LEANDRO AMARAL HERRERA

Caracterização das linhagens tetrasporofíticas e gametofíticas “Edison de Paula” de *Kappaphycus alvarezii* (Rhodophyta) cultivadas na Enseada de Ubatuba, SP

Tese apresentada ao Instituto de Pesquisas Ambientais da Secretaria de Meio Ambiente, Infraestrutura e Logística, como parte dos requisitos exigidos para a obtenção do título de DOUTOR em BIODIVERSIDADE VEGETAL E MEIO AMBIENTE, na Área de Concentração de Plantas Avasculares e Fungos em Análises Ambientais.

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À minha querida esposa

e ao meu filho,

aos meus pais,

com muito amor e carinho!

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LISTA DE ABREVIATURAS

ANOVA	Análise de Variância
APC	Aloficocianina
APTA	Agência Paulista de Tecnologia dos Agronegócios
ATP	Adenosina Trifosfato
BSA	Albumina Sérica Bovina
Ca	Carotenóides
Chla	Clorofila α
DTT	Ditiotreitol
DW	Massa seca (Dry Weight)
EDTA	Ácido Etilenodiamino Tetra-Acético
E_K	Irradiância de Saturação Luminosa
ETR	Taxa de Transporte de Elétrons
ETR_{max}	Taxa de Transporte de Elétrons Máximo
FAO	Food and Agriculture Organization (ONU)
FW	Peso Fresco (Fresh Weight)
G11	Gametófito, ramo número 11
GR	Taxa de Crescimento (Growth Rate)
EMFFI	Fazenda Marinha Experimental do Instituto de Pesca
INMET	Instituto Nacional de Meteorologia
MS	Massa seca
PAR	Radiação Fotossinteticamente Ativa
PC	Ficocianina
PCA	Análise de Componentes Principais
PE	Ficoeritrina
pH	Potencial Hidrogeniônico
PVC	Policloreto de Vinila
SD	Standart Deviation
SM	Material Suplementar
TSP	Proteínas Solúveis Totais
USP	Universidade de São Paulo
α	Eficiência Fotossintetizante
$\Delta F/F_m'$	Rendimento Quântico Efetivo

RESUMO

A macroalga marinha *Kappaphycus alvarezii* (Doty) L.M. Liao (Rhodophyta, Gigartinales) é um importante recurso marinho para a produção comercial de carragenana (polissacarídeo sulfatado), que é utilizada nos setores alimentício, cosmético e farmacêutico. Nas regiões Sul e Sudeste do Brasil, cultivo de *K. alvarezii* demonstra elevado potencial para a geração de renda e o fortalecimento da economia local. Há 30 anos, os cultivos experimentais vêm sendo conduzidos na Fazenda Marinha Experimental do Instituto de Pesca (EMFFI), Enseada de Ubatuba, no estado de São Paulo, enquanto iniciativas comerciais têm se expandido no sul do Rio de Janeiro e em Santa Catarina. Na EMFFI, foram identificadas linhagens espontâneas com distintas colorações, originadas do gametófito marrom-claro "Edison de Paula – EP", cuja caracterização permanece limitada. Este trabalho teve como objetivo avaliar o crescimento, produtividade, parâmetros fotossintetizantes, variáveis bioquímicas (pigmentos, proteínas e carboidratos) e rendimentos da carragenana (teores de 3,6-anidrogalactose e de sulfatos) de quatro linhagens: três gametófitos EP; marrom-claro (E-br), verde-claro (E-lg) e amarelo-esverdeado (E-gy), e o tetrasporófito marrom (T-br), que originou o gametófico EP marrom-claro. Amostras (n = 20) de cada linhagem foram coletadas ao final de cada ciclo de crescimento de 45 dias, ao longo de um ano, em balsa flutuante instalada no ambiente marinho. Os fatores abióticos, incluindo temperatura, salinidade, transparência da água e índices pluviométricos foram monitorados regularmente no entorno da área de cultivo. Inicialmente o estudo identificou que o "efeito de borda" do desenho experimental proposto impactou negativamente as taxas de crescimento, sendo mitigado por um novo arranjo metodológico fundamentado em estatística e controle experimental. Diversos fatores interativos, exógenos e endógenos, determinaram os padrões de sazonalidade, impactando diretamente a taxa de crescimento e produtividade, que foi maior durante o verão. Nesse período, observou-se uma redução nos níveis de proteínas solúveis totais e um aumento nos carotenoides totais, sugerindo adaptações fotoprotetoras. No inverno, os teores de carragenana foram mais elevados. Adicionalmente, nossos resultados destacam a influência da ploidia na determinação das respostas fisiológicas, com maiores quantidades de ficobiliproteínas observadas no tetrasporófito, sugerindo sua influência nas maiores taxas de crescimento e refletindo para o aumento de produtividade. Ademais, os resultados apontam que as linhagens E-lg e E-gy apresentam maior similaridade entre si do que com a linhagem E-br (gametófito genitor), destacando-se que a linhagem E-gy apresenta maior eficiência fotossintetizante. Por outro lado, E-lg apresentou menores taxas de crescimento e concentrações de ficoeritrina. Surpreendentemente, os rendimentos de carragenana e conteúdo de sulfatos foram similares entre as linhagens, e variaram sazonalmente. Os resultados obtidos

evidenciam a importância de integrar fatores genéticos, fisiológicos e sazonais no manejo sustentável de *K. alvarezii*.

Palavras-chave: Algicultura, Carragenana, Gametófitos, Pigmentos, Proteínas, Tetrasporófitos

ABSTRACT

The seaweed *Kappaphycus alvarezii* (Doty) L.M. Liao (Rhodophyta, Gigartinales) is an important marine resource for the commercial production of carrageenan (sulfated polysaccharide) used in the food, cosmetic, and pharmaceutical sectors. Cultivation of *K. alvarezii* in the South and Southeast regions of Brazil demonstrates a high potential for generating income and strengthening the local economy. For 30 years, experimental cultivation has been conducted at the Experimental Marine Farm of the Fisheries Institute (EMFFI), Ubatuba Bay, São Paulo State, while commercial initiatives have expanded to the south of Rio de Janeiro and Santa Catarina state. In the EMFFI, spontaneous strains of different colors originating from the pale-brown gametophyte "Edison de Paula – EP" were identified, but their characterization have remained limited. This study aimed to evaluate the growth, productivity, photosynthetic parameters, biochemical variables (pigments, proteins and carbohydrates) and carrageenan properties (yield and 3,6-anhydrogalactose and sulfate contents) of four strains: three EP gametophytic strains: pale-brown (E-br), light-green (E-lg) and greenish-yellow (E-gy), and the brown tetrasporophyte (T-br), which originated the pale-brown EP gametophyte. Samples (n = 20) of each strain were collected at the end of each 45-day growth cycle over one year, on a floating frame installed in the marine environment. Abiotic factors, including temperature, salinity, water transparency, and rainfall, were regularly monitored around the cultivation area. Initially, the study identified that the "edge effect" of the proposed experimental design negatively impacted growth rates, which was mitigated by a new methodological arrangement based on statistics and experimental control. Several interactive factors, exogenous and endogenous, determined the seasonal patterns, directly impacting growth rate and productivity, which were higher during the summer. During this period, a reduction in the levels of total soluble proteins and an increase in total carotenoids were observed, suggesting photoprotective responses. In the winter, carrageenan levels were higher. Additionally, our results highlight the influence of ploidy in determining physiological responses since higher concentrations of phycobiliproteins were observed in the tetrasporophyte, suggesting their influence on higher growth rates and, consequently, increased productivity. The results show that E-lg and E-gy strains are more similar to each other than to E-br strain (the parental gametophyte), and E-gy strain has higher photosynthetic efficiency. On the other hand, E-lg strain showed lower

growth rates and phycoerythrin concentrations. Surprisingly, the yields of carrageenan and its sulfate contentes were similar among strains, and varied seasonally. The results reinforce the importance of integrating genetic, physiological, and seasonal factors in the sustainable management of *K. alvarezii*.

Keywords: Algiculture, Carrageenan, Gametophytes, Pigments, Proteins, Tetrasporophytes.

1. Introdução geral

1.1 Importância econômica de Kappaphycus alvarezii

Segundo estatísticas da FAO (2024), a produção de aquicultura mundial atingiu um recorde histórico de 37,6 milhões de toneladas anuais de algas frescas. Historicamente, apesar da desaceleração nos últimos anos nas taxas de expansão, o cultivo de algas vermelhas, como *Kappaphycus alvarezii* (Doty) L.M. Liao (Rhodophyta, Gigartinales) e *Eucheuma* spp.tem sido expressivo devido à demanda por carragenana (Hayashi et al., 2011; Paz-Cedeno et al., 2019; FAO 2024).

As Filipinas são responsáveis por mais de 81% da produção global de *K. alvarezii*, totalizando 1,8 milhões de toneladas anuais (FAO, 2022). No Brasil, de acordo com o Ministério da Pesca e Aquicultura, a produção de macroalgas frescas alcançou 666,4 toneladas no ano 2022. Esse cenário reflete uma produção incipiente, impulsionada pelo aumento da produção de *K. alvarezii* nos estados de Santa Catarina e Rio de Janeiro (MPA, 2023).

Os cultivos de *K. alvarezii* estabeleceram-se como uma commodity de elevada relevância na região Ásia-Pacífico (Aslan et al., 2015), evidenciando papel preponderante na produção de hidrocolóides derivados de algas marinhas, dos quais a kappa-carragenana apresenta a maior demanda. (Hurtado et al., 2017).

A kappa-carragenana, hidrocolóide extraído de *Kappaphycus alvarezii*, destaca-se por sua capacidade de formar géis fortes e rígidos (Hayashi et al., 2011; Hurtado et al., 2017; Paz-Cedeno et al., 2019), apresentando propriedades gelificantes e estabilizadoras essenciais para aplicações nas indústrias alimentícia, farmacêutica e nutracêutica (Collén et al., 2014).

Na indústria alimentícia, as carragenanas são amplamente utilizadas como aditivos funcionais, atuando como agentes gelificantes, emulsificantes, espessantes e estabilizadores (Pickering, 2006; Holdt and Kraan, 2011; Porse and Rudolph, 2017).

Do ponto de vista nutricional, *K. alvarezii* é consumida na alimentação humana (Ask and Azanza, 2002; Makkar et al., 2016), sendo considerada um alimento hipocalórico, rico em fibras, vitaminas (A, B1, B12, C) e minerais (Kumar and Kaladharan, 2007; Kumar et al., 2014). Além disso, contém substâncias bioativas, como polissacarídeos, proteínas, lipídios e polifenóis, que conferem propriedades antibacterianas, antivirais e antifúngicas, bem como oligoelementos e uma ampla variedade de metabólitos secundários não encontrados em outros organismos (Kumar et al., 2007; Holdt and Kraan, 2011), além de compostos com ação antioxidante (Araújo et al., 2023). É também fonte natural de tiamina, riboflavina, β-caroteno e tocoferóis (Kumar and Kaladharan, 2007; Kumar et al., 2014).

O amido das florídeas, principal reserva energética das rodofíceas, apresenta elevado interesse biotecnológico devido às suas características estruturais singulares, configurando-se como uma fonte potencial de glicose para processos fermentativos, especialmente em indústrias que demandam substratos de rápida degradação, como na produção de bioetanol (Paz-Cedeno et al., 2019).

Estudos sobre o metabolismo desses polissacarídeos em algas vermelhas têm fornecido importantes *insights* para o desenvolvimento de cultivos voltados à produção de biomassa algácea com aplicações bioenergéticas (Gügi et al., 2015).

Além disso, *K. alvarezii* constitui uma fonte promissora de biocombustíveis de terceira geração, devido ao seu elevado teor de carboidratos (Masarin et al., 2016; Roldán et al., 2017; Solorzano-Chavez et al., 2019), destacando-se também como matéria-prima potencial para a produção de hidrogênio por meio de processos fermentativos (Masarin et al., 2016; Dalbelo, 2017; Rodrigues et al., 2019; Fonseca et al., 2020).

A utilização de *K. alvarezii* como biofertilizante vem ocorrendo em alguns países (Hutado et al. 2021) e, o Brasil tem se consolidado como uma alternativa promissora, impulsionada pela presença de citocininas e microelementos que contribuem para o aumento da produtividade e a melhoria da nutrição das plantas (Pramanick et al., 2014; Gelli et al., 2020). Além disso, extratos de *K. alvarezii*, empregados como bioestimulantes orgânicos, têm demonstrado eficácia em diversas culturas agrícolas, promovendo práticas mais sustentáveis e ambientalmente responsáveis (Paz-Cedeno et al., 2019; Gelli et al., 2020; Gupta and Van Staden, 2021).

No presente estudo, a quantificação precisa da biomassa e dos compostos bioativos de diferentes linhagens, incluindo as variantes espontâneas denominadas “Edison de Paula”, permitirá a identificação de cultivos mais produtivos e economicamente viáveis. Tal abordagem contribuirá para o desenvolvimento de técnicas aprimoradas de cultivo e de extração de metabólitos de interesse biotecnológico.

Estudos experimentais voltados ao cultivo marinho e à caracterização bioquímica de linhagens espontâneas são fundamentais para a seleção de variantes com maior potencial de produtividade e qualidade, representando um avanço estratégico para a consolidação da cadeia produtiva de macroalgas.

1.2 Rhodophyta

O filo Rhodophyta é caracterizado pela combinação de seis características: células eucarióticas; ausência de flagelos; produto de reserva denominado amido das florídeas (α-D-Glucose, com ligações glicosídicas do tipo α(1→4) e pontos de ramificação no C-6, semelhante à amilopectina dos vegetais) armazenado no citoplasma e não nos cloroplastos; ficoeritrina,

ficocianina e aloficocianina como pigmentos acessórios; cloroplastos com tilacóides não agregados (não empilhados); e cloroplastos sem retículo endoplasmático externo (Cole and Sheath, 1990).

As espécies de Rhodophyta podem apresentar diferentes tipos de histórico de vida que são mais complexos do que aqueles apresentados pelos demais grupos de algas por não terem células com flagelos. *Kappaphycus alvarezii* apresenta o histórico de vida do tipo Polysiphonia (Fig. 1-1), que apresenta alternância de gerações isomórficas (tetrasporófito (2n) e gametófito (n) são morfologicamente semelhantes) e mais uma fase diplóide (o carposporófito) crescendo sobre o gametófito feminino, ampliando o número e dispersão de carpósporos o que pode suprir a falta de flagelos (West and Hommersand, 1981).

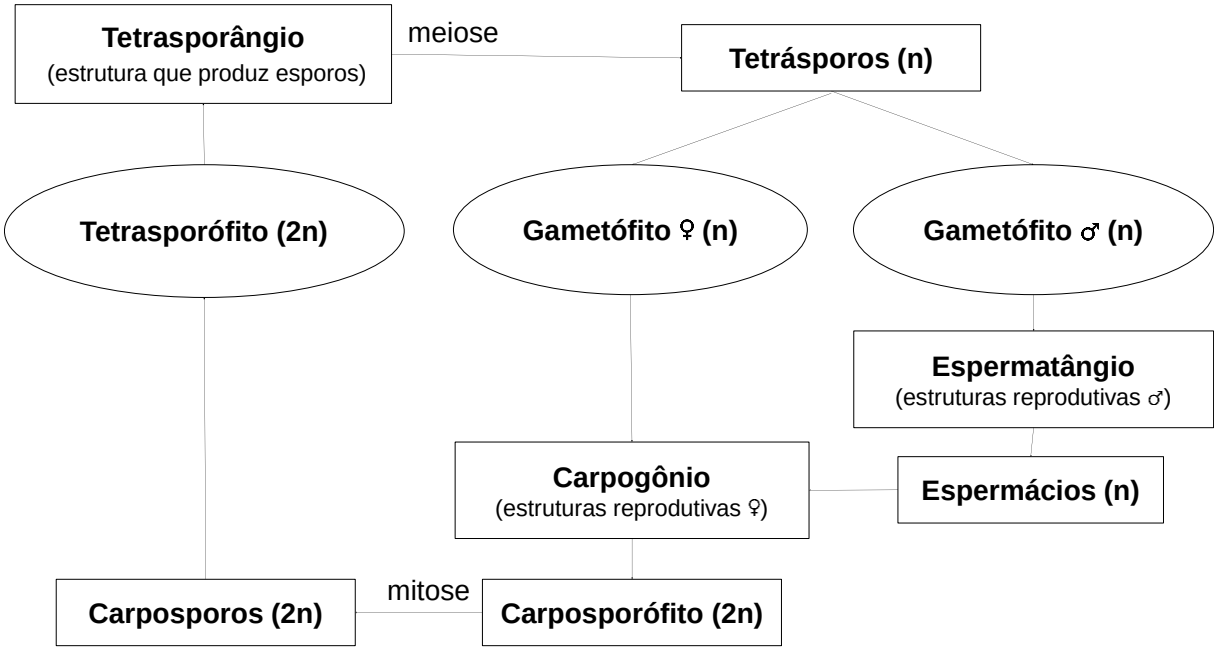

Figura 1-1: Esquema representativo do histórico de vida trifásico, com alternância de gerações isofórmicas, presentes em *Kappaphycus alvarezii* (Roleda et al., 2024 modificado).

Clorofila *a*, carotenóides e ficobiliproteínas são os principais pigmentos fotossintéticos que facilitam a absorção eficiente de luz nas rodofíceas e são partes integrantes de toda a maquinaria fotossintética (Simkin et al., 2022). Os ficobilissomos (Fig. 1-2) estão presentes

nas membranas dos tilacóides das algas vermelhas (Zhao et al., 2016) e funcionam não apenas como antenas para captar a luz, mas também como armazenamento de nitrogênio que é um nutriente limitante do crescimento de organismos fotossintetizantes (Bird et al., 1982).

