novas receitas e produtos foram sendo desenvolvidos, como chips, shakes enriquecidos, biscoitos, purê instantâneo, tagliatelle entre outros, todos produzidos ou enriquecidos com algas (Fleurence, 2016; Rioux et al., 2017). O elevado teor de proteínas encontrados em macroalgas, que pode variar de 3-15% (peso seco) em algas pardas e de 10-47% (peso seco) em algas vermelhas e verdes (Fleurence, 1999), tem chamado a atenção para o uso desses organismos em alimentos vegetarianos e veganos como substitutos de proteínas de origem animal. Algumas macroalgas como a *Porphyra tenera*, conhecida como "nori" e usada em sushi, e a *Palmaria palmata*, conhecida como "dulse" e também muito utilizada na culinária japonesa, podem apresentar teores de proteína (47 e 35% do peso seco, respectivamente) mais elevados do que encontrados na soja, por exemplo (Fleurence, 1999). Alguns dos produtos desenvolvidos visando abastecer principalmente o mercado vegano/vegetariano são óleo de alga, ovos veganos, maionese sem ovo, entre muitos outros (Rioux et al., 2017, foodnavigator-usa.com/feature/news-by-month/08/2016, acessado em 29 de abril de 2018).

Sabe-se que macroalgas podem conter diversos tipos de nutrientes como fibras alimentares, minerais, polissacarídeos, vitaminas (como B1, B12, A, E), e ácidos graxos poliinsaturados (como ômega-3)(Holdt and Kraan, 2011; Wells et al., 2016). A associação entre dietas ricas em algas e menores índices de doenças e benefícios

a saúde, tem levado a entrada de produtos de macroalgas em um novo mercado, o dos alimentos funcionais. Estes tipos de alimento possuem compostos bioativos que visam trazer benefícios à saúde além da nutrição básica. Algas como *Caulerpa lentilifera*, *Ulva fasciata*, *Chondrus ocellatus*, entre outras, possuem elevado teor de fibras alimentares. A ingestão regular destas fibras ajuda a reduzir o risco de doenças como diabetes, doenças cardíacas e câncer (Mohamed et al., 2012). Muitos estudos têm sido realizados buscando identificar o potencial de diferentes compostos presentes nas macroalgas e suas propriedades como agentes anti-inflamatórios, antioxidantes, antivirais, antibactericidas, entre outros (Mohamed et al., 2012; Rioux et al., 2017; Wells et al., 2016). Atualmente, ao menos 145 espécies de macroalgas são utilizadas diretamente para alimentação (Fleurence et al., 2012).

Alimentação animal

Macroalgas vem sendo usadas como alimentação animal por populações costeiras há milênios. Segundo o livro *Bellum Africanum*, de 45 A.C., em tempos de escassez, os Gregos utilizavam macroalgas para alimentação de seus rebanhos (Evans and Critchley, 2013). Na Islândia, ovelhas, cavalos e o gado podiam ser alimentados com macroalgas por até 8 semanas (Makkar et al., 2016). Na primeira guerra mundial, quando não havia ração para os cavalos, algas secas foram usadas pelo exército francês (Hasan, 2017). Na Escócia, ovelhas e gado consomem diferentes espécies de algas quando pastam na costa (Fleurence, 2016).

O uso comercial de macroalgas na ração animal foi pioneiro na Noruega, que iniciou sua produção em escala na década de 1930 (Stévant et al., 2017). Macroalgas podem representar uma excelente fonte alternativa para alimentação animal, pois possuem importantes nutrientes, minerais, carboidratos complexos com atividades probióticas e ácidos graxos poliinsaturados, que podem beneficiar a saúde dos animais e do consumidor (Evans and Critchley, 2013). Macroalgas concentram minerais da água do mar e seu conteúdo pode ser de 10 a 20 vezes maior do que o encontrado em plantas terrestres (Makkar et al., 2016). Estes fatores têm renovado o interesse do setor em desenvolver novos produtos e formulações. Atualmente, a macroalga mais utilizada na alimentação animal é a alga parda *Ascophyllum nodosum*. Esta alga é comumente encontrada na costa norte-ocidental da Europa e no noroeste da América do Norte. Seu extenso uso se deve principalmente à sua abundância, facilidade de coleta e crescimento em áreas próximas a infraestruturas de processamento e, além disso, estudos apontam que esta alga contém elevadas concentrações de minerais (potássio, fósforo, cálcio, sódio, magnésio e enxofre), metais traços e vitaminas (Evans and Critchley, 2013; Holdt and Kraan, 2011).