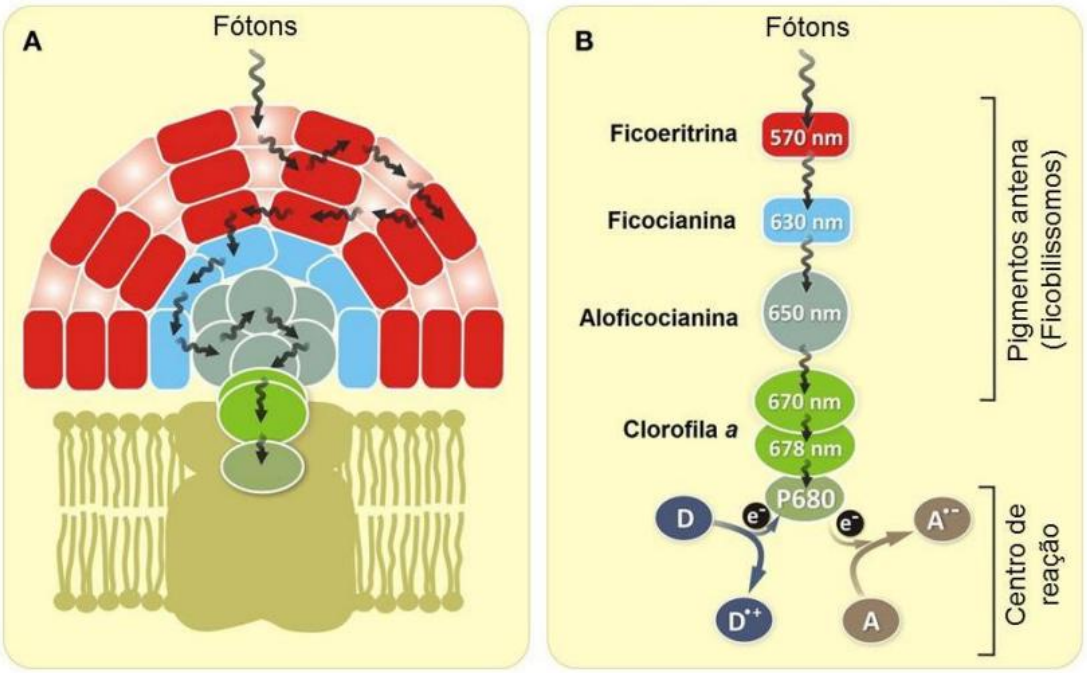

Figura 1-2: Ficobilissomos – Organização estrutural do complexo antena do fotossistema II (FSII) de algas vermelhas (A) e o processo de transferência de energia (B), modificado de Gvindjee and Shevala (2011).

No ambiente marinho, as ficobiliproteínas capturam um amplo espectro de luz e permitem que as algas vermelhas cresçam em águas mais profundas, onde outros comprimentos de onda são atenuados pela coluna de água (Glazer, 1985). A ficoeritrina absorve a cor verde, amarela e vermelha, enquanto a ficocianina absorve a luz azul, verde e amarela. Estes são os espectros de luz que mais penetram o fundo do mar, permitindo às algas vermelhas sobreviverem em condições com baixa irradiância (Hurtado et al., 2017).

As rodofíceas são um grupo de organismos marinhos amplamente estudados por suas propriedades bioquímicas, especialmente em relação aos carboidratos (McHugh, 2003; Duarte

and Noseda, 2015; Hurtado et al., 20121). Os principais carboidratos encontrados nas rodofíceas são os polissacarídeos sulfatados, como agarose, agaropectina e carragenanas (McHugh, 2003; Usov, 2011).

Os galactanos são componentes importantes de muitas paredes celulares vegetais e nas algas marinhas são os principais polissacarídeos em matrizes extracelulares (Ciancia et al., 2020).

Em particular, a maioria das algas vermelhas biossintetizam galactanos sulfatados com uma estrutura que não tem equivalente em plantas terrestres, constituída por alternar unidades b-D-galactopiranosil de 3 ligações (unidade A) e resíduos a-galactopiranosil de 4 ligações (unidade B). Nas algas industrialmente utilizadas como fonte de hidrocolóides, as unidades B pertencem à série D e produzem carrageninas (como em Gigartinales), ou à série L, e são fontes de agarose e/ou polímeros estruturalmente relacionados (por exemplo, Gelidiales, Gracilariales). Em ambos os casos, as últimas unidades aparecem como 3,6-anidro-a-galactose ciclizada em uma certa quantidade (Fig 1-3).

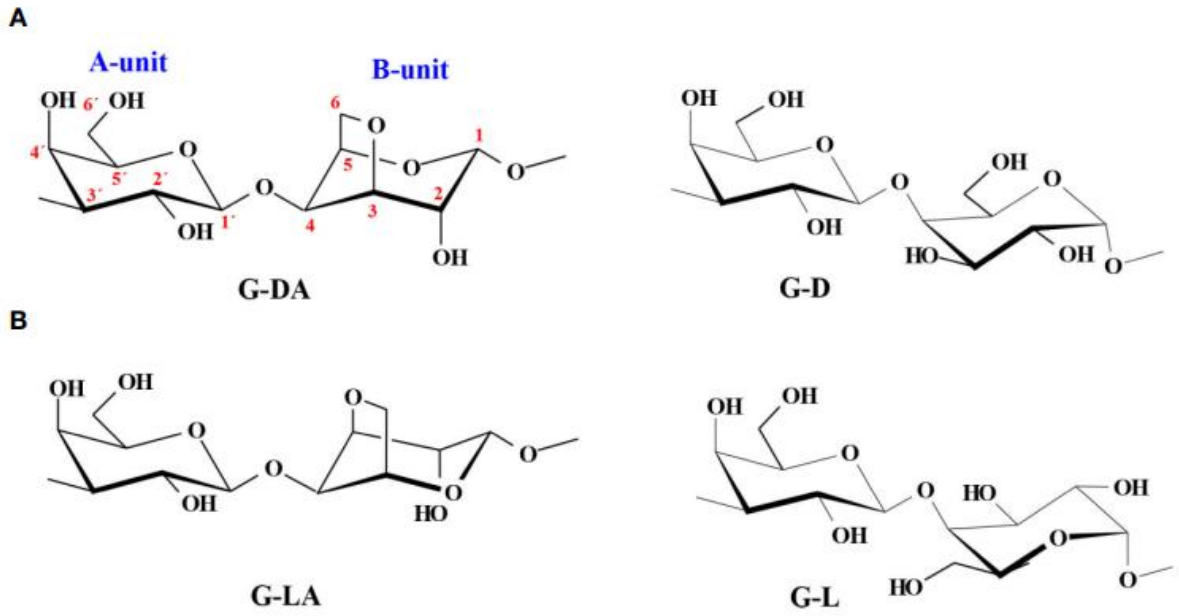

Figura 1-3: Esquemas diasteroméricos alternados de galactanas sulfatadas de algas vermelhas, nomeados de acordo com a nomenclatura atualmente aceita de Knutsen et al., 1994. Esquemas de carragenana (A), Esquemas de agarana (B). O esqueleto de agarana ciclizado (G-LA)

corresponde à estrutura idealizada da agarose. O sistema de numeração é mostrado em vermelho.

Em ambos os casos, as últimas unidades aparecem como 3,6-anidro-a-galactose ciclizada em certas quantidades, que podem ser aumentadas pela ciclização alcalina de unidades de a-galactose 6-sulfato (Ciancia et al., 2020).

Outra característica interessante desses galactanos é a variação nos padrões de sulfatação, que modula seu comportamento físico em soluções aquosas (Hehemann et al. 2012). Embora as carragenanas mais comuns sejam dos tipos κ/ι e λ (com unidades A sulfatadas nas posições 4 e 2, respectivamente), outros tipos de carragenanas foram relatados correspondentes (Ciancia et al., 2020).

Estes polissacarídeos são importantes em algumas funções biológicas e adaptativas das algas ao ambiente marinho, pois mantêm a umidade quando expostas ao dessecamento em marés baixas, a concentração celular interna em ocasiões de chuva, e conferem flexibilidade ao talo (Usov, 2011).

O amido das florídeas é produzido durante o processo de fotossíntese, servindo como uma reserva energética para períodos em que a disponibilidade de luz ou nutrientes no ambiente marinho é limitada, desempenhando um papel crucial no metabolismo das algas vermelhas e em suas adaptações ecológicas (Yoon et al., 2006).

As cadeias de glicose no amido das florídeas são menos organizadas e mais amorfas do que o amido de plantas superiores, o que facilita sua mobilização rápida em momentos de necessidade energética. Essa estrutura mais ramificada também confere a esse amido propriedades físico-químicas diferentes, como maior solubilidade em água e uma digestibilidade mais rápida (Delattre et al., 2016).

Durante a noite ou em condições de estresse ambiental, como variações na temperatura ou na disponibilidade de nutrientes, as algas vermelhas mobilizam o amido florideano para sustentar seu metabolismo (Lee, 2018). Contudo, estudos indicam que, em *Neopyropia haitanensis*, o amido florideano é mantido, enquanto o floridosídeo é metaboliziado sob condições de escuro, sugerindo alterações na interação metabólica da rede "amido das florídeas–floridosídeo" e nas suas bases genéticas (Yu et al., 2021).

1.3 Histórico do cultivo de *Kappaphycus alvarezii* no Brasil

No Brasil, os primeiros experimentos com a macroalga *Kappaphycus alvarezii* foram iniciados a partir de um talo com aproximadamente 2,5 g, proveniente de uma cultura experimental desenvolvida no Japão (USA Marine Institute – University of Kochi). Essa linhagem, considerada saudável e produtiva, teve origem em cultivos comerciais estabelecidos nas Filipinas (Paula and Pereira, 1998; Paula et al., 2001; Gelli et al., 2024).

O talo inicial, de coloração marrom, foi propagado em cultura unialgal sob condições axênicas durante dez meses em laboratório. Posteriormente, os fragmentos foram transferidos para cultivo experimental *ex situ* em ambiente marinho, conduzido em parceria com o Instituto de Pesca, no município de Ubatuba, SP (Paula and Pereira, 1998; Paula et al., 2001).

A metodologia adotada priorizou o transplante de propágulos previamente cultivados em ambiente controlado, reduzindo os riscos de introdução de espécies exóticas e assegurando a avaliação fitossanitária dos talos (Gelli et al., 2024).

Durante o cultivo marinho, observaram-se variações espontâneas na pigmentação dos talos, alternando entre colorações verde, vermelha e marrom, indicativas de plasticidade

fenotípica (Paula et al., 1999). Essas culturas de *K. alvarezii* permanecem sob monitoramento contínuo até os dias atuais (Gelli et al., 2024).

Paralelamente, estudos laboratoriais realizados no Instituto de Biologia da Universidade de São Paulo (USP) investigaram o ciclo de vida da espécie sob condições controladas (Paula et al., 2001). O talo marrom original, identificado como pertencente à fase esporofítica tetrasporófita, produziu tetrásporos que originaram gametófitos com distintas características de coloração, morfologia e taxa de crescimento (Paula et al., 1999).

No entanto, a germinação *in vitro* dos tetrásporos apresentou elevada taxa de mortalidade entre dois e quatro dias após a liberação, sendo que apenas 20 indivíduos foram cultivados com sucesso, mantendo-se viáveis por mais de um ano (Paula et al., 1999). Esses resultados evidenciaram o potencial biotecnológico das progênies de tetrásporos como material genético para a seleção de novas linhagens.

Algumas dessas progênies foram propagadas experimentalmente no mar, em Ubatuba, SP. Após sete meses de cultivo, apenas dois indivíduos sobreviveram, apresentando baixas taxas de crescimento. Essas linhagens também exibiram modificações morfológicas, como aumento no número de ramificações e alterações na pigmentação (Paula et al., 1999). Entre os indivíduos sobreviventes, um gametófito foi selecionado em razão de sua estabilidade e características desejáveis, sendo denominado “G11”, cuja ploidia foi confirmada por meio de microscopia de fluorescência confocal (Zitta et al., 2012). Posteriormente, em homenagem ao pesquisador responsável, essa linhagem foi nomeada “Edison de Paula” (Hayashi, 2007).

Embora se espere maior variabilidade genética entre progênies de tetrásporos em comparação às de carpósporos, a amplitude de variação fenotípica observada nesse estudo revelou-se incomum e aparentemente sem precedentes entre as espécies de algas conhecidas (Paula et al., 1999).

Ao final desses estudos, demonstrou-se que três linhagens com diferentes colorações — marrom, verde e vermelha — apresentaram taxas de crescimento e rendimentos de carragenana semelhantes. Em contrapartida, a linhagem "G11" destacou-se por seu menor crescimento, com maior teor de 3,6-anhidrogalactose e força de gel superior (Paula et al., 1999; Hayashi et al., 2008).

O cultivo comercial de *K. alvarezii* no Brasil teve início em 1998, na Baía da Ilha Grande, RJ, utilizando propágulos importados da Venezuela (Gelli et al., 2024). Contudo, apenas em 2007 foi publicada a regulamentação oficial para o cultivo da espécie no país (Brasil, 2007).

Os cultivos experimentais enfrentaram limitações impostas por fatores econômicos, como o baixo retorno financeiro, e por entraves burocráticos associados à regularização das áreas destinadas ao cultivo (Brasil, 2020). Em Santa Catarina, entretanto, fazendas experimentais de *K. alvarezii* foram recentemente estabelecidas em áreas anteriormente destinadas ao cultivo de moluscos bivalves, demonstrando a viabilidade do aproveitamento dessas áreas para sistemas de policultivo (Gelli et al., 2024).

Apesar do elevado potencial do Brasil para o desenvolvimento da maricultura de algas marinhas, o setor ainda se encontra em estágio incipiente. No litoral norte do Estado de São Paulo, esforços recentes resultaram na elaboração de um mapa de ordenamento territorial específico para a instalação de fazendas marinhas, visando à expansão sustentável da atividade e à maximização do uso racional da zona costeira (Gelli et al., 2024).

1.4 Aspectos ambientais do cultivo de Kappaphycus alvarezii

A macroalga *Kappaphycus alvarezii* foi introduzida experimentalmente no litoral norte do estado de São Paulo em 1995, com o propósito de suprir a crescente demanda por seus subprodutos. Essa iniciativa foi impulsionada pela projeção de esgotamento dos bancos naturais da alga nativa *Hypnea pseudomusciformis* Nauer, Cassano & Oliveira, pela dependência de matérias-primas importadas derivadas de macroalgas e pelas dificuldades técnicas associadas ao cultivo de espécies nativas (Paula and Pereira, 1998; Oliveira, 2005; Reis et al., 2007a, b; Hayashi and Reis, 2012).

A introdução de *K. alvarezii* na costa brasileira (Processo IBAMA/MMA nº 02027.009179/1996-11) foi precedida por estudos abrangentes sobre seu potencial econômico, aspectos biológicos e ecológicos, técnicas de cultivo e riscos ambientais associados, com apoio do Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Paula, 2001; Paula et al., 2002; Paula and Pereira, 1989).

Com o objetivo de mitigar possíveis impactos ambientais, foram implementados protocolos de avaliação de risco e programas de monitoramento, voltados à detecção da instalação da espécie no ambiente e à prevenção da introdução de organismos associados indesejáveis (Oliveira, 2005; Castelar et al., 2009).

Os cultivos experimentais foram conduzidos durante o inverno, estação menos favorável ao desenvolvimento da espécie, e os resultados demonstraram a viabilidade da área para o estabelecimento de cultivos comerciais (Hayashi, 2007).

Após 29 anos de cultivo contínuo de *K. alvarezii* na Baía de Ubatuba, não foram observadas evidências de sua dispersão por fragmentos vegetativos ou propágulos reprodutivos, indicando baixo potencial de invasão (Bulboa et al., 2008; Araújo et al., 2020).

Ainda assim, destaca-se a necessidade de manter um monitoramento ambiental sistemático e permanente, essencial para prevenir impactos e garantir a sustentabilidade da aquicultura (Araújo et al., 2020, 2023).

O cultivo de macroalgas também proporciona benefícios ambientais indiretos, como a remoção de nutrientes dissolvidos em excesso, contribuindo para a mitigação da eutrofização em ambientes costeiros (Marinho-Soriano et al., 2002).

Estudos de Hayashi et al. (2008) evidenciaram a eficiência de *K. alvarezii* na absorção de nutrientes, caracterizando-a como biofiltro potencial em sistemas integrados de cultivo multitrófico.

Além disso, os cultivos funcionam como atratores biológicos, oferecendo substrato, abrigo e alimento a diversos organismos marinhos, como peixes e tartarugas (Reis et al., 2004; Oliveira, 2005).

Em comparação a matérias-primas vegetais terrestres, as macroalgas apresentam vantagens significativas: não requerem áreas agrícolas, evitam competição com culturas alimentares, dispensam o uso de insumos nitrogenados e possuem baixos teores de lignina, o que reduz a necessidade de pré-tratamentos onerosos para a liberação de açúcares, favorecida por sua baixa recalcitrância (Dalbelo, 2017)

1.5 Algicultura e sua importância econômica e social

A sustentabilidade social nas cadeias de valor da pesca e da aquicultura consolidou-se como tema central para a comunidade internacional e demais partes interessadas, refletindo uma crescente preocupação com os impactos sociais dessas atividades (FAO, 2022).

A aquicultura tem sido promovida como setor estratégico, com elevado potencial de crescimento e capacidade para fortalecer o protagonismo de mulheres e jovens, especialmente ao ampliar o poder de decisão das mulheres sobre o consumo e a oferta de alimentos nutritivos (Cervantes-Godoy et al., 2014; FAO, 2022).

Em reconhecimento à relevância desses temas, a Assembleia Geral das Nações Unidas declarou o ano de 2022 como o "Ano Internacional da Pesca e da Aquicultura de Pequena Escala" (IYAFA 2022). Durante esse período, as ações institucionais buscaram ampliar a conscientização global, promover o empoderamento de pequenos produtores e evidenciar os benefícios de políticas e práticas inclusivas que favoreçam a gestão sustentável da pesca artesanal e da aquicultura.

A implementação de uma aquicultura ordenada e responsável configura-se como estratégia eficaz para a geração de emprego e renda. Essa abordagem amplia a produtividade das zonas costeiras por meio da utilização racional e do manejo sustentável dos recursos naturais, favorece a permanência dos produtores em suas comunidades de origem, protege o meio ambiente, fortalece cadeias produtivas associadas — como o turismo e a própria aquicultura — e contribui para a redução da pressão sobre os estoques pesqueiros (Giffoni et al., 1998; Fagundes et al., 2004; Pereira and Rocha, 2015).

As regiões litorâneas compreendidas entre os estados de São Paulo e Rio de Janeiro apresentam características ambientais e geográficas favoráveis à implantação da maricultura. A proximidade com os principais centros consumidores, aliada à presença de enseadas e pequenas baías abrigadas, confere a essas áreas um elevado potencial para o desenvolvimento de cultivos marinhos (Fagundes et al., 2004; Nogueira, 2018).

No estado de São Paulo, destacam-se comunidades tradicionais costeiras que enfrentam processos acelerados de transformação social e declínio progressivo dos recursos pesqueiros.

Esse cenário ressalta a necessidade de alternativas sustentáveis de produção, entre as quais a aquicultura se apresenta como ferramenta promissora de adaptação e resiliência (Nogueira, 2018). Entretanto, a implantação da algicultura no estado ocorre de forma gradual e planejada, considerando aspectos técnicos, ambientais e sociais (Gelli, 2019).

1.6 Conclusão

Considerando a diversidade fenotípica das linhagens de *Kappaphycus alvarezii* cultivadas na Fazenda Marinha Experimental do Instituto de Pesca (EMFFI), hipotetiza-se que as linhagens gametofíticas "Edison de Paula" apresentem diferenças significativas nas respostas fisiológicas em comparação à linhagem tetrasporofítica. Espera-se, assim, identificar linhagens com desempenho superior e maior potencial biotecnológico para cultivos comerciais.

O objetivo principal deste estudo é identificar, entre as linhagens selecionadas, linhagem tetrasporofítica "Tetrasporófito marrom – T-br" e as gametofíticas "Edison de Paula marrom-claro – E-br", "Edison de Paula verde-claro – E-lg" e "Edison de Paula amarelo-esverdeada – E-gy", aquelas com melhor desempenho produtivo em termos de biomassa e subprodutos, por meio da avaliação das respostas fisiológicas de crescimento, atividade fotossintetizantes, variáveis bioquímicas e teores de carragenanas.

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2. Capítulo 1

Artigo proposto segundo as normas da Revista “Boletim do Instituto de Pesca”

Preliminary tests to establish the experimental design for the cultivation of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) strains in Ubatuba Bay, Brazil

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2.1 ABSTRACT

The seaweed *Kappaphycus alvarezii* (Doty) L.M. Liao (Rhodophyta, Gigartinales) has been cultivated experimentally at the Marine Experimental Farm of the Fisheries Institute (MEFFI) in Ubatuba. This seaweed, along with its various strains, is an important commercial source of carrageenan. The study aimed to evaluate the experimental design by analyzing the growth rates on the floating frame (n = 10) in four color strains of *K. alvarezii*: three gametophytes strains, light-green (E-lg), yellowish-green (E-gy), and pale-brown "Edison de Paula" (E-br), which derived from the brown tetrasporophyte (T-br) also evaluated. Initially, the experimental design included seedlings arranged in two floating raft, with 16 lines containing 10 seedlings each (secured using the "tie-tie" method). Each row contained 70 - 100 g of a single strain, resulting in four sample units for each strain replanted at each harvest over 30 days. However, this design exhibited an "edge effect," which could influence the outcomes of subsequent analyses. The experiment has been revised in the new setup. Each strain was represented by 20 seedlings that were placed together in a single floating frame and randomly distributed across eight lines, becoming 80 randomly distributed seedling positions and a 45-day growth cycle while maintaining other experimental conditions.

Keywords: mariculture, floating raft, edge effect

2.2 INTRODUCTION

The seaweed *Kappaphycus alvarezii* (Doty) L.M.Liao (Rhodophyta, Gigartinales) from Southeast Asia was introduced in Brazil in 1995 by the Fishing Institute of São Paulo in partnership with the Institute of Biosciences of the University of São Paulo (Paula et al., 1999; Reis et al., 2007) when considering the difficulties for the cultivation of native carrageenans (Oliveira, 1990; Paula et al., 2002), for carrageenan extraction (Ask & Azanza, 2002; Paula et al., 2002).

Since then, *K. alvarezii* has been cultivated in an open environment. It is subject to seasonal variations (Ask and Azanza, 2002; Hurtado et al., 2015; Hayashi et al., 2007a) due to

climatic actions and can also be influenced by its physical arrangement (cultivation protocols) in the cultivation structures.

Some cropping systems were used based on field and laboratory experiments (Ask & Azanza, 2002). Among these, the “tie-tie” system, noted by Doty & Alvarez (1975), emerged in different forms, including fixed longlines and floating rafts. This system became predominant mainly due to its simplicity, the availability and low cost of materials, and the healthy growth of the plants (Trono, 1989; Ask, 1999). Over the years, there have been few variations in cultivation protocols (Ask & Azanza, 2002).

In Ubatuba, a cultivation period of approximately 40 to 45 days is recommended. While longer periods may tolerate some plant breakage and material loss due to larger sizes achieved, it is advisable to grow the plants close to the surface. Maintaining a planting density 12 plants m^{-2} can help avoid self-shading among neighboring plants (Hayashi et al., 2007b).

To anticipate the final proposed experiment results it was necessary to observe whether the arrangement of macroalgae within the cultivation structures was influencing growth rates. This observation could lead to satisfactory results.

2.3 EXPERIMENTAL DESIGN

Samples of *Kappaphycus alvarezii* strains will come from experimental cultures developed at the Experimental Marine Farm of the Fisheries Institute, in Ubatuba Bay, São Paulo state, Brazil (23°27′5.8″S; 45° 02′49.3″W), (Fig. 2-1).

Figure 2-1: Initial experimental design. Experimental Marine Farm of the Fisheries Institute (EMFFI), in Ubatuba Bay, São Paulo state, Brazil

The determination of the experimental design was evaluated through the growth rates of the strains of the seaweed *Kappaphycus alvarezii* tetrasporophyte (T-br) brown and the gametophytes “Edison de Paula” (E-br) Pale-brown; Light green (E-lg) and greenish-yellow (E-gy) were used for longline with floting frame (Solorzano-Chavez et al., 2019), already established on site (Gelli, personal communication) in the experimental unit of seaweed cultivation at the Fisheries Institute, Ubatuba-SP.

Two frames (3 m X 2.5 m) were inserted between polypropylene cables of the “long-line”, formed by PVC tubes with "cap" at the ends (floats) in the transversal direction to the flow of the water current, the seedlings were placed in the 8 lines and each line 10 moorings

(tie-tie) containing a single strain, totaling 16 lines with 4 sampling units of each strain. The positioning of the strains was previously drawn (Fig. 2-2).

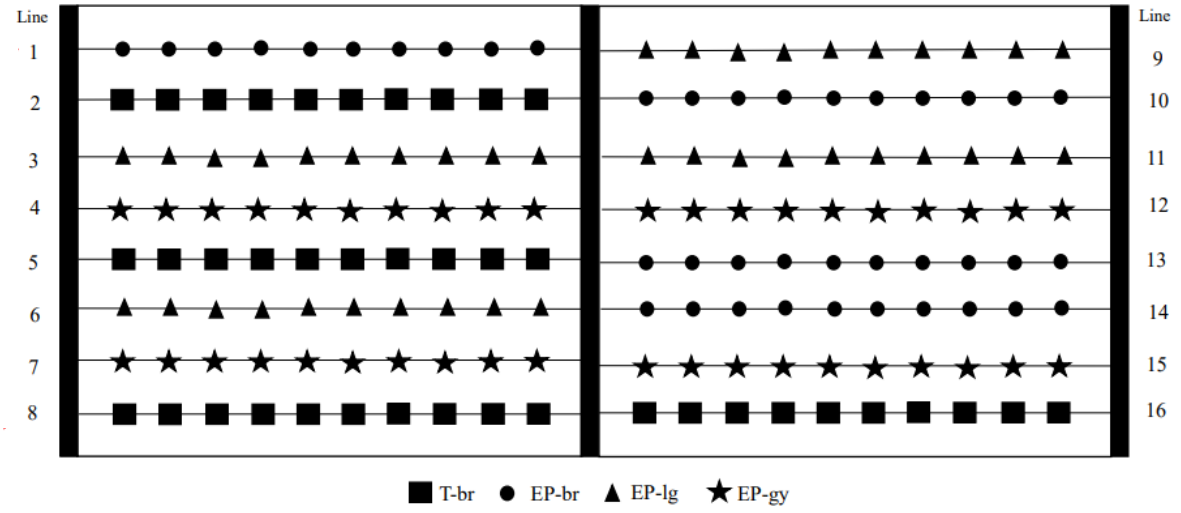

Figure 2-2: Initial spatial distribution of the strains of *Kappaphycus alvarezii* strains. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula (EP-br); Light-green Edison de Paula (EP-lg); Greenish-yellow Edison de Paula (EP-gy), (January/2022 – July/2022).

Seedlings were placed in distance of 20 cm on the row and 27 cm apart between rows, the density was12 plants m^{-2} (Hayashi et al., 2007b) and seedlings of 70 to 100 g weight were replanted in each harvest , where they remained at a maximum depth of 50 cm from the surface of the water for a growth period of 30 days (growth cycle).

Growth Rate

Fresh mass was recorded at 30-day intervals (growth cycles). Weight data were determined for everyone (n = 40) with a precision electronic balance. The growth rates (GR, % d^{-1}) were calculated according to the formula: $GR = [(Bf/Bi)^{1/t} - 1] \times 100\ \%$; Bi = initial mass, Bf = final mass at the harvest, t = time in days (Yong et al. 2013).

2.4 RESULTS

Table 2-1 presents the growth rate variations observed for *Kappaphycus alvarezii* strains over a six-month period, from January to July 2022.

Table 2-1: Growth rates of *Kappaphycus alvarezii* strains cultivated from January to June/2022, in the Experimental Marine Farm of the Fisheries Institute (EMFFI), Ubatuba Bay, São Paulo state. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula gametophyte (E-br); Light-green Edison de Paula gametophyte (E-lg); Greenish-yellow Edison de Paula gametophyte (E-gy). Values represent average ± standard deviation (n = 40).

	Strains (GR, % d^{-1})			
Growth Cycles	**T-br**	**E-br**	**E-lg**	**E-gy**
January/2022	7.57 ± 2.65	3.11 ± 0.47	2.07 ± 0.44	1.68 ± 0.35
February/2022	8.90 ± 1.67	4.48 ± 0.52	3.63 ± 0.50	3.95 ± 0.43
March/2022	5.06 ± 0.80	2.82 ± 1.14	1.65 ± 0.35	2.44 ± 0.21
April/2022	3.92 ± 0.80	1.88 ± 0.32	1.21 ± 0.22	1.15 ± 0.08
May/2022	3.57 ± 0.32	1.93 ± 0.22	1.49 ± 0.09	1.13 ± 0.16
June/2022	3.35 ± 0.34	1.82 ± 0.25	1.84 ± 0.21	1.01 ± 0.17

Additionally, summarizes the average growth rates for each strain over the total sample period in each row in the floating frame, (Table 2-2).

Table 2-2: Average of growth rates per row of *Kappaphycus alvarezii* strains cultivated from January to June/2022, in the Experimental Marine Farm of the Fisheries Institute (EMFFI), Ubatuba Bay, São Paulo state. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula (E-br); Light-green Edison de Paula (E-lg); Greenish-yellow Edison de Paula (E-gy). Values represent average ± standard deviation (n = 60).

Strains	Line	% d^{-1}	Strains	Line	% d^{-1}
T-br	2	6.24 ± 2.79	E-br	1	2.21 ± 0.77
	5	5.86 ± 2.63		10	2.44 ± 0.85
	8	4.00 ± 1.32		13	2.94 ± 1.07
	16	5.48 ± 2.47		14	3.12 ± 1.40
E-lg	3	2.21 ± 0.86	E-gy	2	1.57 ± 0.61
	6	2.04 ± 1.09		7	1.58 ± 0.74
	9	1.84 ± 0.65		12	1.83 ± 0.92
	11	1.84 ± 0.68		15	1.92 ± 0.76

The data distribution (normality) initially proposed organizations and evidenced differences in the growth rates of the same strain. Tukey test to observe significant differences between the average of growth rates between the row for each strain (Table 2-3).

In the experimental design, it was observed that growth rates varied among the strains placed on the floating raft, and their position on the frame influenced these differences. T-br strain, line 8, located at the edge, exhibited slower growth compared to lines 2 and 6, as well as line 16. E-br strain, line 1, also showed slower growth than lines 13 and 14, which were positioned more toward the center of the floating raft (Table 2-3).

Table 2-3: Statistical analysis of growth rates of each cultivation line 1 (L1); line 2 (L2); line 3 (L3); line 4 (L4); line 5 (L5); line 6 (L6); line 7 (L7); line 8 (L8); line 9 (L9); line 10 (L10); line 11 (L11); line 12 (L12); line 13 (L13); line 14 (L14); line 15 (L15); line 16 (L16); (Tukey test, $p \leq 0.05$) of *Kappaphycus alvarezii* strains. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula (E-br); Light-green Edison de Paula (E-lg); Greenish-yellow Edison de Paula (E-gy), (January/2022 – June/2022, n = 60).

		L2	L5	L8	L16
T-br	L2		0.8961	**0.0002***	0.5003
	L5	0.9933		**0.0029***	0.8977
	L8	5.9620	4.9690		**0.0269***
	L16	1.9800	0.9872	3.9820	

		L1	L10	L13	L14
	L1		0.5637	**0.0140***	**0.0010***
E-br	L10	1.8390		0.3071	0.0643
	L13	4.2950	2.4560		0.8759
	L14	5.3570	3.5190	1.063	
		L3	L6	L9	L11
	L3		0.8059	0.2706	0.4093
E-lg	L6	1.2700		0.7978	0.9147
	L9	2.5610	1.2920		0.9938
	L11	2.1902	0.9225	0.3690	
		L2	L7	L12	L15
	L2		0.9889	0.1983	0.0896
E-gy	L7	0.4497		0.3459	0.1788
	L12	2.8010	2.3510		0.9826
	L15	3.3250	2.8750	0.5242	

*Bold, significant p-values ($p \leq 0.05$).

2.5 DISCUSSION

The growth rate (GR) data of *Kappaphycus alvarezii* strains showed that brown tetrasporophyte had higher GR (5.42 ± 2.31 % d^{-1}) when compared with gametophytic strains (E-br: 2.67 ± 1.05 % d^{-1}; E-lg: 1.98 ± 0. 85 % d^{-1}; E-gy: 1.73 ± 0.81 % d^{-1}), corroborating the results of previous studies in which tetrasporophytes presented higher growth rates than gametophytes in the same cultivation site (Hayashi et al., 2007a; Solorzano-Chavez et al., 2019).

To reduce the “edge effect”, which negatively influenced the results, the experimental design was modified. The edge effect is one of the main bias factors in experiments with fragmented ecosystems, as environmental and biological conditions at the edges differ from

those in the interior, affecting organisms and abiotic variables (Laurance, 2000; Harper et al., 2005). Meta-analyses show that the intensity of these effects varies according to the type of edge (natural or anthropogenic), reinforcing their relevance in experimental design (Magura, 2017).

Thus, methodological strategies such as excluding plots near the edges, using continuous models (Ewers & Didham, 2006), and bias simulations (Van Meter et al., 2010) are essential to ensure the robustness and validity of research.

2.6 CONCLUSION

The modification in the experimental design aims to achieve satisfactory results by working with a smaller volume of biomass during the production cycles. The methodology proposed by Levin (1987) and Triola (1999) determined a new appropriate sample size, according to the equation:

$$n = \left(\frac{Z\alpha/2 \cdot \sigma}{E} \right)^2$$

Where: n = number of individuals in the sample; $Z\alpha$ = critical value corresponding to the desired degree of confidence; σ = population standard deviation of the studied variable; E = Maximum estimate error margin (maximum difference between the sample mean (X) and the true population means).

This approach, based on the previously obtained results, facilitated the calculation of a new sample size. To ensure statistical reliability for the project with a confidence level of 95% ($p \leq 0.95$), a minimum sample size of 13 units was determined based on the maximum occurrence in the standard deviation (SD) along the same row, without accounting for edge effects between strains. As a result, we proposed using 20 seedlings (n = 20) from each strain

for the new sampling design. Total of 80 seedlings will be placed in a floating frame, and they will be rearranged by random selection every 45 days during the production cycle (Fig. 2-3).

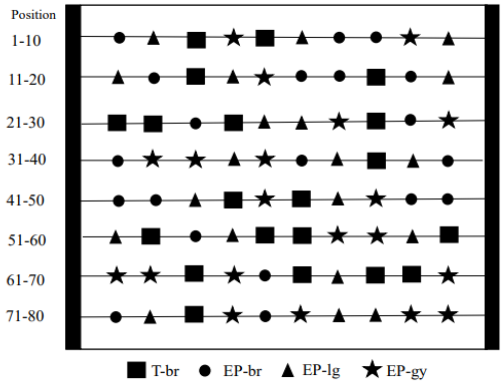

Figure 2-3: Proposal for experimental cultivation of *Kappaphycus alvarezii* strains, random spatial distribution within the frame. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula (E-br); Light-green Edison de Paula (E-lg); Greenish-yellow Edison de Paula (E-gy), (n = 20).

Within this cultivation floating raft will be planted, 80 seedlings from 70 to 100 g (strains; T-br, E-br, E-lg, and E-gy, n = 20) along eight rows using the mooring system commonly called "Tie-Tie" (12 plants m^{-2}) (Hayashi et al. 2007b). For each rope embedded in the raft, ten seedlings will be planted at a 20 cm distance on the cable and 27 cm between them and maintained at a depth of approximately 50 cm.

Harvests occurred every 45 days, resulting in a total of 8 consecutive growth cycles (cycle 1: August-September/2022, cycle 2: October-November/2022, cycle 3: November-December/2022, cycle 4: January-February/2023, cycle 5: February -March/2023, cycle 6: April-May/2023, cycle 7: May-June/2023, and cycle 8: July-August/2023). After 45 days, the seaweeds will be harvested, cleaned, and weighed, and new seedlings will be planted. Concomitantly data to evaluate growth rates will be obtained. At each harvest, the seedlings will be randomly positioned through seedling draws and subsequently inserted into the floating frame (from 1 to 80 position).

In conclusion, the analysis revealed that the differences in growth rates were influenced by the position of the seedlings within the floating raft in the initial experimental design, highlighting a phenomenon known as the "edge effect." In response, a new experimental design was proposed and implemented. This new methodological approach, grounded in statistical principles and experimental control, led to increased reliability and reduced bias in the collected data.

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3. Capítulo II

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Variation in growth, photosynthesis, proteins and pigments of gametophytic and tetrasporophytic color strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) cultivated in Brazilian subtropical waters

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3.1 Abstract

Kappaphycus alvarezii (Doty) L.M. Liao (Rhodophyta) is a widely studied species that provides raw material for carrageenan production, however, mass production struggles to maintain species viability owing to low genetic variability. New strains of *K. alvarezii* with different phenotypes have emerged from sea cultivation in Ubatuba bay, southeastern Brazil. The present study aimed to evaluate growth rates, photosynthesis, and pigment and protein concentrations in four *K. alvarezii* strain, including the brown tetrasporophyte (T-br) and three gametophytic strains: "Edison de Paula" pale-brown gametophyte (E-br), which originated new strains, named light-green (E-lg) and greenish yellow (E-gy) gametophytes. These strains were cultivated in a floating raft system for one year at the Experimental Marine Farm of the Fisheries Institute. T-br tetrasporophyte showed higher growth rates and higher phycobiliprotein concentrations than the gametophytic strains. Maximum electron transport rates (ETR_{max}) and irradiance saturation (E_k) varied among strains and seasons, and the E-lg and E-gy gametophytes had higher ETR_{max} and E_k than the T-br tetrasporophyte and E-br gametophyte. Results of principal component analyses (PCA) evidenced three groups: E-lg and E-gy gametophytes with higher ETR_{max} and E_k, T-br tetrasporophyte and E-br gametophyte formed distinct groups related to phycobiliprotein concentrations. In conclusion, our results corroborate the significant differences between T-br tetrasporophyte and the three gametophytic strains of *K. alvarezii,* and results of PCA showed two distinct groups of gametophytic strains, although E-lg and E-gy gametophytes were originated from the E-br gametophyte. Furthermore, novel strains are needed to increase genetic diversity and to expand the cultivation of *K. alvarezii* in the region.