Estudos utilizando macroalgas na alimentação de peixes sugerem que essa fonte

de proteína pode aumentar o ganho de peso e a deposição de proteínas e triglicerídeos nos músculos. Além disso, uma dieta enriquecida com macroalgas poderia melhorar a resistência ao estresse e doenças nos peixes (Fleurence et al., 2012; Mustafa et al., 1995). Na pecuária, o uso de macroalgas na ração levou ao aumento da produção de leite no gado, da taxa de crescimento em cordeiros, e da melhora na cor da gema nos ovos (Holdt and Kraan, 2011).

Cosméticos

Os oceanos são fontes extremamente ricas de produtos bioativos, muitos com características não encontradas em organismos terrestres. Já foram isolados mais de 7,000 produtos naturais marinhos, sendo 25% extraídos de algas (Kijjoa and Sawangwong, 2004). Durante seu desenvolvimento, as macroalgas geram uma grande quantidade de compostos químicos conhecidos como "compostos bioativos", fazendo com que estes organismos possuam um grande potencial para uso no setor de cosméticos. Extratos oriundos de macroalgas podem ser utilizados em uma grande variedade de produtos como sabonetes, xampu, pasta de dentes, hidratantes corporais, maquiagem, protetor solar, entre muitos outros (Pimentel et al., 2017; Wang et al., 2015).

Macroalgas são organismos sésseis, expostos a grandes variações ambientais que levaram ao desenvolvimento de mecanismos adaptativos, como a produção de compostos bioativos com atividade antioxidante. Em cosméticos, estes compostos ajudam a retardar o envelhecimento da pele, inflamações cutâneas, câncer de pele e na proteção contra raios ultravioleta (filtros solar) (Biba, 2014; Cardozo et al., 2007). Podemos citar como exemplo de macroalgas com potencial para uso em cosméticos a macroalga vermelha *Chondrus cripsus*, que é rica em polissacarídeos e minerais como manganês, zinco, cálcio e magnésio que possuem ação hidratante, condicionante, calmante e cicatrizante (Kim et al., 2008); *Asparagopsis spp.*, que já é cultivada na França para produção de extratos para o tratamento de pele (Leal et al., 2016); *Fucus vesiculosus*, cujo extratos podem ser usados para reduzir e melhorar a aparência de olheiras, e estimular a produção de colágeno, reduzindo rugas e linhas de expressão (Sun and Chavan, 2014). Algas pardas, em geral, possuem muitas vitaminas, minerais e ácidos graxos essenciais, incluindo ômega 3 e 6, conhecidos por auxiliar na regeneração e saúde da pele (Kim et al., 2008). O principal mercado de cosméticos produzidos a partir de macroalgas é a França, que utiliza aproximadamente 5 toneladas (peso fresco) de algas para atender sua demanda (Kim et al., 2008).

A mudança no padrão de consumo dos clientes, que buscam produtos ambientalmente corretos, oriundos de fontes renováveis e sem adição de compostos

sintéticos tem estimulado a busca por produtos utilizando macroalgas (Ariede et al., 2017; Wang et al., 2015). Embora o efeito cosmético desses compostos bioativos venha cada vez mais sendo descrito em diversos estudos e patentes, muitos destes produtos ainda não chegaram ao mercado consumidor (Ariede et al., 2017). Alguns dos principais entraves para a comercialização são os elevados custos para identificação e extração do composto bioativo, o desenvolvimento de técnicas de cultivo para obtenção de maiores rendimentos e concentração dos compostos. Além disso, um elevado nível de padronização, eficácia e rastreabilidade dos produtos são necessárias (Hafting et al., 2015).