Keywords: "Edison de Paula" gametophytes, photosynthetic performance, total soluble protein, phycobiliproteins, Ubatuba Bay

3.2 Introduction

Kappaphycus alvarezii (Doty) L.M.Liao and *Eucheuma* J. Agardh hold the highest position in the global production volume rankings for cultivated macroalgae (Hurtado et al. 2019). *K. alvarezii* biomass can be used for carrageenan extraction (Solorzano-Chaves et al. 2019), bioethanol production (Roldán et al. 2017), or as foliar biofertilizers (Gelli et al. 2020).

The historic of the introduction of *Kappaphycus alvarezii* in Brazil was described in details by Gelli et al. (2024). In summary, *K. alvarezii* was introduced in Brazil in 1995 by Professor Édison José de Paula of São Paulo University, and thallus segments of brown tetrasporophyte were provided by Professo Masao Ohno, from experimental cultivation in Usa Bay, Kochi, Shikoku Island, Japan (Paula et al. 1998). Unialgal culture of *K. alvarezii* was initiated from a 2.5 g brown tetrasporophytic branch in the Laboratory of Marine Algae, Institute of Biosciences, São Paulo University (Paula et al. 1999), and this strain was originated from commercial cultivation in the Philippines (Paula and Pereira 1998; Paula et al. 1999). After 10 months of laboratory cultivation, 20 branches (2.0-4.0 g) were transferred to sea cultivation (Paula et al. 1999), and the process of *K. alvarezii* domestication has been ongoing since 1995-1996 through the cultivation of brown tetrasporophytic strain into the Experimental Marine Farm of Fisheries Institute, Ubatuba bay, São Paulo State, Brazil (Gelli et al. 2024). During the period of December 1995–May 1996, three fertile tetrasporophytic branches (~10 cm, 50 g fresh weight) were collected monthly (Paula et al. 1999), and cultivated in laboratory controlled conditions for tetraspore liberation. After 1-2 days, released tetraspores were cultivated giving rise to tetrasporelings (Paula et al. 1999; Paula et al. 2001). Although a large number of tetraspores were produced, high percentage of mortality was observed, and only 21 tetrasporelings survived, and 20 individuals with different color and morphology remaining viable for at least two years under laboratory conditions (Paula et al.

1999). Firstly, tetrasporelings were grown fixed to the bottom of Petri dishes, but they were transferred to Erlenmeyer flasks after one month, and grown together during 4 months. Afterwards, they were transferred to separate flasks and cultivated individually (Paula et al. 1999), and for this reason, fertilization did not occur and cystocarps were not developed. Differently, Hinaloc and Roleda (2021) cultivated tetraspore progeny in pool, and observed sexual differentiation in male and female gametophytes as well as phenotypic variation in morphology and color. Paula et al. (1999) reported that only two gametophytes (Pl. 11 and Pl. 18) survived a full year at sea cultivation. Finally, only the P11 gametophyte (initially named G11) has been cultivated in the Ubatuba bay currently (Gelli et al. 2024). Afterwards, the G11 gametophyte was named "Edison de Paula" gametophyte to honour Prof. Dr. Edison José de Paula, who isolated and cultivated this strain (Hayashi 2007). The ploidy of brown tetrasporophyte and "Edison de Paula" pale-brown gametophyte was confirmed by Zitta et al. (2012) using a method with confocal fluorescence microscopy.

Gelli et al. (2024) reported that during the domestication process of *K. alvarezii* by clonally propagation of original parental brown tetrasporophyte and Edison de Paula pale-brown gametophyte, spontaneous growth of branches with distinct color from parental plant was observed, and these branches were clonally propagated originating new color strains. Currently, 12 color strains (9 tetrasporophytic strains and 3 gametophytic strains) are being cultivated in the Experimental Marine Farm of Fisheries Institute, Ubatuba bay (Gelli et al. 2024). Furthermore, temperature was a determinant factor on the generation of new strains of *K. alvarezii* in the region (Gelli et al. 2023). During the harvesting process, the appearance of branches with different color was observed, and these branches were isolated and propagated to generate new color strains. The original brown tetrasporophyte originated red and green tetrasporophytic strains, which originated other color strains. In 2013, "Edison de Paula" pale-brown gametophyte developed light-green branches, which were excised and clonally

propagated originating the light-green (E-lg) gametophyte (Fig. 1), and greenish-yellow branches originated the corresponding E-gy gametophyte (Gelli 2013).

Variations in the colors of *K. alvarezii* may lead to new germplasm banks for vegetatively propagated individuals; therefore, the characterization of new strains is a strategy designed to increase the production of carrageenan (Tan et al. 2022). Araújo et al. (2023) reported differences in the chemical composition of *K. alvarezii* strains cultivated in Ubatuba Bay, suggesting distinct potential applications for each strain. Choosing a suitable site for growing seaweed is essential since abiotic factors and their interactions in the cultivation area affect their development and, consequently, their productivity (Bulboa and Paula 2006; Roleda and Hurd 2019; Solorzano-Chavez et al. 2019). Furthermore, it is fundamental to highlight the importance of *ex situ* gene banking to the conservation of eucheumatoid genetic resources (Gonzaga et al. 2025), as well as the improvement of carragenan extraction techniques for enhancing efficiency and reducing environmental impacts (Mendes et al. 2025).

In Rhodophyta, light-harvesting complexes consist of phycobiliproteins that assemble into phycobilisomes on thylakoid surfaces, collecting light and transferring it to the mechanisms that convert light energy into chemical energy, thereby enabling the organism to adjust its structure in response to irradiance fluctuations (Liu et al. 2024). Thus, red algae can adjust the composition and density of their light rods when the environment changes (Liu et al. 2024) which means that chromatic variations in strains selected in the present study may influence their physiological responses owing to pigmentar characteristics. Furthermore, distinct physiological responses between diploid-haploid organisms were reported in several studies that predicted differences based on ploidy, reproductive phase, and complexity of life history (Hughes and Otto 1999; Albecker et al. 2021; Faria and Plastino 2023).

The present study aimed to investigate the growth, photosynthesis (*in vivo* chlorophyll *a* fluorescence), pigment concentrations, and total soluble proteins of the new gametophytic strains of *K. alvarezii*. We hypothesized that the light-green (E-lg) and greenish yellow (E-gy) gametophytic strains would perform like the pale-brown gametophytic strain (E-br), but differently from the brown tetrasporophytic strain (T-br). To test this hypothesis, we evaluated different physiological and biochemical responses of different color strains of *K. alvarezii* cultivated over one year in Ubatuba bay.

3.3 Material and Methods

Algal material and cultivation site

Four color strains of *Kappaphycus alvarezii* were studied: the brown tetrasporophyte (T-br; Fig. 2a), the gametophytic pale-brown "Edison de Paula" strain (E-br; Fig. 2b), and the gametophytic light-green (E-lg, Fig. 2c) and greenish-yellow (E-gy, Fig. 2d) "Edison de Paula" strains. The last two strains originated from the gametophytic pale-brown "Edison de Paula" strain (E-br).

The four strains were cultivated at the Ubatuba Bay (Experimental Marine Farm of the Fisheries Institute), in São Paulo state, Brazil (23°27′5.8″S 45° 02′49.3″W) (Fig. 3), in a floating raft system (Gelli et al. 2024), which was assembled with two parallel cables, which were kept on the surface by floats made of 3 m-PVC tubes. The cables were separated at a distance of 2.5 m, forming a frame, and secured at the ends by iron anchors weighing approximately 50 kg. Within this floating raft system, 80 plants (70-100 g fresh weight, FW) were attached (20 plants of each strain; T-br, E-br, E-lg, and E-gy) along eight ropes, using the "tie-tie" system. For each rope of the floating raft, ten plants were tied at distance of 20 cm from the cable and 27 cm between them, and the depth of approximately 50 cm from the

surface. The positioning of the plants on the rope was determined randomly by drawing the numbers that correspond to each position of the floating raft (1 to 80), as shown in the schematic illustration (Fig. 4a-b). Branches of T-br strain (n=20) were positioned in the first 20 numbers drawn, followed by the E-br, E-lg e E-gy strains. This procedure was repeated in each 45 day-growth cycle, when seaweeds were harvested, and new plants were tied. A total of 8 consecutive growth cycles were conducted (cycle 1: August-September/2022, cycle 2: October-November/2022, cycle 3: November-December/2022, cycle 4: January-February/2023, cycle 5: February -March/2023, cycle 6: April-May/2023, cycle 7: May-June/2023, and cycle 8: July-August/2023).

Growth rates based on fresh mass variation of 20 plants of each strain (n=20) were determined for the 8 consecutive growth cycles. Biochemical and photosynthesis analyses were conducted in four plants of each strain (n=4), which were randomly selected by drawing the numbers of their positioning on the rope in each growth cycle. These analyses were performed in the following season and growth cycle: Winter: cycle 1 (August-September/2022), Spring: cycle 3 (November-December/2022), Summer: cycle 5 (February-March/2023), and Autumn: cycle 7 (May-June/2023).

Abiotic Data

Abiotic data (water transparency, temperature and salinity) were recorded in working days at around 9:00 a.m. from August 15th, 2022, to August 17th, 2023, without replicates. Considering that the number of working days varied among the months, we took into consideration the shortest month, February of 2023, which had 24 working days. To standardize the other months to the same data number (n=24), we excluded data of the initial and final days of the month until to reach an equal number of data.

Water transparency was measured using a Secchi Disk, and temperature and salinity were measured using an alcohol thermometer and a refractometer, respectively (n=24). Rainfall index was based on the monthly average recorded by three meteorological stations (n=3) closest to the cultivation site, and information available at the National Institute of Meteorology (INMET).

Growth Rate (GR)

At the end of each 45 day-growth cycle, seaweeds were harvested, cleaned to eliminate epiphytes, and weighed. Fresh mass (FW) of each color strain (n=20) was recorded using a precision electronic balance. The growth rates (GR, % d^{-1}) were calculated as GR = $[(Bf/Bi)^{1/t} - 1]$ x 100 %, where Bi = initial mass, Bf = final mass at the harvest, and t = time in days (Yong et al. 2013).

Photosynthetic performance

Photosynthetic performance was evaluated by *in vivo* chlorophyll *a* fluorescence, and measurements were made 4 h after sunrise (n = 4), using an underwater Diving-PAM fluorometer (Walz®, Germany). Apical segments were arranged on a closed compartment to avoid overlapping and acclimated in the dark for 10 min for exposure to PSII. We used 12 levels of irradiance to construct electron transport rate (ETR) × photosynthetically active radiation (PAR) curves: 7, 25, 50, 72, 107, 147, 210, 301, 460, 688, 1,038 and 1,750 µmol photons m^{-2} s^{-1}. Exposure time of apical segments in each irradiance was 0.8 s interspersed by 15 s, and the "light curve" option on the equipment was selected to obtain "fast light curves" (Suggett et al. 2011). These data were used to construct Electron Transport Rate (ETR) x Irradiance (I) curves (Suggett et al. 2011). ETR was calculated as rETR=*ΔF/Fm'* x EPAR x

0.15, where (*ΔF/Fm'* = Y) represents the measured effective photochemical efficiency, EPAR is the incident PAR irradiance expressed in µmol photons m^{-2} s^{-1}, and 0.15 is the fraction of incident light absorbed directly by PSII, equivalent to red algae (Figueroa et al. 2003). Absorbance is the fraction of incident light absorbed by algae, and the ETR x PAR curves were calculated according to the model by Platt et al. (1982) as P = PS*[1-exp(-α*I/PS)]*[exp(-β*I/PS)], where P = photosynthesis, PS = photosynthetic saturation, α = photosynthetic efficiency, β = photoinhibition, and I = irradiance. The following photosynthetic parameters were calculated (Schreiber 2004): photosynthetic efficiency (α), maximum electron transport rate (ETR_{max}), saturation irradiance (E_k), and effective photochemical efficiency ($\Delta F/F_m'$).

Extraction and quantification of photosynthetic pigments

Phycobiliprotein extractions were carried out at 4 °C according to Kursar et al. (1983) modified by Plastino and Guimarães (2001). Apical segments of each strain (500 mg FW, n=4) were powdered in liquid nitrogen, followed by the addition of 4 mL of phosphate buffer 50 mM, pH 5.5. Crude extracts were centrifuged at 20,550 rpm for 20 min. The supernatant containing phycobiliproteins, including phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC), was separated, and kept in sealed vials at 4 °C until reading in spectrophotometer (Epoch 2-BioTek; BioTek Instruments, Winooski, Vermont, USA). Chlorophyll *a* (Chla) and total carotenoids (Ca) were extracted after dissolving the pellet from the previous procedure in methanol (addition of 4 mL) and then centrifuging at 10,730 rpm for 15 min. PE, PC, and APC concentrations were calculated according to Kursar et al. (1983), and Chla and Ca concentrations were calculated according to Torres et al. (2014). Results were expressed in µg g^{-1} (FW).

Extraction and quantification of total soluble proteins

Algal mass (100 mg FW, n=4) was powdered in liquid nitrogen and carried in phosphate buffer (0.2 M, pH 8.0, 5 mM EDTA, 1 mM DTT) in the proportion of 1 g fresh mass per 10 mL g^{-1}. After extraction, the homogenates were centrifuged (12,000 rpm; 4 °C; 15 minutes). Total soluble proteins (TSP) were determined by UV-vis spectrophotometry (Shimadzu – UV 1800, 595 nm), and Coomassie Blue solution (Bio-Rad) was added according to Bradford (1976). Protein concentration was determined using bovine serum albumin (BSA, Bio-Rad, standard curve), and results were expressed in mg g^{-1} (FW).

Data analysis

The assumptions of normality and homogeneity of variances were tested using the Shapiro-Wilk and Levene tests, respectively. When necessary, logarithmic transformation was employed (x = log (x + 1)) and tested again (Zar 1996). Abiotic factors (temperature, seawater transparency, salinity and rainfall) were analyzed by one-way factorial ANOVA. Growth rate (GR), *in vivo* chlorophyll *a* fluorescence (ETR_{max}, α, E_k, $\Delta F/F_m'$), pigment concentrations (PE, PC, APC, Chla, Ca), and TPS were analyzed by two-way factorial ANOVA (independent variables: strains and growth cycles). When ANOVA results were significant, Tukey's *a posteriori* multiple comparison test was used to establish significant differences among the independent variables (GR, ETR_{max}, E_k, and PC). In other cases, Tukey's *a posteriori* test was used to establish significant differences in a single independent variable owing to the lack interaction between independent variables: Growth Cycles (α, $\Delta F/F_m'$, Ca, and TSP) and Strains (PE and APC). Principal Component Analysis (PCA) was also performed on abiotic

data and all variables analyzed. Statistical analyses were performed using the Past 4.03 program (Oyvind Hammer), considering $p < 0.05$.

3.4 Results

Abiotic Data

Significant differences were observed in abiotic data among the different growth cycles of *Kappaphycus alvarezii* strains throughout the cultivation period, from August/2022 to August/2023 (Supplementary Information, Tables SI 1-2). Temperature, water transparency, salinity and rainfall showed a seasonal variation (Fig. 5a-d). The highest temperature averages were recorded during the summer, at the growth cycles 4 and 5 (25.4 ± 1.5 ºC; 25.7 ± 0.9 ºC, respectively), as well as the highest seawater transparency average of 298 ± 53 cm (Fig. 5a, b). On the other hand, during the winter, seawater temperatures were lower (19.9 ± 0.8 ºC), as well as the seawater transparency (144.2 ± 58.4 cm). Furthermore, the lowest salinity average (31.3 ± 4.2) was recorded in the spring as a result of increased rainfall averages (390 ± 32 mm in December) (Fig. 5c-d, Supplementary Information, Tables SI 1-2).

Growth Rates

Growth rates (GR) varied among different *K. alvarezii* strains and growth cycles ($F_{2,32} = 6.6$, $p = 0.000$), and when comparing the growth rates of different strains in each growth cycle, the tetrasporophytic strain (T-br) displayed higher growth rates than the gametophytic strains (Fig. 6, Table 1). The E-lg strain showed lower growth rates in autumn (cycle 6, Fig. 6). Comparing growth rates among different growth cycles, the four strains showed seasonal variation throughout the cultivation period. Overall, all strains presented higher growth rates

in the summer (cycle 5, Fig. 6), and lower growth rates were observed at the end of winter (cycle 1, Fig. 6). Comparing each strain throughout the growth cycles, T-br showed the highest growth rates in cycle 5 (6.7 ± 0.7 % d^{-1}) compared to cycle 1 (4.4 ± 0.5 % d^{-1}), while E-br showed the highest growth rate in cycle 5 (4.8 ± 0.7 % d^{-1}) compared to the cycle 1 (2.5 ± 0.4 % d^{-1}), and E-lg showed the highest growth rate in cycle 5 (4.3 ± 0.8 % d^{-1}) compared to cycle 1 (2.3 ± 0.3 % d^{-1}). E-gy showed the highest growth rate in cycle 5 (4.4 ± 0.7 % d^{-1}) compared to cycle 1 (2.7 ± 0.4 % d^{-1}) (Fig. 6).

Photosynthetic performance

Comparing the photosynthesis (ETR) and irradiance curves (PxI curves) among the four *K. alvarezii* strains (Fig. 7a-d), the tetrasporophyte (T-br) and the E-br gametophyte had similar patterns, unlike the E-gy and E-lg gametophytes (Fig. 7a-d). Generally, the latter two gametophytic strains had PxI curves with higher ETR than the T-br and E-br strains, except in the winter (Fig. 7a).

Maximum electron transport rates (ETR_{max}) varied among different *K. alvarezii* strains and seasons (Table 1). Comparing the ETR_{max} among the four strains in each season, it was evident that the tetrasporophyte (T-br) had lower ETR_{max} than the E-gy gametophyte, except in the winter when E-lg gametophyte had higher ETRmax (Table 1; Fig. 8a). Comparing ETR_{max} of each strain throughout the seasons, the T-br strain presented ETR_{max} between winter (2.5 ± 0.3 µmol e m^{-2} s^{-1}) and autumn (3.1 ± 0.7 µmol e m^{-2} s^{-1}), while the E-br strain presented ETR_{max} between spring (1.8 ± 1.0 µmol e m^{-2} s^{-1}) and winter (3.6 ± 1.1 µmol e m^{-2} s^{-1}), and the E-lg strain presented ETR_{max} between spring (2.4 ± 0.1 µmol e m^{-2} s^{-1}) and winter (9.5 ± 4.0 µmol e m^{-2} s^{-1}). The E-gy strain presented ETR_{max} between spring (5.3 ± 2.7 µmol e m^{-2} s^{-1}) and summer (14.3 ± 3.0 µmol e m^{-2} s^{-1}) (Fig. 8a).

Photosynthetic efficiency (α) of the four *K. alvarezii* strains varied only among different seasons (Table 1). The strains had higher α in the summer in comparison to spring and autumn (Table 1; Fig. 8b). The averages of α varied as follows: winter (0.05 ± 0.02), spring (0.04 ± 0.02), summer (0.06 ± 0.02), and autumn (0.04 ± 0.01) (Fig. 8b).

Irradiance saturation (E_k) varied among strains and seasons (Table 1). After comparing the E_k of different *K. alvarezii* strains in each season, the E-lg gametophyte had higher E_k, but only at the end of winter (Table 1; Fig. 8c). Comparing E_k of each strain throughout the seasons, the T-br tetrasporophyte presented lower E_k in the winter (53.9 ± 29.0 µmol photons m^{-2} s^{-1}) and higher E_k in the spring (101.1 ± 11.5 µmol photons m^{-2} s^{-1}), while the E-br gametophyte presented E_k variation between 46.4 ± 113.7 µmol photons m^{-2} s^{-1} (autumn) and 88.0 ± 27.5 µmol photons m^{-2} s^{-1} (winter). The E_k of the E-lg gametophyte varied from 58.4 ± 10.6 µmol photons m^{-2} s^{-1} (spring) to 215.2 ± 93.6 µmol photons m^{-2} s^{-1} (winter), while E_k values of E-gy gametophyte varied from 144.8 ± 60.2 µmol photons m^{-2} s^{-1} (winter) to (209.0 ± 40.7 µmol photons m^{-2} s^{-1} (summer) (Fig. 8c).

Effective photochemical efficiency ($\Delta F/F_m'$) varied among different seasons (Table 1), and the four strains had lower $\Delta F/F_m'$ during the spring when compared to values observed in other seasons (Table 1; Fig. 8d). Comparing $\Delta F/F_m'$ throughout the seasons, the averages of the four strains were as: winter (0.49 ± 0.06), spring (0.38 ± 0.08), summer (0.44 ± 0.07), and autumn (0.51 ± 0.05) (Fig. 8d).

Photosynthetic pigment and protein concentrations

Phycoerythrin (PE) concentrations varied among different *K. alvarezii* strains, but not among seasons (Table 1). The highest average PE concentrations were observed in T-br tetrasporophyte (164.8 ± 18.4 µg g^{-1} FW), intermediate values were observed in E-br and E-

gy gametophytes (106.8 ± 8.8 µg g^{-1} FW, 94.5 ± 34.1 µg g^{-1} FW), and E-lg gametophyte had the lower PE concentrations (43.8 ± 9.9 µg g^{-1} FW), (Table 1; Fig. 9a).

Phycocyanin (PC) concentrations varied among different strains and seasons (Table 1, Fig. 9b). During the cultivation period, the T-br tetrasporophyte had higher PC concentration compared to all gametophytic strains (Table 1; Fig. 9b), varying from 103.2 ± 10.9 µg g^{-1} FW (summer) to (139.7 ± 43.0 µg g^{-1} FW (spring), while the E-br strain showed PC concentrations between (44.1 ± 7.4 µg g^{-1} FW (spring) and (62.7 ± 22. µg g^{-1} FW (winter). The E-lg and Egy gametophytes showed, respectively, lower PC concentrations: 33.7 ± 10.1 µg g^{-1} FW in autumn and 54.4 ± 12.3 µg g^{-1} FW in spring, 16.1 ± 4.7 µg g^{-1} FW (spring) and 30.8 ± 12.3 µg g^{-1} FW autumn) (Fig. 9b).

Allophycocyanin (APC) concentrations varied among different strains (Table 1). The highest average APC concentrations were observed in the T-br tetrasporophyte (58.1 ± 8.5 µg g^{-1} FW) when compared to the gametophytic strains (24.1 ± 6.2 µg g^{-1} FW, 29.6 ± 10.2 µg g^{-1} FW, and 33.2 ± 15.0 µg g^{-1} FW for E-br, E-lg, and E-gy strains, respectively) (Table 1; Fig. 9c).

Chlorophyll *a* (Chla) concentrations of the four *K. alvarezzi* did not vary among strains and seasons, and the average was 39.0 ± 13.4 µg g^{-1} FW (Table 1). However, total carotenoids (Ca) concentrations varied among different seasons (Table 1), and the higher Ca concentrations were observed in summer (1.08 ± 0.48 µg g^{-1} FW) than the other seasons (Table 1; Fig. 9d).

Total soluble protein (TSP) concentrations presented by the four *K. alvarezii* varied among different seasons (Table 1), with the highest TSP concentrations observed in the spring compared to other seasons. In the winter and autumn, the strains had intermediate

concentrations, while the lowest TSP concentrations were recorded in the summer (Table 1; Fig. 10).

Principal Component Analysis

Principal component analysis (PCA) was performed to evaluate the correlation of variables measured (growth and metabolite concentrations), abiotic factors of cultivation site and the four *K. alvarezzi* strains (Table 2, Fig. 11). The first two principal components accounted for 77.926% of total variance (62.198% for axis 1 and 15.728% for the axis 2). Axis 1 was mainly influenced by phycobiliproteins (PC, 0.5864; PE, 0.4489; APC, 0.3246) in the positive side, and E_k (-0.3951) and ETR (-0.3776) in the negative side (Table 1). Axis 2 was mainly influenced by APC (0.7285), ETR (0.5185), and E_k (0.3430) in the positive side. PCA resulted in three groups: 1. T-br tetrasporophytes grouped on the positive side of axis 1 which was related to the higher phycobiliprotein concentrations (PE, PC and APC), 2. E-br gametophytes grouped also in the positive side of axis 1 but in the negative side of axis 2 due to the higher PE and PC concentrations but lower APC concentrations, and 3. E-lg and E-gy gametophytes grouped on the negative side of axis 1 due to lower phycobiliprotein concentrations and in the positive side of axis 2, which was related to higher electron transport rates (ETR) and saturation irradiance (E_k) (Table 2, Fig. 11).

3.5 Discussion

The four *Kappaphycus alvarezii* strains cultivated in Ubatuba bay, southeastern Brazil, showed a seasonal variation in growth rates, photosynthetic efficiency, effective photochemical efficiency, and concentrations of carotenoids and total soluble proteins. In general, growth rates of the four strains were higher during the summer and lower during the

winter, and these results are related to seasonal variations of temperature, as reported to T-br tetrasporophyte by Paula et al. (2002) and Paula and Pereira (2003), and also to T-br tetrasporophyte and E-br gametophyte (cited as G11 strain) by Hayashi et al. (2007a), and Solorzano-Chavez et al. (2019). Similarly, growth rates of *K. alvarezii* color strains in the tropical waters of Yucatán, Mexico, were also affected by temperature (Muñoz et al. 2004).

In the same way of growth responses, the photosynthetic efficiency of the four *K. alvarezzi* strains were higher in the growing season (summer). On the other hand, the values of effective photochemical efficiency of the four *K. alvarezzi* strains were lower in higher temperatures (summer and spring), and similar results were reported to tropical specimens of *Hypnea pseudomusciformis* Nauer, Cassano & M.C. Oliveira (Rhodophyta) submitted to laboratory simulation of marine heatwaves with increase of 4^{o}C and 6^{o}C in the temperature annual average (Nauer et al. 2022).

Concentrations of total soluble proteins of the four *K. alvarezii* strains were also lower during the summer, and these results could be explained by the high nitrogen metabolism associated with higher growth rate of *K. alvarezii* strains during this season summer. The protein concentrations obtained for the four *K. alvarezii* in the present study were lower than those reported by Navarte et al. (2022) ranging from 1.02 % to 4.61 % for ten strains of *K. alvarezii* cultivated under land-based hatchery conditions in the Philippines.

Carotenoids of the four *K. alvarezzi* varied seasonally, with higher concentrations in the summer when the water transparency was higher than in the other seasons. These results can be explained by the key role of carotenoids to protect photosynthetic organisms from the harmful effects of excess exposure to light (Hashimoto et al. 2016, Borburema et al. 2022).

Growth rates also varied among different *K. alvarezii* strains, and tetrasporophytic strain (T-br) had higher growth rates than the other three gametophytic strains. In our study,

T-br tetrasporophyte showed the growth rates ranging from 4.4 ± 0.5 % d^{-1} to 6.7 ± 0.7 % d^{-1}, and growth rates for E-br gametophyte ranged from 2.5 ± 0.4 % d^{-1} to 4.8 ± 0.7 % d^{-1}, and these ranges of growth rates were lower than those reported by other studies performed with the same strains and cultivation site (Table 3). Hayashi et al. (2007b) determined that the best cultivation conditions for brown tetrasporophyte of *K. alvarezii* in Ubatuba bay (growth cycle of 28 days, and density of 12 plants m^{-2}), and obtained growth rates of 5.2-7.2 % d^{-1}. Notably, Solorzano-Chavez et al. (2019) reported higher growth rates, 5.3-9.0 % d^{-1} for brown tetrasporophyte and 3.3-6.6 % d^{-1} for pale-brown gametophyte (cited as G11 strain) of *K. alvarezii*. Therefore, our study reported lower average growth rates for T-br tetrasporophyte and E-br gametophyte, which could be related to the cultivation system, as the longer growth cycle (45 days), and variations in environmental factors during the cultivation period.

The higher growth rates observed in T-br tetrasporophyte than the three gametophytic strains could be related to the ploidy, As in other studies with red algae, ploidy determined physiological differences among diploid and haploid phases in *Gracilaria* species (Destombe et al. 1989; Engel et al. 2001), and in *Gracilariopsis tenuifrons* (C.J.Bird & E.C.Oliveira) Fredericq & Hommersand (Faria and Plastino 2023). In contrast, the growth rates of laboratory-cultured diploid tetrasporophytes did not differ significantly from those of the haploid gametophytic progeny of *K. alvarezii* from the Philippines (Hinaloc and Roleda 2021).

Phycobiliprotein concentrations, as phycoerythrin (PE) and allophycocyanin (APC) of the *K. alvarezii* varied among the strains, but not among seasons, however phycocyanin (PC) concentrations varied among strains and seasons. In general, higher phycobiliprotein concentrations were observed in T-br tetrasporophyte, while intermediate and lower concentrations were observed in gametophytic strains. However, the agarophyte *Gracilaria caudata* J. Agardh showed different responses depending on the collecting site, phase and

irradiance levels under laboratory conditions (Faria et al. 2017). For example, under low irradiance, female gametophytes had higher PE and PC concentrations than tetrasporophytes in *G. caudata* collected in the northeastern and southeastern Brazil (Faria et al. 2017). Therefore, in the present study, the phycobiliprotein concentrations of the four *K. alvarezii* strains are related to the thallus color instead of diploid or haploid phases. However, different results were observed in red and green morphotypes of *K. alvarezii*, which showed similar phycoerythrin (PE) levels, but the phycocyanin (PC) and allophycocyanin (APC) concentrations were 2-fold higher in the green than in the red morphotype (Aguirre-Von-Wobeser et al. 2001).

Although the present study demonstrated that temperature contributed to variations in photosynthesis efficiency (α) and effective photochemical efficiency (*ΔF/Fm'*), no evidence showed better photosynthesis performance at elevated temperatures, possibly owing to the narrow range of temperature during the period studied, similar to other studies with red algae (Liu et al. 2017; Fujimoto et al. 2015; Vo et al. 2015; Faria and Plastino 2022).

Photosynthetic parameters, as maximum electron transport rates (ETR_{max}) and irradiance saturation (E_k) varied among different *K. alvarezii* strains and seasons, and the E-lg and E-gy gametophytes had higher ETR_{max} and E_k than T-br tetrasporophyte and E-br gametophyte. In PCA, these photosynthetic parameters grouped E-lg and E-gy gametophytes in the positive side of axis 2, and on the negative side of axis 1 due to lower phycobiliprotein concentrations. On the other hand, T-br tetrasporophytes and E-br gametophytes formed distinc groups related to higher phycobiliprotein concentrations.

In conclusion, results of PCA did not support the hypothesis of similarity on among the three gametophytic strains of *K. alvarezzi*, although the E-lg and E-gy gametophytes were originated from the E-br gametophyte. The E-lg and E-gy gametophytes were grouped

separately mainly due to higher ETR_{max} and E_k than T-br tetrasporophyte and E-br gametophyte. Furthermore, our results corroborate the significant differences between T-br tetrasporophyte and gametophytic strains of *K. alvarezii* cultivated in Ubatuba bay, indicating that tetrasporophytic strain is more suitable for commercial cultivation. However, novel strains with different physiological and biochemical characteristics are needed to increase the genetic diversity and to expand the cultivation of *K. alvarezii* in the region.

3.6 References

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Declarations

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Conflict of interest

The authors declare that they have no competing interests. NSY is member of Editorial Board of the Journal of Applied Phycology, and the peer-review process for this article was independently handled by another member of the journal editorial board.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Data availability statement

All data generated or analyzed during this study are included in this published article.

Code availability

“Not applicable”.

Authors' contributions

Leandro Herrera: conceptualization, methodology, investigation, formal analysis, writing – original draft.

Valéria C. Gelli: conceptualization, methodology, investigation, funding acquisition, writing – review & editing.

André V. F. Faria: conceptualization, methodology, writing – review.

Estela M. Plastino: conceptualization, writing – review & editing.

Nair S. Yokoya: conceptualization, funding acquisition, writing – review & editing.

All authors contributed to the article and approved the submitted version.

Table 3-1 Results analyzed by two-way ANOVA to examine the influence of independent variables on the two dependent variables (strains and periods) of *K. alvarezii* cultivated in Ubatuba Bay, southeastern Brazil. Strains: Brown tetrasporophyte, T-br; Pale-brown Edison de Paula, E-br; Light-green Edison de Paula, E-lg; Greenish-yellow Edison de Paula, E-gy). Periods: cycle 1: August-September/2022 (Winter), November-December/2022 (Spring), February-March/2023 (Summer), May-June/2023 (Autumn)

Variable	**Source of variation**	**df**	**F (2,16)**	***p*-value**
Maximum electron transport rate (ETR_{max})	Period	3	8.62	**0.000 ***
	Strain	3	32.7	**0.000 ***
	Interaction	9	4.50	**0.000 ***
Photosynthetic efficiency (α)	Period	3	5.6	**0.002 ***
	Strain	3	1.0	0.410
	Interaction	9	1.8	0.282
Saturation irradiance (E_k)	Period	3	0.2	0.881
	Strain	3	15.6	**0.000 ***
	Interaction	9	2.6	**0.001 ***
Effective photochemical efficiency (**$\Delta F/F_m'$**)	Period	3	14.4	**0.000 ***
	Strain	3	4.8	**0.005 ***
	Interaction	9	1,2	0.338
Phycoerythrin (PE)	Period	3	1.7	0.178
	Strain	3	44.6	**0.000 ***
	Interaction	9	2.0	0.064
Phycocianin (PC)	Period	3	1.0	0.410
	Strain	3	102.8	**0.000 ***
	Interaction	9	2.6	**0.016 ***
Allophycocinin (APC)	Period	3	2.5	0.072
	Strain	3	10.7	**0.000 ***
	Interaction	9	0.9	0.533
Chlorophyll *a* (Chla)	Period	3	2.8	0.050
	Strain	3	2.4	0.078
	Interaction	9	1.3	0.268
Carotenoids (Ca)	Period	3	3.3	**0.028 ***

	Strain	3	1.4	0.265
	Interaction	9	1.1	0.363
Total soluble proteins (TPS)	Period	3	17.3	**0.000** *
	Strain	3	4.6	0.776
	Interaction	9	1.2	0.292

* bold, significant correlation (p-value < 0.05)

Table 3-2: Pearson´s correlation coefficients among abiotic factors and variables analyzed in color strains of *Kappaphycus alvarezii* cultivated in Ubatuba bay during four growth cycles: August-September/2022 (Winter), November-December/2022 (Spring), February-March/2023 (Summer), and May-June/2023 (Autumn). Color strains: Brown tetrasporophyte, T-br; Pale-brown Edison de Paula, E-br; Light-green Edison de Paula, E-lg; Greenish-yellow Edison de Paula, E-gy

Abiotic factors/Metabolites (abbreviations)	**Principal Components**	
	Axis 1	**Axis 2**
Water temperature (TE)	0.0024	0.0535
Salinity (SA)	0.0026	0.0381
Water transparency (TR)	0.0072	0.1235
Growth rates (GR)	0.1601	0.1814
Electron transport rate (ETR)	**-0.3776 ***	**0.5185 ***
Photosynthetic efficiency (α)	-0.0018	0.0075
Saturation irradiance (E_k)	**-0.3951 ***	**0.3430 ***
Effective photochemical efficiency (*ΔF/Fm´*)	-0.0116	-0.0022
Phycoerythrin (PE)	**0.4489 ***	0.0726
Phycocianin (PC)	**0.5864 ***	0.0218
Allophycocinin (APC)	**0.3246 ***	**0.7285 ***
Chlorophyll *a* (Chla)	0.1531	0.1112
Carotenoids (Ca)	0.0333	0.0347
Total soluble proteins (TSP)	-0.0110	-0.1046
Explicability (%)	62.198	15.728

* bold, significant importance of the variable on the axes

Table 3-3 Studies performed with brown tetrasporophyte (T-br strain) and pale-brown "Edison de Paula" gametophyte (E-br strain, formely named G11 strain) of *Kappaphycus alvarezii* cultivated at Experimental Marine Farm of the Fisheries Institute, Ubatuba bay, southeastern Brazil

Growth rate (% d^{-1})		**Cultivation period Month/Year**	**Temperature (°C)**	**Growth cycle (days)**	**References**
T-br strain	**E-br strain**				
6.1 – 8.9	3.3 – 7.2[a]	March/1997-February/1998	-	30	Paula et al. (1999)
4.1 – 8.2	-	October/1995-October/1996	19 - 28	27 – 37	Paula and Pereira (2003)
5.0 – 6.5	1.3 – 4.8	February/2001-December/2001	20 - 28	30	Hayashi et al. (2007a)
-	5.2 – 7.2	-	-	28	Hayashi et al. (2007b)
10.9[b]	-	-	-	40	Hayashi et al. (2008)
6.3	-	-	-	30	Roldán et al. (2017)
5.3 – 9.0	3.3 – 6.6	August/2013-June/2014	20 - 31	30	Solorzano-Chavez et al. 2019)
4.3 – 6.7	2.2 – 4.8	August/2022-July/2023	19 - 26	45	Present study

[a] after 7 days; [b] cultivation in tanks and later in open-sea cultivation

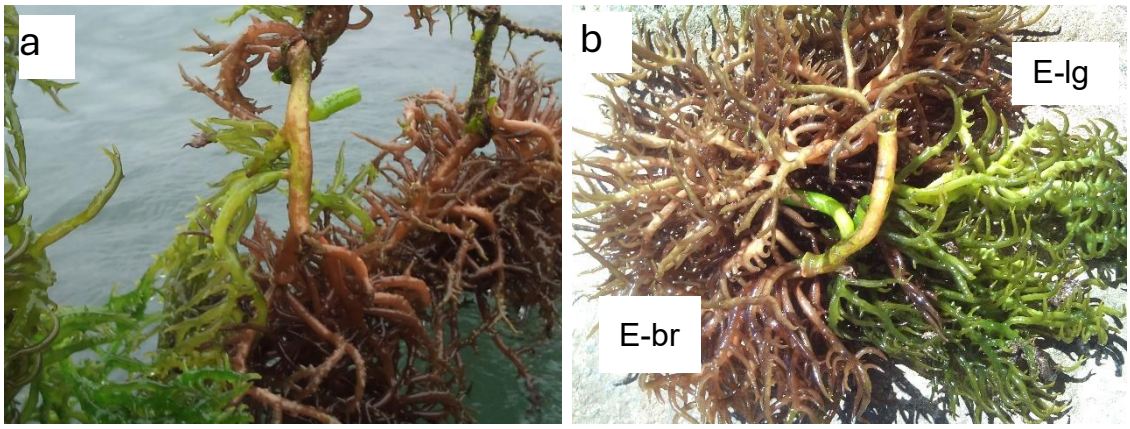

Fig 3–1 Edison de Paula gametophyte of *Kappaphycus alvarezii* cultivated in the Experimental Marine Farm of the Fisheries Institute, Ubatuba bay, southeastern Brazil. (a) Pale-brown Edison de Paula gametophyte with light-green branches, which originated from the pale-brown branches. (b) Detailed of one specimen with both pale-brown branches and light-green branches, which were isolated and propagated separately, resulting in two strains: pale-brown Edison de Paula gametophyte (E-br) and light-green Edison de Paula gametophyte (E-lg). Photos: V. C. Gelli´s personal archive, (2013).