Fármacos

As macroalgas são os recursos marinhos mais amplamente estudados e nas últimas três décadas o interesse e descoberta de compostos bioativos extraídos desses organismos tem crescido exponencialmente (Smit, 2004; Wang et al., 2017). Estudos revelaram que alguns desses compostos podem possuir propriedades terapêuticas e atuar no combate de doenças como câncer, diabetes, hipertensão, possuir atividades anti-virais, bactericidas, neuroprotetoras e etc. (Mohamed et al., 2012). Ao longo da história, macroalgas foram usadas com fins medicinais por populações das mais diversas tradições, como chineses e japoneses, que utilizavam diversas espécies de algas na medicina tradicional. Na Europa do século 18, vermífugos foram preparados a partir de espécies de *Laminaria spp.*, romanos que usavam cataplasmas de*Fucus vesiculosus* para o tratamento de dores nas articulações e com fins cosméticos (Hasan, 2017), entre muitos outros exemplos. Na Irlanda, ainda hoje são usados chás tradicionais feitos com Irish moss (*Chondrus crispus*) para tratar resfriados, broquite e tosses crônicas (Holdt and Kraan, 2011).

Estudos sugerem que compostos extraídos de algas pardas, como floroglucinol podem apresentar atividades anti-inflamatórias, anti-tumoral e anti-diabética (Wang et al., 2015). *Laminaria spp.* contém até 13 vezes mais cálcio do que o leite (Hasan, 2017). Já fucoidans, também extraídos de algas pardas, são excelentes anticoagulantes e podem prevenir trombose (Mohamed et al., 2012). Extratos ricos em fenol oriundos de algas como *Alaria spp.*, *Ascophyllum spp.*, *Palmaria spp.*, *Ulva spp.* podem atuar como agentes antioxidantes, bem como anti-diabéticos ao inibir determinadas enzimas digestivas (Hasan, 2017).

Carotenóides, tradicionalmente utilizados para pigmentação, podem atuar como antioxidantes, na prevenção de câncer e melhora na resposta do sistema imune (Kim et al., 2008). O elevado teor de fibras alimentares solúveis presentes em espécies como *Eucheuma cottonii*, *Caulerpa lentillifera*, *Sargassum polycystum*, *Ahnfeltiopsis concinna*, *Gayralia oxysperma*, *Chondrus ocellatus* e *Ulva fasciata* podem auxiliar

na redução de colesterol (Mohamed et al., 2012). Alguns polissacarídeos sulfatados extraídos de algas vermelhas apresentaram atividades anti-viral em doenças como vírus da imunodeficiência humana (HIV), vírus herpes simplex (HSV) tipo 1 e 2 e vírus sincicial respiratório (RSV) (Smit, 2004).

Contudo, apesar das pesquisas e esforços acadêmicos e empresariais, poucos fármacos derivados de macroalgas chegaram ao mercado consumidor (Smit, 2004). Uma das principais barreiras para o desenvolvimento e disponibilização destes produtos são os custos de produção, o caráter inovador dos produtos, dado que a maioria dos medicamentos disponíveis no mercado é baseada em organismos terrestres, falta de tecnologias de cultivo que atendam os requerimentos do setor e a falta de regulamentação específica (Holdt and Kraan, 2011).

Biocombustíveis

Atualmente, a população mundial gira em torno 7 bilhões de pessoas, e espera-se que em 2050 sejamos 9,6 bilhões (Kawai and Murata, 2016). Esse crescimento populacional, o aumento da longevidade e a elevação no padrão de vida (Alaswad et al., 2015; Chemodanov et al., 2017) aumentam a pressão sobre diversos setores, entre eles o energético. Visando atender a essas demandas e reduzir o impacto ambiental causado pelo setor, fontes renováveis de energia vêm sendo cada vez mais adotadas.

O uso de biocombustíveis é globalmente difundido como alternativa ao uso de combustíveis fósseis. As duas fontes de biocombustíveis utilizadas atualmente são as fontes alimentares, que são culturas agrícolas com finalidades energéticas como milho e cana-de-açúcar, e fontes não alimentares, que utilizam resíduos de biomassa, como aparas agrícolas e o bagaço de cana-de-açúcar. Porém, o cultivo terrestre para produção de biocombustíveis exerce uma grande pressão no ambiente, com uso intensivo do solo, demanda por água potável e uso de agroquímicos, além de competir com cultivos alimentares (Fernand et al., 2017). Com o crescimento da demanda por alimentos, estes conflitos tendem a se agravar e novas alternativas precisam ser encontradas.

O uso de macroalgas como fonte de biocombustíveis apresenta muitas vantagens quando comparado às fontes atualmente utilizadas. Algumas dessas vantagens são a ausência de lignina encontrada nas algas, a não competição por área com culturas alimentares, macroalgas não necessitam de água potável nem de fertilizantes, apresentam elevadas taxas de crescimento e podem fixar gás carbônico (Chen et al., 2015; Kawai and Murata, 2016; Suutari et al., 2014). Alguns requisitos devem ser levados em consideração para a escolha da macroalga a ser estudada para produção de biocombustíveis, como cultivo sustentável e a disponibilidade de biomassa em

grandes quantidades (ao longo de todo ano ou a maior parte dele), para atender a demanda do setor (Alaswad et al., 2015). Segundo dados compilados por Jiang et al. (2016), aproximadamente metade dos trabalhos investigando o uso potencial de macroalgas no setor energético focam na alga parda *Laminaria japonica*, seguido de diversas espécies de *Sargassum sp.* Entre as algas vermelhas e verdes, espécies de *Gracilaria spp.* e *Ulva spp.* são as mais intensamente estudadas. Estas espécies possuem diversos usos biotecnológicos e já são intensamente cultivadas e tradicionalmente conhecidas, principalmente em países asiáticos.

Embora o uso de macroalgas como fonte de biocombustíveis seja proeminente, esta tecnologia ainda é limitada por barreiras tecnológicas e pela baixa relação custo-benefício. Estudos estimam que o custo para produção de bioetanol a partir de macroalgas, seja de $0,50/kg (em dólares por peso seco) contra $0,16 para o milho (Chen et al., 2015). Quanto à tecnologia, os protocolos de produção ainda estão sendo desenvolvidos, principalmente tendo como base aqueles usados na produção de biocombustíveis convencionais, sendo necessária muita pesquisa para seu desenvolvimento. Por estas razões, a produção de biocombustíveis a partir de macroalgas não é economicamente viável, ainda estando limitada a estudos em escala laboratorial e em mesocosmos (Alaswad et al., 2015; Jiang et al., 2016; Suutari et al., 2014).

A produção de macroalgas na América Latina

Na América Latina as espécies de macroalgas representam de 4,9 a 8,7% da biodiversidade marinha , sendo encontrada no Brasil a maior biodiversidade – 10,6 espécies por 100 km de costa (Rebours et al., 2014). Os principais países produtores são Argentina, Brasil, Chile, México e Peru (Rebours et al., 2014). A exceção do Chile, informações sobre a produção de macroalgas na América Latina são dispersas. O Chile é o maior produtor de macroalgas, com uma produção, em 2015, de 11952 toneladas (FAO, 2018), sendo 97,6% oriunda da coleta na natureza e apenas 2,4% de cultivos (Camus et al., 2016; Hayashi et al., 2013). As principais espécies de macroalgas comercializadas são *Gracilaria chilensis*, usada na produção de ágar e *Macrocystis pyrifera*, principalmente para a extração de alginato e alimentação de abalone (Camus et al., 2016).

A produção comercial de macroalgas na Argentina tem por volta de 40 anos e as principais espécies coletadas são: *Macrocystis pyrifera*, *Lessonia vadosa*, *Gracilaria gracilis*, *Gigartina skottsbergii*, *Sarcothalia sp.*, and *Porphyra columbina* (Zaixo et al., 2006). Toda macroalga utilizada é coletada na Patagônia, principalmente na província de Chubut. Essas algas são cultivadas para produção de carragenana, alginato, consumo humano, nutraceuticos, cosméticos e fucoidans (Rebours et al.,

2014).

No México, o comércio de macroalgas é estabelecido desde 1960, contudo, ainda hoje, essa atividade é totalmente baseada na coleta na natureza, principalmente para a produção de carragenana (Hayashi et al., 2013; Valderrama et al., 2015). No México as quatro principais espécies mais exploradas são *Macrocystis pyrifera*, *Gelidium robustum*, *Chondracanthus canaliculatus*, *Gracilariopsis lemaneiformis* (Rebours et al., 2014).

No Peru as informações disponíveis sobre a produção de macroalgas são escassas. Sabe-se que *Chondracanthus chamissoi* e *Gracilaria lamaneiformis* são coletadas para a produção de ficocolóides, principalmente carragenana, e pequenas quantidades de *Porphyra columbina*, para consumo humano (Hayashi et al., 2013).