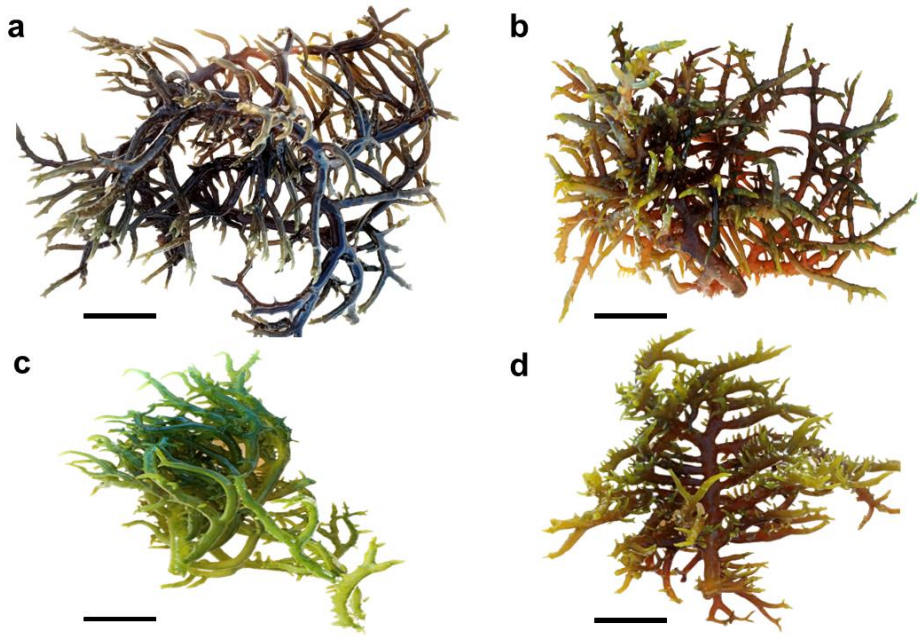

Fig 3–2 Macroscopic morphology of four color strains of *Kappaphycus alvarezii* cultivated in the Experimental Marine Farm of the Fisheries Institute, Ubatuba bay, southeastern Brazil. (a) Brown tetrasporophyte; (b) Pale-brown Edison de Paula gametophyte; (c) Light-green Edison de Paula gametophyte; (d) Greenish-yellow Edison de Paula gametophyte. Scale bar = 5 cm.

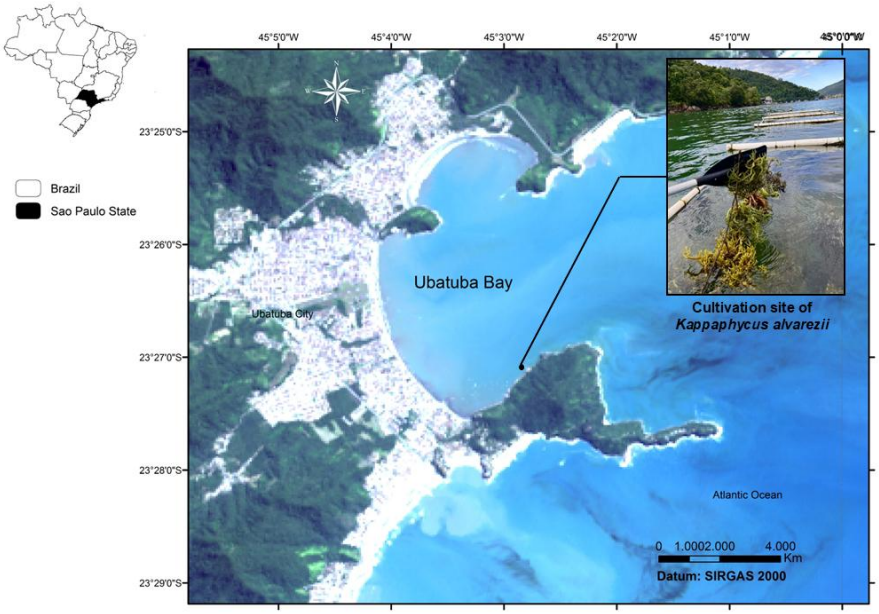

Fig 3–3 Cultivation site of *Kappaphycus alvarezii* strains in the Experimental Marine Farm of the Fisheries Institute, Ubatuba Bay, São Paulo state, southeastern Brazil.

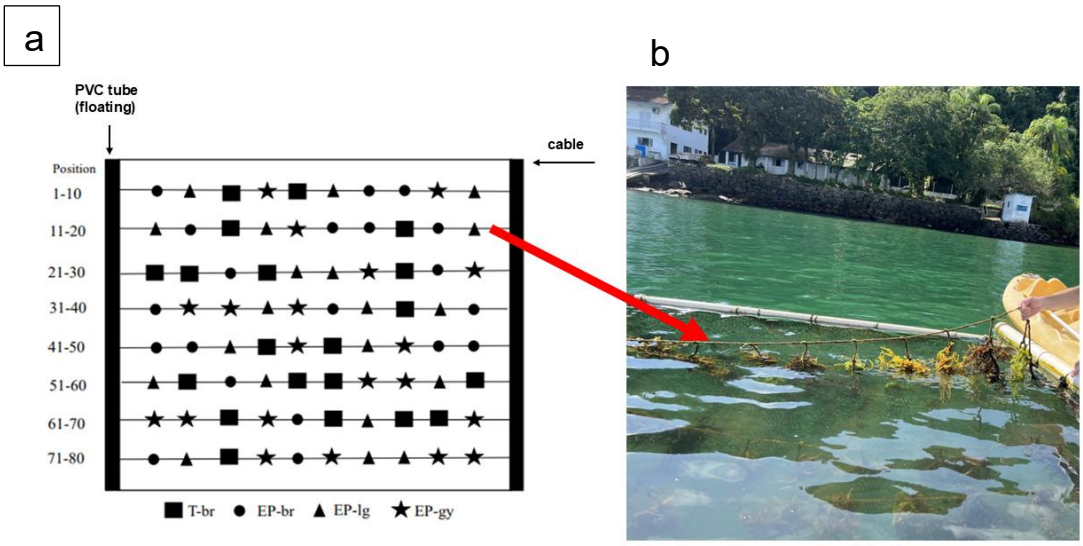

Fig 3–4 Floating raft cultivation system of *Kappaphycus alvarezii* color strains in Ubatuba bay, southeastern Brazil. (a) Schematic illustration of the positioning of each color strain (n=20) on the ropes. (b) General aspect of the floating raft showing one rope with plants of different color strains (red arrow). Brown tetrasporophyte (■ T-br); Pale-brown Edison de Paula gametophyte (● E-br); Light-green Edison de Paula gametophyte (▲E-lg); Greenish-yellow Edison de Paula gametophyte (★ E-gy).

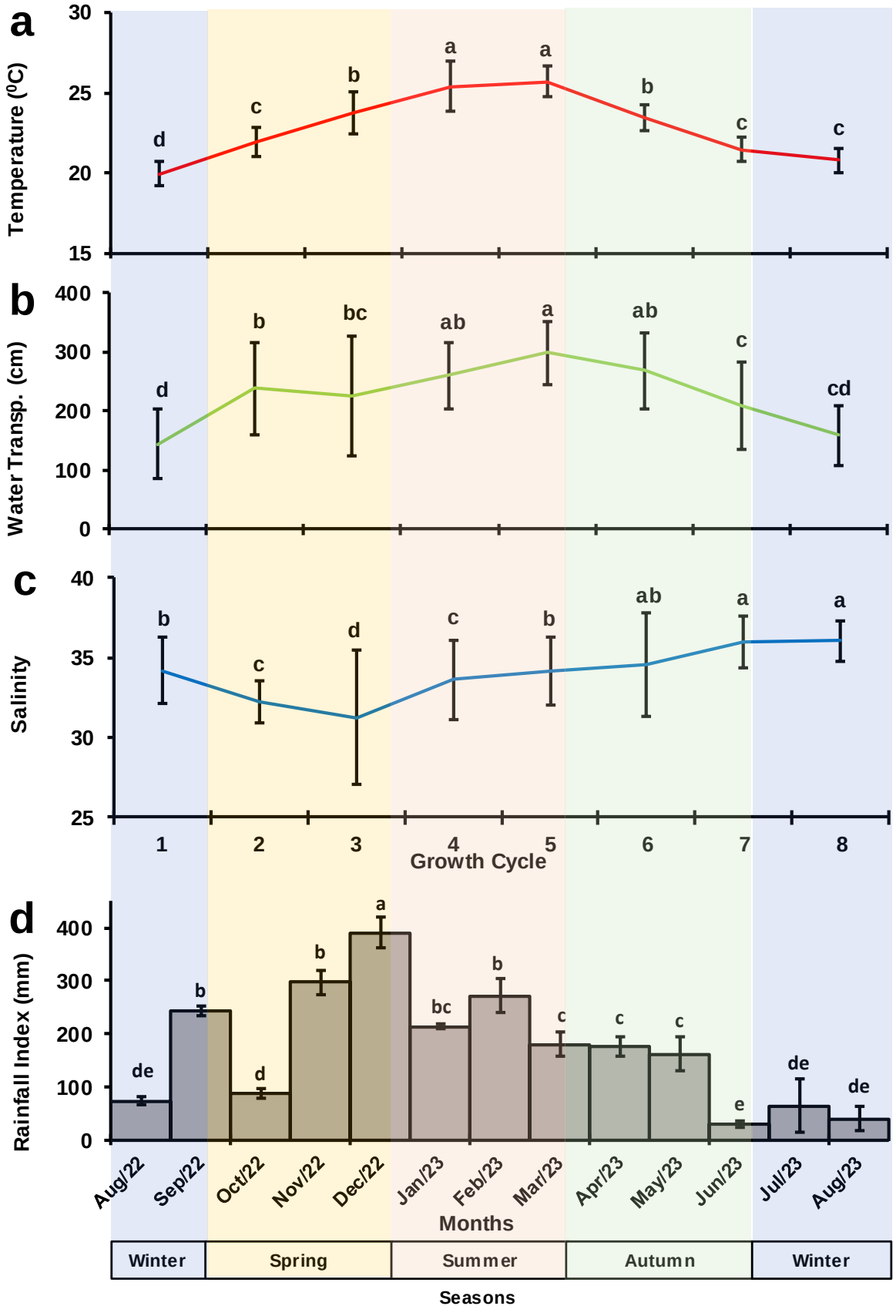
a
Temperature (°C)
30
25
20
15
d
c
b
a
a
b
c
c
b
Water Transp. (cm)
400
300
200
100
0
d
b
bc
ab
a
ab
c
cd
c
Salinity
40
35
30
25
b
c
d
c
b
ab
a
a
1
2
3
4
5
6
7
8
Growth Cycle
d
Rainfall Index (mm)
400
300
200
100
0
de
b
d
b
a
bc
b
c
c
c
e
de
de
Aug/22
Sep/22
Oct/22
Nov/22
Dec/22
Jan/23
Feb/23
Mar/23
Apr/23
May/23
Jun/23
Jul/23
Aug/23
Months
Winter
Spring
Summer
Autumn
Winter
Seasons

Fig 3–5 Variations of Temperature (a), Water Transparency (b), and Salinity (c) in the cultivation site of *Kappaphycus alvarezii* in Ubatuba bay. Data represent averages ± SD (n = 24) recorded during the eight 45 day-growth cycles (cycle 1: August-September/2022, cycle 2: October-November/2022, cycle 3: November-December/2022, cycle 4: January-February/2023, cycle 5: February -March/2023, cycle 6: April-May/2023, cycle 7: May-June/2023, and cycle 8: July-August/2023). Rainfall Index (d) was represented by monthly averages ± SD (n=3) recorded by the three meteorological stations closest to the cultivation site. Different letters represent significant differences among growth cycles or months (one-way ANOVA, Tukey´s multiple comparison test, $p < 0.05$).

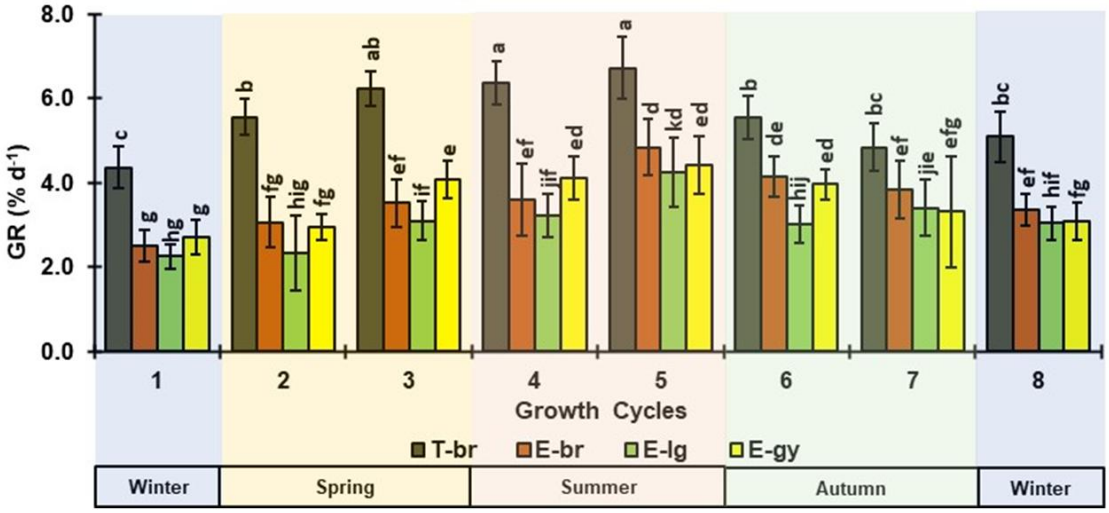

Fig 3–6 Growth rates (GR) of *Kappaphycus alvarezii* color strains cultivated in Ubatuba bay, from August/2022 to August/2023, comprising the eight 45 day-growth cycles (Mean ± SD, n = 20). Brown tetrasporophyte (T-br); Pale-brown Edison de Paula gametophyte (E-br); Light-green Edison de Paula gametophyte (E-lg); Greenish-yellow Edison de Paula gametophyte (E-gy). Growth cycles (1: August-September/2022, 2: October-November/2022, 3: November-December/2022, 4: January-February/2023, 5: February-March/2023, 6: April-May/2023, 7: May-June/2023, and 8: July-August/2023. Different letters represent significant differences among growth cycles or months (Two-way ANOVA, Tukey´s multiple comparison test, $p < 0.05$).

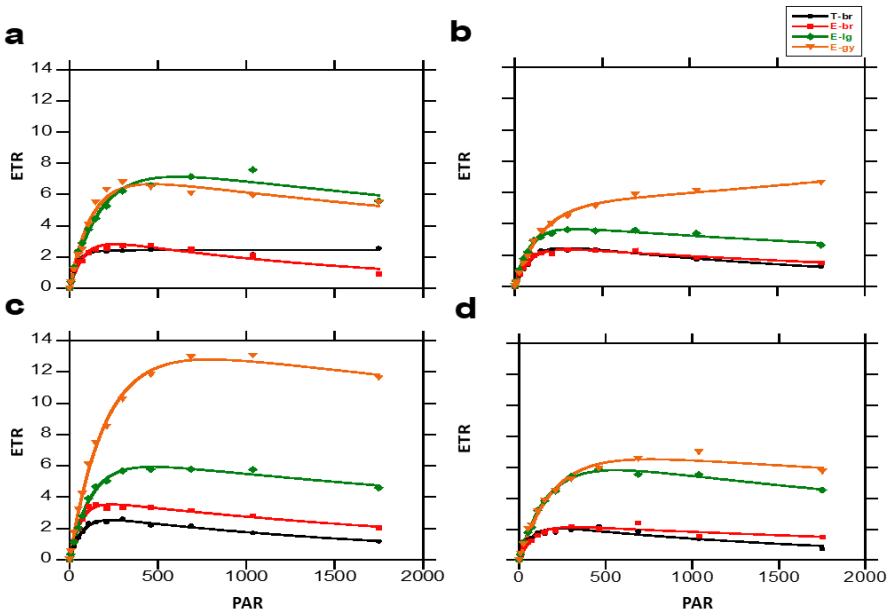

Fig 3–7 Photosynthesis X Irradiance curves of the four *Kappaphycus alvarezii* strains cultivated in Ubatuba bay for the 45 day-growth cycle in each season. Photosynthesis based on data of *in vivo* chlorophyll *a* fluorescence (Electron Transport Rate, ETR) measured at the end of 45 day-growth cycle, and Photosynthetically Active Radiation (PAR – µmol photons m^{-2} s^{-1}). (a) cycle 1: August-September/2022 (Winter), (b) cycle 3: November-December/2022 (Spring), (c) cycle 5: February-March/2023 (Summer), and (d) cycle 7: May-June/2023 (Autumn). Brown tetrasporophyte (T-br); Pale-brown Edison de Paula gametophyte (E-br); Light-green Edison de Paula gametophyte (E-lg); Greenish-yellow Edison de Paula gametophyte (E-gy).