No Brasil, a produção de macroalgas é principalmente oriunda de coletas realizadas na natureza. Contudo, o cultivo de *Kappaphycus sp.* tem sido realizado no país há pelo menos 20 anos (Hayashi et al., 2013). Outras duas espécies comercialmente exploradas são *Gracilaria sp.* e *Hypnea sp.* (E. et al., 2006).Segundos dados da FAO (2018), em 2015 o Brasil produziu 730 toneladas de macroalgas (peso fresco).

Na América Latina, com exceção do Chile, a exploração econômica das macroalgas ainda é pouco conhecida e para seu desenvolvimento desafios como tecnologia, mão-de-obra qualificada e falta tradição no uso de macroalgas precisam ser superadas (Rebours et al., 2014). Além disso, é fundamental a criação de planos de manejo e regulamentação que possam garantir o desenvolvimento sustentável dessa atividade econômica.

Vantagens e desvantagens

Desvantagens

Macroalgas absorvem nutrientes como nitrogênio e fósforo presentes no ambiente marinho, mas também podem acumular metais pesados como arsênico, cobre, zinco entre outros (Makkar et al., 2016). Para que se evitem tais contaminações é necessário realizar o monitoramento regular da qualidade do ambiente de cultivo ou coleta, e da composição das macroalgas, principalmente quando utilizadas para alimentação humana e animal. Assim como ocorre com as plantas terrestres, o valor nutricional e composição bioquímica podem variar entre as espécies e grupos de macroalgas, estação do ano e localização geográfica (Craigie, 2010; Fernand et al., 2017). Este é um dos principais desafios para o desenvolvimento de produtos com maior valor agregado como fármacos, que necessitam de um rigoroso nível de padronização. Para atender a essas demandas, técnicas de cultivo, extração dos

compostos e armazenamento da biomassa precisam ser desenvolvidos (van Hal et al., 2014).

Em lugares como a América Latina e alguns países da Europa, a produção de macroalgas ainda está em seus primeiros estágios e políticas públicas e legislação ainda estão em desenvolvimento (Rebours et al., 2014). A falta de controle para a coleta de macroalgas na natureza pode levar ao uso predatório e a exaustão desse recurso. É interessante notar que existe uma diferença entre a produção de biomassa e o depósito de patentes registradas pelos países. Entre os maiores produtores encontram-se países como Filipinas, Vietnã, China e Japão, enquanto os dois primeiros países possuem um número de registros de patentes irrelevante, os últimos são líderes no rank de registro de patentes (Mazarrasa et al., 2013).

Vantagens

As macroalgas possuem uma ampla distribuição geográfica e elevado teor nutricional, o que as posicionam como uma alternativa para enfrentar o desafio global de alimentar uma população cada vez mais numerosa, sem acrescentar mais pressão aos recursos ambientais já combalidos. Em 1999, o Fórum Nacional de Macroalgas realizado pelo Ministério de Recursos Naturais e Marinhos da Irlanda, determinou que o desenvolvimento da aquacultura de macroalgas era atividade fundamental para atender mercados emergentes e criar postos de trabalhos altamente qualificados em áreas costeiras no país (Werner et al., 2004). Em países em desenvolvimento, o cultivo de macroalgas pode ser uma valiosa fonte de renda para comunidades costeiras afetadas pela pesca comercial (Buschmann et al., 2017).

Dentro do cultivo de macroalgas, uma técnica que vem sendo difundida é o Sistema de Cultivo Multitrófico Integrado (IMTA, na sigla em inglês). Nesse sistema, as macroalgas são cultivadas em fazendas para criação de peixes, crustáceos ou moluscos (Al-Hafedh et al., 2014; Radulovich et al., 2015). Este cultivo integrado leva a uma maior diversificação da produção, atendendo mais de um mercado e gerando assim uma maior renda para os aquacultores. Outro fator importante são os serviços ambientais prestados. Nesse sistema, as macroalgas absorvem os nutrientes oriundos da produção pesqueira, atuando como biofiltros e removendo o excesso de nutrientes. Ainda captam o gás carbônico atmosférico através da fotossíntese, ajudando a reduzir a concentração desse gás de efeito estufa no ambiente. Além disso, o processo fotossintético das macroalgas é altamente eficiente, (6-8%), muito superior às plantas terrestres (1,8-2,2%) (Chen et al., 2015; Fernand et al., 2017).

O aumento do cultivo de macroalgas em até 14% por ano poderia gerar 500 milhões de toneladas de biomassa (em peso seco) em 2050, aumentando em 10% a oferta atual de alimentos, gerando renda e melhorando a qualidade ambiental

(Yarish, 2016).

Biorrefinarias

Um dos principais gargalos para o desenvolvimento de bioprodutos extraídos de macroalgas são os elevados custos com tecnologia, tanto para a produção de biomassa como para a extração dos compostos desejados (Holdt and Kraan, 2011; Jiang et al., 2016). A solução mais proeminente para essas questões seria a implementação do conceito de biorrefinarias. Similar a refinarias de petróleo, onde as diferentes frações dos produtos derivados são extraídos, na biorrefinaria múltiplos compostos podem ser obtidos através do fracionamento da biomassa utilizada (Gerardo et al., 2015).

Esse conceito visa otimizar o uso de recursos, minimizar custos e maximizar os lucros (Sander et al. 2016), extraindo em um só local produtos de elevado valor agregado, como fármacos, e comodities como biocombustíveis e biomassa para alimentação animal e fertilizantes (Jiang et al., 2016; Trivedi et al., 2013). Além disso, não há descarte de biomassa, já que cada fração é aproveitada. Uma importante vantagem do conceito de biorrefinaria, são os serviços ambientais prestados, como a mitigação da emissão de gases do efeito estufa, a substituição do uso de combustíveis fósseis, através da produção de biocombustíveis que não competem por terras aráveis ou água doce, a biorremediação de ambientes marinhos, entre outros (Buschmann et al., 2017; Fernand et al., 2017; Trivedi et al., 2013). Atualmente existem diversas iniciativas para implementação e desenvolvimento de biorrefinarias na Europa (Kraan, 2010).

Referências Bibliográficas

Al-Hafedh, Y. S., Alam, A., and Buschmann, A. H. (2014). Bioremediation potential, growth and biomass yield of the green seaweed, *Ulva lactuca* in an integrated marine aquaculture system at the Red Sea coast of Saudi Arabia at different stocking densities and effluent flow rates. *Reviews in Aquaculture*, 7(3):161–171.

Alaswad, A., Dassisti, M., Prescott, T., and Olabi, A. (2015). Technologies and developments of third generation biofuel production. *Renewable and Sustainable Energy Reviews*, 51:1446–1460.

Ariede, M. B., Candido, T. M., Jacome, A. L. M., Velasco, M. V. R., de Carvalho, J. C. M., and Baby, A. R. (2017). Cosmetic attributes of algae - a review. *Algal Research*, 25:483–487.

Biba, E. (2014). Protection: The sunscreen pill. *Nature*, 515(7527):S124–S125.

Buschmann, A. H., Camus, C., Infante, J., Neori, A., Israel, Á., Hernández-González, M. C., Pereda, S. V., Gomez-Pinchetti, J. L., Golberg, A., Tadmor-Shalev, N., and Critchley, A. T. (2017). Seaweed production: overview of the global state of exploitation, farming and emerging research activity. *European Journal of Phycology*, 52(4):391–406.

Camus, C., Infante, J., and Buschmann, A. H. (2016). Overview of 3 year precommercial seafarming of *Macrocystis pyrifera* along the Chilean coast. *Reviews in Aquaculture*, 10(3):543–559.

Cardozo, K. H., Guaratini, T., Barros, M. P., Falcão, V. R., Tonon, A. P., Lopes, N. P., Campos, S., Torres, M. A., Souza, A. O., Colepicolo, P., and Pinto, E. (2007). Metabolites from algae with economical impact. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 146(1-2):60–78.

Chemodanov, A., Jinjikhashvily, G., Habiby, O., Liberzon, A., Israel, A., Yakhini, Z., and Golberg, A. (2017). Net primary productivity, biofuel production and CO 2 emissions reduction potential of *Ulva sp.* (Chlorophyta) biomass in a coastal area of the Eastern Mediterranean. *Energy Conversion and Management*, 148:1497–1507.