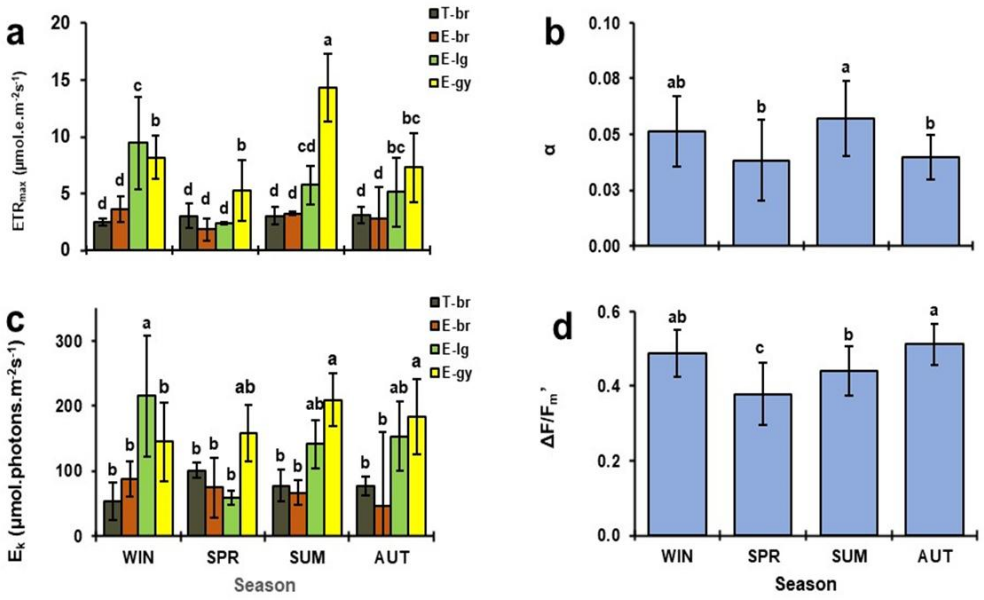

Fig 3–8 Photosynthetic parameters (mean ± SD, n=4) based on *in vivo* chlorophyll a fluorescence of *Kappaphycus alvarezii* strains cultivated in Ubatuba bay for the 45 day-growth cycle in each season. (a) Maximum electron transport rate (ETR_{max}); (b) Photosynthetic Efficiency (α), (c) Saturation Irradiance (E_k); and (d) Effective Photochemical Efficiency ($\Delta F/F_m'$). Blue columns represent the averages of the four strains together since there were no significant differences among them. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula gametophyte (E-br); Light-green Edison de Paula gametophyte (E-lg); Greenish-yellow Edison de Paula gametophyte (E-gy). Winter (WIN), Spring (SPR), Summer (SUM), Autumn (AUT). Different letters represent significant differences among growth cycles or months (Two-way ANOVA, Tukey´s multiple comparison test, $p < 0.05$).

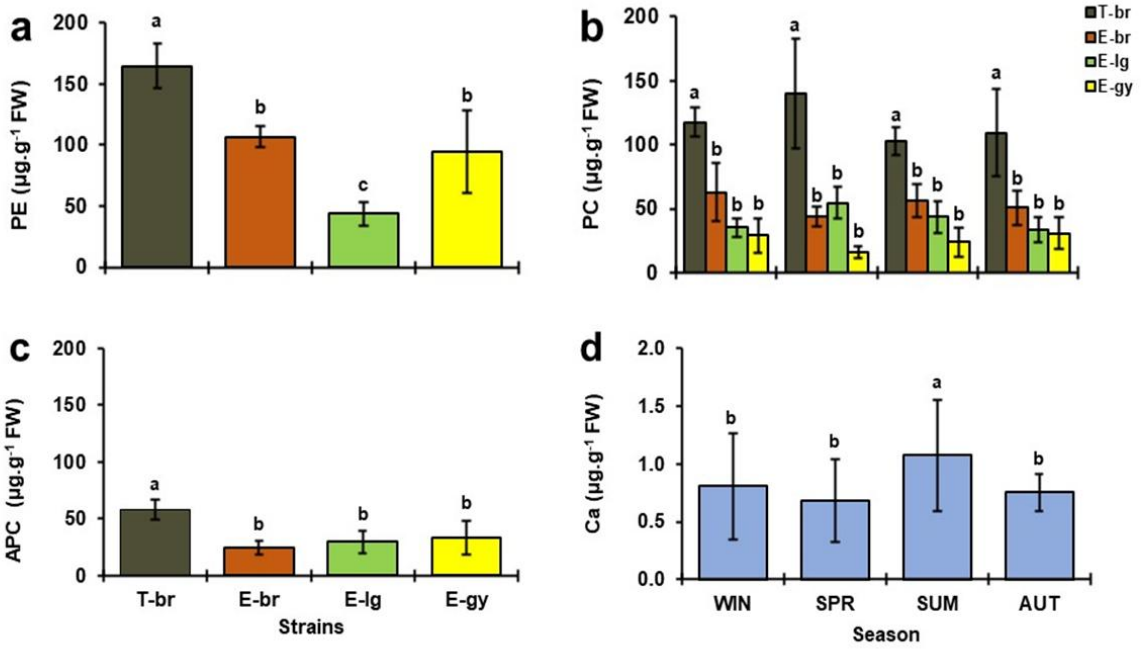

Fig 3–9 Pigment concentrations (mean ± SD, n=4) determined by *in vivo* chlorophyll *a* fluorescence of *Kappaphycus alvarezii* strains cultivated in Ubatuba bay for the 45 day-growth cycle in each season. (a) Phycoerythrin (PE) and (c) allophycocyanin concentrations (APC): the columns represent the averages of the four seasons together since there were no significant differences among the seasons. (b) Phycocyanin concentration (PC), and (d) Total Carotenoids concentrations (Ca) - blue columns represent the averages of the four strains together since there were no significant differences among them. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula gametophyte (E-br); Light-green Edison de Paula gametophyte (E-lg); Greenish-yellow Edison de Paula gametophyte (E-gy). Winter (WIN), Spring (SPR), Summer (SUM), Autumn (AUT). Different letters represent significant differences among growth cycles or months (Two-way ANOVA, Tukey´s multiple comparison test, $p < 0.05$).

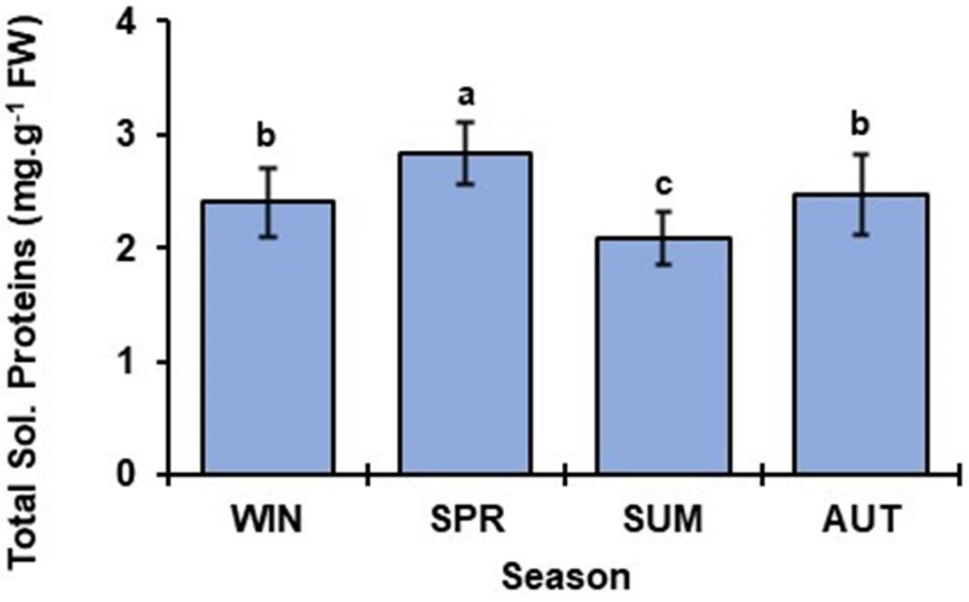

Fig 3–10 Total soluble protein concentrations (mean ± SD, n=4) of *Kappaphycus alvarezii* strains cultivated in Ubatuba bay for 45 day-growth cycle in each season. The blue columns represent the averages of the four strains together since there were no significant differences among them. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula gametophyte (E-br); Light-green Edison de Paula gametophyte (E-lg); Greenish-yellow Edison de Paula gametophyte (E-gy). Seasons: Winter (WIN), Spring (SPR), Summer (SUM), Autumn (AUT). Different letters represent significant differences among seasons (Two-way ANOVA, Tukey´s multiple comparison test, $p < 0.05$).

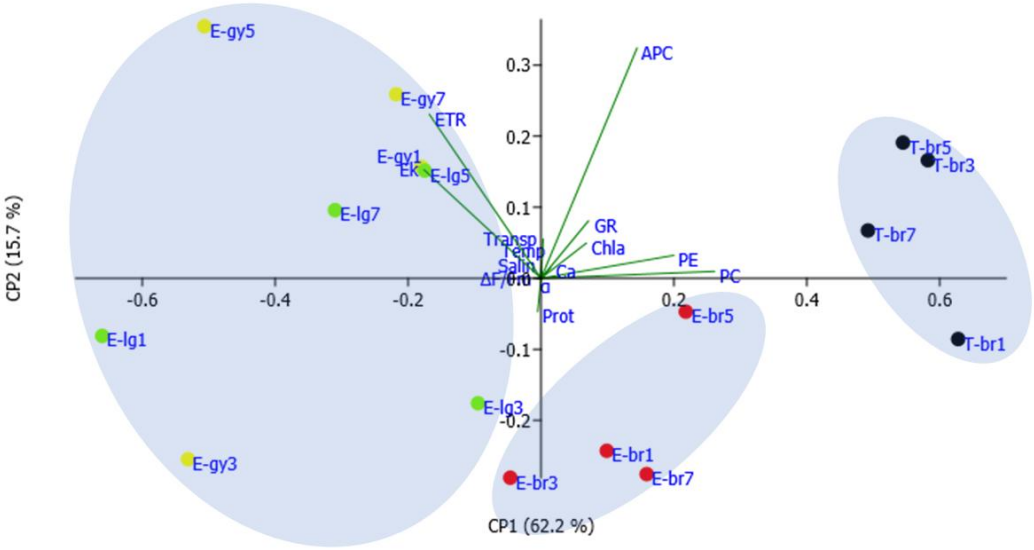

Fig 3–11: Scatter diagram of plots of the first two principal component analysis axes of data on temperature (Temp), salinity (Salin), water transparency (Transp), growth rate (GR), maximum electron transport rate (ETR_{max}), photosynthetic efficiency (α), saturation irradiance (E_k), effective photochemical efficiency (*ΔF/Fm´*), phycoerythrin (PE), phycocianin (PC), allophycocianin (APC), chlorophyll *a* (Chla); total carotenoids (Ca), and total soluble proteins (Prot) of *Kappaphycus alvarezii* strains cultivated in Ubatuba bay. Brown tetrasporophyte (black dots ●, T-br); Pale-brown Edison de Paula gametophyte (red dots ●, E-br); Light-green Edison de Paula gametophyte (green dots ●, E-lg); Greenish-yellow Edison de Paula gametophyte (yellow dots ●,E-gy) cultivated for 45 day-growth cycles: 1 = August-September/2022 (Winter), 3 = November-December/2022 (Spring), 5 = February-March/2023 (Summer), 7 = May-June/2023 (Autumn).

3.7 Supplementary material

Variation on growth, photosynthesis, proteins and pigments of gametophytic and tetrasporophytic color strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartilanes) cultivated in Brazilian subtropical waters

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Table S1 Summary of analyses of variance (one-way ANOVA) of abiotic data (water temperature, salinity and water transparency) measured in working-days at *Kappaphycus alvarezii* cultivation site (Experimental Marine Farm of the Fisheries Institute, Ubatuba bay, southeastern Brazil). Minimum (Min.) and, maximum (Max.) values, and mean ± SD (n = 24) for eight 45 day-growth cycles (cycle 1: August-September/2022, cycle 2: October-November/2022, cycle 3: November-December/2022, cycle 4: January-February/2023, cycle 5: February -March/2023, cycle 6: April-May/2023, cycle 7: May-June/2023, and cycle 8: July-August/2023). F statistical analysis of variance (ANOVA) of the ratio between the variation between the groups and the variation within the groups, and *p* statistically significant difference between the groups, at the 5% significance level.

Environmental factors	Growth cycles	Min.	Max.	Mean ±SD	F	*p*
Temperature (°C)	1	18.0	21.0	19.9 ± 0.8	103.7	**0.0000**
	2	20.0	23.0	22.0 ± 0.9		
	3	21.0	26.0	23.8 ± 1.3		
	4	21.5	27.0	25.4 ± 1.5		
	5	24.0	27.0	25.7 ± 0.9		
	6	22.0	25.0	23.4 ± 0.9		
	7	20.0	22.5	21.5 ± 0.7		
	8	19.0	22.0	20.8 ± 0.8		
Water Transparency (cm)	1	40	290	144 ± 58	14.4	**0.0000**
	2	70	360	238 ± 78		
	3	70	400	225 ± 101		
	4	150	335	261 ± 57		
	5	170	30	298 ± 53		
	6	100	340	268 ± 64		
	7	100	360	209 ± 74		
	8	100	260	159 ± 50		
Salinity (PSU)	1	30.0	37.0	34.2 ± 2.1	314.6	**0.0000**
	2	29.0	34.0	32.3 ± 1.3		
	3	21.0	35.0	31.3 ± 4.2		

4	25.0	36.0	33.6 ± 2.5
5	26.0	36.0	34.2 ± 2.1
6	25.0	37.0	34.5 ± 3.3
7	32.0	37.0	36.0 ± 1.6
8	32.0	37.0	36.0 ± 1.2

bold, significant correlation ($p < 0.05$)

Table S2 Summary of one-way ANOVA of monthly rainfall index (minimum (Min.) and maximum (Max.) values, mean ± SD, n = 3). Monthly rainfall average measured by the three meteorological stations closest to the *Kappaphycus alvarezii* cultivation site (Experimental Marine Farm of the Fisheries Institute, Ubatuba bay, southeastern Brazil). F statistical analysis of variance (ANOVA) of the ratio between the variation between the groups and the variation within the groups, and *p* statistically significant difference between groups at the 5% significance level.

Month / Year	**Rainfall Index (mm)**		**Mean ± SD**	**F**	***p***
	Min.	Max.			
August/2022	66	80	73 ± 7	94.8	**0.000**
September/2022	234	254	243 ± 10		
October/2022	81	98	87 ± 10		
November/2022	273	315	297 ± 22		
December/2022	366	425	390 ± 32		
January/2023	207	219	214 ± 6		
February/2023	249	294	272 ± 22		
March/2023	167	197	181 ± 15		
April/2023	162	187	175 ± 13		
May/2023	123	201	162 ± 39		
June/2023	28	32	30 ± 2		
July/2023	46	82	64 ± 18		
August/2023	20	65	40 ± 23		

Bold, significant correlation ($p < 0.05$); SD, standard deviation

4. Capítulo III

Artigo proposto segundo as normas da Revista “Journal of Applied Phycology”

Productivity, carbohydrates and carrageenan characteristics of gametophytic and tetrasporophytic color strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) cultivated in Brazilian subtropical waters

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4.1 Abstract

Kappaphycus alvarezii (Doty) L.M. Liao (Rhodophyta, Gigartinales) is a key source of κ-carrageenan, widely used in food, cosmetic, and pharmaceutical industries. Since 1995, this species has been cultivated at the Experimental Marine Farm of the Instituto de Pesca in Ubatuba Bay, São Paulo, Brazil. Over three decades of cultivation, spontaneous color variants

have emerged from the original brown tetrasporophyte and Edison de Paula (EP) pale-brown gametophyte. This study aimed to characterize two new gametophytic strains light-green (E-lg) and yellowish-green (E-gy), and compare them with the pale-brown EP gametophyte (E-br) and brown tetrasporophyte (T-br). Seedlings (n = 20 per strain) were cultivated on floating rafts in a “tie-tie” system, with 45-day growth cycles. Seasonal assessments included productivity, water content, total soluble carbohydrates, carrageenan and sulfate yields, and 3,6-anhydrogalactose content. Abiotic factors (temperature, salinity, transparency) were also monitored. Productivity was highest for T-br during summer (8.5 ± 2.9 g m^{-2} d^{-1}), while all strains showed reduced growth in cooler months. T-br and E-gy strains had significantly higher total carbohydrate levels in spring, and carrageenan yields peaked in winter (41.6 ± 4.8 % DW), inversely correlated with temperature and salinity. Carrageenan composition was similar across strains, with average 3,6-anhydrogalactose content of 42.7 ± 3.7 % and seasonal peaks in sulfate content (29.8 ± 2.6 %). These results highlight seasonal influences on biochemical composition and suggest potential for selecting strains with enhanced carrageenan or carbohydrate yields.
Keywords: growth, seasonality, polysaccharides, 3,6-anhydrogalactose content, sulfate contente, Ubatuba Bay.

4.2 Introduction

Most rhodophytes synthesize sulfated galactan in their cell walls, forming specific linear polymers. These galactans produce carrageenan and variation in sulfation patterns modulate the physical characteristics of these polysaccharides in aqueous solutions, influencing their functional properties. In addition, these Galatians play crucial adaptive roles, such as moisture retention, cell concentration regulation, and structural strengthening in seaweeds (Usov 2011; Ciancia et al. 2020).

Florid starch, produced during photosynthesis, is a vital energy reserve during periods of low light or nutrient scarcity and is essential for the metabolism and ecological red algae adaptations (Yoon et al., 2006). Its less organized and more amorphous structure, compared to starch from higher plants, facilitates rapid energy mobilization, in addition to conferring greater water solubility and digestibility (Delattre et al. 2016).

Starch from flowers has attracted interest in biotechnology due to its amorphous and highly branched structure. This makes it a promising glucose source for fermentation, especially in industries that require fast-processing substrates, such as bioethanol production (Paz-Cedeno et al., 2019).

Carrageenans are extracted from several species of red algae, including *Chondrus crispus* Stackhouse, *Hypnea musciformis* (Wulfen) Lamouroux, *Eucheuma denticulatum* (Burman) Collins & Hervey and *Kappaphycus alvarezii* (Doty) Doty (Stanley 1987; Hayashi et al. 2007).

Carrageenan is a generic name given to the family of sulfated polysaccharides composed of β-D-galactose and 3,6-α-anhydro-D-galactose subunits (Hayashi et al. 2007a; Van de Velde 2008; Ciancia et al. 2020), extracted from red algae (Véliz et al. 2017) and, together with alginates and agars, form a group of biopolymeric complex substances that are called phycocolloids. These natural polymers have the ability to form thermoreversible gels or viscous solutions in saline solutions. This gelling capacity gives these polysaccharides several applications (Van de Velde et al. 2002; Dalbelo 2017).

The seaweed *Kappaphycus alvarezii* (Doty) L.M.Liao (Rhodophyta, Gigartinales) is originally from Southeast Asia and was introduced in Brazil in 1995 by the Fisheries Institute of São Paulo in partnership with the Biosciences Institute of the University of São Paulo (Paula et al. 1999; Reis et al. 2007) when considering the difficulties for the cultivation of native carrageenan algae (Oliveira 1990; Paula et al. 2002), as well as in several other

countries with the same commercial purposes (Paula et al. 2001; Nunes 2010), to carrageenan extraction (Ask and Azanza 2002; Paula et al. 2002).