Chen, H., Zhou, D., Luo, G., Zhang, S., and Chen, J. (2015). Macroalgae for biofuels production: Progress and perspectives. *Renewable and Sustainable Energy Reviews*, 47:427–437.

Craigie, J. S. (2010). Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology*, 23(3):371–393.

Dillehay, T. D., Ramirez, C., Pino, M., Collins, M. B., Rossen, J., and Pino-Navarro, J. D. (2008). Monte verde: Seaweed, food, medicine, and the peopling of south america. *Science*, 320(5877):784–786.

E., M.-S., W.S.C., M., and M.A.A., C. (2006). Some aspects of the growth of *Gracilaria birdiae* (Gracilariales, Rhodophyta) in an Estuary in Northeast Brazil. *Aquaculture International*, 14(4):327–336.

Evans, F. D. and Critchley, A. T. (2013). Seaweeds for animal production use. *Journal of Applied Phycology*, 26(2):891–899.

FAO (2018). *The state of world fisheries and aquaculture 2018 : meeting the sustainable development goals*. Food and Agriculture Organization of the United Nations, Rome.

Fernand, F., Israel, A., Skjermo, J., Wichard, T., Timmermans, K. R., and Golberg, A. (2017). Offshore macroalgae biomass for bioenergy production: Environmental aspects, technological achievements and challenges. *Renewable and Sustainable Energy Reviews*, 75:35–45.

Fleurence, J. (1999). Seaweed proteins. *Trends in Food Science & Technology*, 10(1):25–28.

Fleurence, J. (2016). Seaweeds as food. In *Seaweed in Health and Disease Prevention*, pages 149–167. Elsevier.

Fleurence, J., Morançais, M., Dumay, J., Decottignies, P., Turpin, V., Munier, M., Garcia-Bueno, N., and Jaouen, P. (2012). What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture? *Trends in Food Science & Technology*, 27(1):57–61.

Gerardo, M. L., Hende, S. V. D., Vervaeren, H., Coward, T., and Skill, S. C. (2015). Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants. *Algal Research*, 11:248–262.

Hafting, J. T., Craigie, J. S., Stengel, D. B., Loureiro, R. R., Buschmann, A. H., Yarish, C., Edwards, M. D., and Critchley, A. T. (2015). Prospects and

challenges for industrial production of seaweed bioactives. *Journal of Phycology*, 51(5):821–837.

Hasan, M. M. (2017). Algae as nutrition, medicine and cosmetic: the forgotten history, present status and future trends. *World Journal of Pharmacy and Pharmaceutical Sciences*, pages 1934–1959.

Hayashi, L., Bulboa, C., Kradolfer, P., Soriano, G., and Robledo, D. (2013). Cultivation of red seaweeds: a Latin American perspective. *Journal of Applied Phycology*, 26(2):719–727.

Holdt, S. L. and Kraan, S. (2011). Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology*, 23(3):543–597.

Jiang, R., Ingle, K. N., and Golberg, A. (2016). Macroalgae (seaweed) for liquid transportation biofuel production: what is next? *Algal Research*, 14:48–57.

Kawai, S. and Murata, K. (2016). Biofuel production based on carbohydrates from both brown and red macroalgae: Recent developments in key biotechnologies. *International Journal of Molecular Sciences*, 17(2):145.

Kijjoa, A. and Sawangwong, P. (2004). Drugs and cosmetics from the sea. *Marine Drugs*, 2(2):73–82.

Kim, S.-K., Ravichandran, Y. D., Khan, S. B., and Kim, Y. T. (2008). Prospective of the cosmeceuticals derived from marine organisms. *Biotechnology and Bioprocess Engineering*, 13(5):511–523.

Kraan, S. (2010). Mass-cultivation of carbohydrate rich macroalgae, a possible solution for sustainable biofuel production. *Mitigation and Adaptation Strategies for Global Change*, 18(1):27–46.

Leal, M. C., Rocha, R. J. M., Rosa, R., and Calado, R. (2016). Aquaculture of marine non-food organisms: what, why and how? *Reviews in Aquaculture*, 10(2):400–423.

Makkar, H. P., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F., and Ankers, P. (2016). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology*, 212:1–17.

Mazarrasa, I., Olsen, Y. S., Mayol, E., Marbà, N., and Duarte, C. M. (2013). Rapid growth of seaweed biotechnology provides opportunities for developing nations. *Nature Biotechnology*, 31(7):591–592.

Milledge, J., Smith, B., Dyer, P., and Harvey, P. (2014). Macroalgae-derived biofuel: A review of methods of energy extraction from seaweed biomass. *Energies*, 7(11):7194–7222.

Mohamed, S., Hashim, S. N., and Rahman, H. A. (2012). Seaweeds: A sustainable functional food for complementary and alternative therapy. *Trends in Food Science & Technology*, 23(2):83–96.

Mustafa, G., Wakamatsu, S., aki Takeda, T., Umino, T., and Nakagawa, H. (1995). Effects of algae meal as feed additive on growth, feed efficiency, and body composition in red sea bream. *Fisheries science*, 61(1):25–28.

Neushul, P. (1989). Seaweed for war: California's world war I kelp industry. *Technology and Culture*, 30(3):561.

Pimentel, F., Alves, R., Rodrigues, F., and Oliveira, M. P. P. (2017). Macroalgae-derived ingredients for cosmetic industry—an update. *Cosmetics*, 5(1):2.

Radulovich, R., Neori, A., Valderrama, D., Reddy, C., Cronin, H., and Forster, J. (2015). Farming of seaweeds. In *Seaweed Sustainability*, pages 27–59. Elsevier.

Rebours, C., Marinho-Soriano, E., Zertuche-González, J. A., Hayashi, L., Vásquez, J. A., Kradolfer, P., Soriano, G., Ugarte, R., Abreu, M. H., Bay-Larsen, I., Hovelsrud, G., Rødven, R., and Robledo, D. (2014). Seaweeds: an opportunity for wealth and sustainable livelihood for coastal communities. *Journal of Applied Phycology*, 26(5):1939–1951.

Rhein-Knudsen, N., Ale, M., and Meyer, A. (2015). Seaweed hydrocolloid production: An update on enzyme assisted extraction and modification technologies. *Marine Drugs*, 13(6):3340–3359.

Rioux, L.-E., Beaulieu, L., and Turgeon, S. L. (2017). Seaweeds: A traditional ingredients for new gastronomic sensation. *Food Hydrocolloids*, 68:255–265.

Smit, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology*, 16(4):245–262.

Stévant, P., Rebours, C., and Chapman, A. (2017). Seaweed aquaculture in norway: recent industrial developments and future perspectives. *Aquaculture International*, 25(4):1373–1390.

Suutari, M., Leskinen, E., Fagerstedt, K., Kuparinen, J., Kuuppo, P., and Blomster, J. (2014). Macroalgae in biofuel production. *Phycological Research*, 63(1):1–18.

Trivedi, N., Gupta, V., Reddy, C., and Jha, B. (2013). Enzymatic hydrolysis and production of bioethanol from common macrophytic green alga *Ulva fasciata* delile. *Bioresource Technology*, 150:106–112.

Valderrama, D., Cai, J., Hishamunda, N., Ridler, N., Neish, I. C., Hurtado, A. Q., Msuya, F. E., Krishnan, M., Narayanakumar, R., Kronen, M., Robledo, D., Gasca-Leyva, E., and Fraga, J. (2015). The economics of *Kappaphycus* seaweed cultivation in developing countries: A comparative analysis of farming systems. *Aquaculture Economics & Management*, 19(2):251–277.

van Hal, J. W., Huijgen, W., and López-Contreras, A. (2014). Opportunities and challenges for seaweed in the biobased economy. *Trends in Biotechnology*, 32(5):231–233.

Wang, H.-M. D., Chen, C.-C., Huynh, P., and Chang, J.-S. (2015). Exploring the potential of using algae in cosmetics. *Bioresource Technology*, 184:355–362.

Wang, H.-M. D., Li, X.-C., Lee, D.-J., and Chang, J.-S. (2017). Potential biomedical applications of marine algae. *Bioresource Technology*, 244:1407–1415.

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., Smith, A. G., Camire, M. E., and Brawley, S. H. (2016). Algae as nutritional and functional food sources: revisiting our understanding. *Journal of Applied Phycology*, 29(2):949–982.

Yarish, C. (2016). *Seaweed Aquaculture for Food Security, Income Generation and Environmental Health in Tropical Developing Countries*. World Bank.