Chemical variations between the types of carrageenan determine different rheological properties and therefore different industrial applications (Véliz et al. 2017). The main commercially available carrageenans are kappa, iota and lambda types (Glicksman 1987). Kappa-carrageenan with a harder and more brittle gel; iota-carrageenan with a more elastic and softer gel; and lambda does not form a gel, only liquids with a high viscosity. (McHugh 2003; Bixler and Porse 2011).

Commercial kappa-carrageenan is obtained predominantly by extracting the macroalgae *Kappaphycus alvarezii* in proportions that can exceed 65% by weight of dry mass of algae (Véliz et al. 2017), as well as *Eucheuma denticulatum* is the main species for the production of iota-carrageenan (Campo et al. 2009).

The present study aims to understand the biomass quantification, extraction and quantifications of carbohydrates contents, extraction and quantification of carrageenan contente, and quantification of 3,6-anhydrogalactose and total sulfate from carrageenan of the new gametophytic strains of *K. alvarezii*. The hypothesis is that the light-green and yellowish-green gametophytic strains will perform similarly to the pale-brown gametophytic strain but differently from the brown tetrasporophytic strain. To test this hypothesis, we evaluated different parameters, such as productivity, carrageenan yields (levels of 3,6-anhydrogalactose and sulphate in carrageenan), of different strains cultivated over a period of one year in Ubatuba Bay. The results were correlated with abiotic data (temperature, water transparency, and salinity) from the same period to evaluate seasonal variations.

4.3 Materials and Methods

Algal material and cultivation site

Four color strains of *Kappaphycus alvarezii* were studied: the brown tetrasporophyte (T-br; Fig. 2a), the gametophytic pale-brown “Edison de Paula” strain (E-br; Fig. 2b), and the gametophytic light-green (E-lg, Fig. 2c) and greenish-yellow (E-gy, Fig. 2d) “Edison de Paula” strains. The last two strains originated from the gametophytic pale-brown “Edison de Paula” strain (E-br).

The four strains were cultivated at the Ubatuba Bay (Experimental Marine Farm of the Fisheries Institute), in São Paulo state, Brazil (23°27′5.8″S 45° 02′49.3″W), in a floating raft system (Gelli et al. 2024), which was assembled with two parallel cables, which were kept on the surface by floats made of 3 m-PVC tubes. The cables were separated at a distance of 2.5 m, forming a frame, and secured at the ends by iron anchors weighing approximately 50 kg. Within this floating raft system, 80 plants (70-100 g fresh weight, FW) were attached (20 plants of each strain; T-br, E-br, E-lg, and E-gy) along eight ropes, using the “tie-tie” system. For each rope of the floating raft, ten plants were tied at distance of 20 cm from the cable and 27 cm between them, and the depth of approximately 50 cm from the surface. The positioning of the plants on the rope was determined randomly by drawing the numbers that correspond to each position of the floating raft (1 to 80), as shown in the schematic illustration (Fig. 4a-b). Branches of T-br strain (n=20) were positioned in the first 20 numbers drawn, followed by the E-br, E-lg e E-gy strains. This procedure was repeated in each 45 day-growth cycle, when seaweeds were harvested, and new plants were tied.

Harvests occurred every 45 days, resulting in a total of 8 consecutive growth cycles (cycle 1: August-September/2022, cycle 2: October-November/2022, cycle 3: November-December/2022, cycle 4: January-February/2023, cycle 5: February -March/2023, cycle 6: April-May/2023, cycle 7: May-June/2023, and cycle 8: July-August/2023). After 45 days, the seaweed was harvested, cleaned, weighed, and new seedlings were planted. At each harvest,

the seedlings were randomly positioned through seedling drawers and subsequently inserted into the floating raft (from 1 to 80), (Fig 4-2).

Productivity based on fresh mass variation of 20 plants of each strain (n=20) were determined for the 4 every two growth cycles, corresponding to 3-month intervals (Cycle 1: August/15/2022 – September/04/2022, Cycle 2: November/17/2022 – December/03/2022, Cycle 3: February/16/2023 – March/04/2023, Cycle 4: May/18/2023 – June/04/2023. Carbohydrate concentrations, carrageenan yield, and the contents of 3,6-anhydrogalactose and total sulfates in the extracted carrageenan analyses were conducted in three plants of each strain (n=3), which were randomly selected by drawing the numbers of their positioning on the rope in each growth cycle. These analyses were performed in the following season and growth cycle: Winter: cycle 1 (August-September/2022), Spring: cycle 3 (November-December/2022), Summer: cycle 5 (February-March/2023), and Autumn: cycle 7 (May-June/2023).

Abiotic Data

Abiotic data were measured daily at around 9:00 a.m. from August 15th, 2022, to August 17th, 2023, during the working days. To standardize the number of data of each growth cycle, we took into consideration the shortest month, February 2023, which has the shortest duration. We excluded data of initial and final days of the other months until to obtain an equal number of collection days. Ultimately, this resulted in a total of 24 consecutive collection days for each month.

Water transparency was measured using a Secchi Disk, and temperature and salinity were measured using a thermometer of alcohol and refractometer, respectively (n = 24).

Biomass quantification (Productivity)

Productivity was calculated according to the equation: Productivity = $[(Bf-Bi)^1/t]$ x [(Ms/Bf)/A], where: Bf = Final fresh biomass (g), Bi = Initial fresh biomass (g), t = number of days of cultivation, Ms = dry mass (g); A = total area (m^2). Productivity results were expressed in g m^{-2} d^{-1} (n = 20) (Hurtado and Agbayani 2002).

Determination of thallus water content

Approximately 300 g of fresh weight (FW) of the strains (n = 3) were dried in an oven at a maximum temperature of 50°C until reaching constant dry weight (DW). The percentage of water content (WC) was determined by the equation WC = [1-(AP/AP)] x 100 (Solorzano-Chavez et al. 2019). The results were expressed as a percentagem and used for subsequent calculations.

Extraction and quantifications of carbohydrates contents

Approximately 200 mg (dry weight – DW) were weighed to determine the dry matter mass and then subjected to carbohydrate extraction according to Carvalho et al. (1998) as modified. The samples were pulverized in a mortar, placed in 80% ethanol, and kept in a water bath at 80ºC for 1 h, then centrifuged (8000 rpm, 10 min, 4 ºC). The precipitates were re-extracted in 80% ethanol twice. The ethanolic supernatants were pooled and reserved. The final residues were extracted twice in water at 60ºC for 1 h and centrifuged (1082 rpm, 15 min, 4 ºC). The quantitative analysis of total soluble sugars of the macroalgae extracts was performed using the phenol-sulfur colorimetric method, determined by Dubois et al. (1956) using glucose (SIGMA) as standard. The absorbance reading was performed in a spectrophotometer (490 nm). The estimated sugar levels will be through calculations based on the equation of the straight line that will be generated from the glucose standard curve. In this

analysis, (n = 4) were performed for each color strain. The results of the ethanolic and aqueous extracts were expressed in mg g^{-1} DW (n = 4).

Extraction and quantification of Carrageenan content

Approximately 0.4 g (dry weight – DW, relative humidity less than 20%) were soaked with 40 ml of KOH (6%) for 24 h at room temperature to obtain the "cold" alkaline transformation, according to Masarin et al. (2016). Then, the samples were abundantly washed with water, bleached in the sun for at least 6 h, and subsequently dried at a maximum temperature of 60 °C until their weight was constant. The material was again suspended in 80 ml of distilled water and subsequently crushed. The crushed material was subjected to a water bath at 65 °C for 2 h (Masarin et al. 2016) and hot-filtered through nylon fabric. The resulting filtrate (carrageenan solubilized in water) was dried in an oven at a maximum temperature of 60 °C, according to the method described by Hayashi et al. (2007a). Carrageenan quantification results were expressed as % DW (n = 3).

Quantification of 3,6-anhydrogalactose from carrageenan

Approximately 10 mg of dry carrageenan (n = 3) were dissolved in 40 mL of distilled water in a water bath (85ºC) until complete dissolution (90 to 120 min). The samples will be cooled in an ice-water bath and the volume will be completed to 50 mL with distilled water (stock solution). In a test tube, 400 µL of the stock solution 400 µL of distilled water, 100 µL of 5% thymol, and 1 mL of 0.5% ferric chloride will be added. The samples will be homogenized, heated in a water bath for 13 min at 80ºC, and cooled in an ice-water bath. Then, 2 mL of 98% ethanol will be added. After these procedures, the samples will be analyzed in a spectrophotometer (635 nm). Their absorbances were recorded to calculate the

amount of 3,6-anhydrogalactose (%), using the calibration curve, through the use of a fructose standard (Sigma) (12-120 µg), according to the methodology described by Matsuhiro (1995) and Saito (1997).

Quantification of total sulfate from carrageenan

Approximately 35 mg of dry carrageenan (n = 3) were moistened in 100 µL of 95% ethanol, followed by the addition of 500 µL of 0.5 N HCl. The samples will be hydrolyzed in boiling water for 2 h. The volume will be completed to 10 mL with distilled water. The samples will be centrifuged at 12,000 rpm for 15 min at room temperature, discarding the precipitate and storing the supernatant (stock solution). In a test tube, 1 mL of the stock solution, 1 mL of distilled water, and 200 µL of 0.5 N HCl will be added, followed by gentle shaking. Then, 100 µL of the barium chloride-gelatin solution will be added. The samples will be shaken again, keeping them at room temperature for 30 min. After this period, the samples will be analyzed in a spectrophotometer (550 nm) and their absorbances recorded to calculate the amount of sulfate (%) using a calibration curve, through an anhydrous sodium sulfate standard, according to Saito (1997).

Statistical analysis

The assumptions of normality and homogeneity of variances were tested using the Sharipo-Wilk and Levene tests, respectively. Total soluble carbohydrates and carrageenan yields, sulfates, 3-6-anhydrogalactose content and were analyzed by ANOVA (two-way) (independent variables: Strains and Seasons). In all cases, the Tukey a posteriori test was used to establish statistical differences between treatments. Pearson's correlation analysis was performed by correlating abiotic data (temperature, seawater transparency and salinity) with

all measured parameters. Statistical analyses were performed using the Past 4.03 program (Oyvind Hammer), considering $p < 0.05$.

4.4 Results

Abiotic Data

Our study revealed significant differences in the climate between the different seasons throughout the study period, (Table 4-1). The highest temperature means were recorded during the summer (25.7 ± 0.9 ºC). These results were accompanied by the highest average seawater transparency of 298 ± 53 cm (cycle 3). During winter (cycle 1), seawater temperatures were lower (19.9 ± 0.8 ºC) and the lowest seawater transparencies were also measured (144.2 ± 58.4 cm). Furthermore, autumn (cycle 4) recorded the highest average salinities (36.0 ± 1.6).

Productivity

The productivity varied among different strains and seasons (Table 4-3). When comparing the produtictivity of different strains in each season, tetrasporophytic strain (T-br) displayed higher growth rates than the gametophytic strains. Overall, all strains presented higher productivity in the summer, and lower productivity at the winter, and Autumn, (Fig 4-4). Comparing each strains throughout the seasons, T-br showed the highest productivity in the spring and summer (276.0 ± 47.3 – 372.4 ± 126.0 g m^{-2} d^{-1}) compared to the winter and autumn (112.2 ± 27.4 – 155.0 ± 42.9 g m^{-2} d^{-1}); E-br showed the highest productivity in the spring, summer, and autumn (75.0 ± 21.4 – 156.5 ± 47.0 g m^{-2} d^{-1}) compared to the winter (38.2 ± 9.5 g m^{-2} d^{-1}); E-lg showed the highest productivity in the summer (117.2 ± 52.4 g m^{-2} d^{-1}) compared to the winter (32.4 ± 6.3 g m^{-2} d^{-1}); E-gy showed the highest productivity in the spring and summer (97.6 ± 21.4 – 124.5 ± 39.3 g m^{-2} d^{-1}) compared to the winter (43.6 ± 12.0 g m^{-2} d^{-1}), (Fig 4-4).

Determination of thallus water content

The results showed similarities among strains and growth cycles (Table 4-2), and the results were used to determine total soluble carbohydrate concentrations and carrageenan yield.

Total carbohydrate content

Total soluble carbohydrate contents showed differences between the strains and interactions (Table 4-3). Tetrasporophyte (T-br) and gametophyte (E-gy) presented higher total soluble carbohydrate contents at the spring. On winter, they gametophytes presented higher total soluble carbohydrate contents than autumn. Comparing the total soluble carbohydrate contents in each strain throughout the season (Fig. 4-5), T-br presented contents between spring (251.0 ± 41.0 mg g^{-1} DW) and summer (131.0 ± 4.8 mg g^{-1} DW); E-br presented contents between winter (209.0 ± 35.6 mg g^{-1} DM) and summer (131.3 ± 37.0 mg g^{-1} DW); E-lg presented contents between winter (200.0 ± 7.0 mg g^{-1} DW) and summer (108.4 ± 18.5 mg g^{-1} DW); E-gy presented levels between spring (232.7 ± 89.8 mg g^{-1} DW) and autumn (124.6 ± 12.4 mg g^{-1} DW), (Fig 4-5).

Carbohydrates – Ethanolic extraction

The results showed significant differences between strains and interactions (Table 4-3). The gametophyte (E-lg) strain showed higher levels compared to all gametophytes in winter and lower in spring, (Fig 4-6a). Comparing the levels of alcohol-soluble carbohydrates in each strain throughout the season, T-br showed levels between spring (26.5 ± 5.5 mg g^{-1} DW) and summer (25.1 ± 2.4 mg g^{-1} DW); E-br showed levels between winter (54.2 ± 10.1 mg g^{-1} DW) and autumn (15.6 ± 3.2 mg g^{-1} DW); E-lg presented contents between winter (54.3 ±

10.1 mg g^{-1} DW) and spring (4.9 ± 6.4 mg g^{-1} DW); E-gy presented contents between winter (50.3 ± 12.9 mg g^{-1} DW) and autumn (7.7 ± 2.8 mg g^{-1} DW), (Fig 4-6a).

Carbohydrates – Aqueous extraction

The results showed significant differences between strains and interactions (Table 4-3). The tetrasporophyte (T-br) strain in spring showed the highest levels of carbohydrates soluble in water and the gametophyte E-gy strain the lowest levels in summer (Fig 4-6). Comparing the carbohydrate levels throughout the seasons, T-br showed levels between spring (226.5 ± 43.4 mg g^{-1} DW) and summer (105.9 ± 7.1 mg g^{-1} DW); E-br presented contents between winter (177.6 ± 30.4 mg g^{-1} DW) and summer (109.3 ± 28.2 mg g^{-1} DW); E-lg presented contents between summer (147.9 ± 23.3 mg g^{-1} DW) and winter (100.9 ± 18.0 mg g^{-1} DW); E-gy presented contents between spring (189.2 ± 80.7 mg g^{-1} DW) and summer (104.1 ± 8.2 mg g^{-1} DW), (Fig 4-6b).

Extraction and quantification of Carrageenan content

The carrageenan contents varied across seasons (Tab 4-3). All strains showed higher carrageenan in the winter, (Fig 4-7). Comparing average carrageenan contents of the strains throughout the seasons: Winter (41.6 ± 4.8 % DW), Spring (30.4 ± 3.8 % DW), Summer (32.4 ± 5.0 % DW), and Autumn (30.6 ± 6.8 % DW), (Fig 4-7).

3,6-anidrogalactose of carrageenan quantification

The 3,6-anhydrogalactose levels showed similarities between the strains and seasons (Table 4-3), and the average 3,6-anhydrogalactose was 42.7 ± 3.7% of carrageenan.

Total sulfates of carrageenan quantification

The total sultates contents varied across only different seasons (Tab 4-3). After comparing the value of total sulfates contents in different growth cycles, the summer and autumn, had a lowers total sulfates compared to the spring (Table 4-1; Fig 4-8). Comparing total sulfates contents throughout the seasons, the average results of the strains were: winter (26.9 ± 5.4 %), spring (29.8 ± 2.6 %), summer (22.7 ± 7.1 %), and autumn (24.4 ± 5.8 %), (Fig 4-8).

Pearson correlation

The following correlations were observed among various factors: Abiotic data indicated a positive correlation between temperature and water transparency, as well as with productivity. Total Carbohydrates were negatively correlated with salinity. carrageenan content showed negative correlations with both temperature and water transparency.

4.5 Discussion

Our data corroborate the hypothesis that the new light-green and yellow-green color variants are similar to the pale-brown strain of the “Edison de Paula” gametophytes in the evaluated characteristics, demonstrating similarity in their productivity, total carbohydrate, carrageenan and 3,6-anhydrogalactose and sulfate contents of the carrageenan.

The results supported the hypothesis that diploid organisms are more productive and that seasonality in seawater temperature during winter (19.9 ± 0.8 ^{o}C) and summer (25.7 ± 0.9 ^{o}C) correlated with productivity and carrageenan contents.

Seasonality responses were shaped by multiple interacting factors, both exogenous and endogenous (Pereira and Gomes 2009). Seasonal temperature variations in the study had a direct influence on productivity, increasing in the summer, as well as the carrageenan yield

was inversely correlated to productivity, in addition to the higher carrageenan sulfate contents in the spring.

Terada et al. (2015) reported that seawater temperature during the cultivation of *K. alvarezii* was lower than the ideal range. Additionally, epiphytes is presence in seaweed production is common and can diminish productivity (Hurtado et al. 2007; Mulyaningrum et al. 2019). Few studies in the same cultivation site relate to productivity. Productivity gains when applying a density of 6.7 plants m^{-2} in the studied strains in the same cultivation site showed results of 46 to 181 g m^{-2} d^{-1} for T-br and 20 to 71 g m^{-2} d^{-1} for E-br (Solorzano-Chávez et al. 2019). This highlights the consistency of our results when considering the density factor applied in our study.

Abiotic factors such as water temperature and salinity, luminosity, water movement, and nutrients also impact the biochemical contents of polysaccharides (Tanniou et al., 2015; Stiger-Poureau et al. 2016) and influence the development of *K. alvarezii* (Rupert et al. 2022) with temperature appearing to be the most critical factor for carrageenan production (Glenn and Doty 1992; Heriyanto et al. 2018) until reaching the ideal temperature between 28 – 31 ^{o}C (Orbita 2013), at which point the production rate drops rapidly (Bulboa et al. 2008, Kumar et al. 2020). Studies establish carbohydrate contents around 66% for *K. alvarezii* in the Philippines (Meinita et al. 2012; Abd-Rahim et al. 2014), in addition to seasonal variations in the same cultivation site from 122.92 ± 15.11 mg g^{-1} DW (Summer 2017) to 231.79 ± 16.86 mg g^{-1} DW (Winter, 2017) (Araújo et al., 2023).

Our results demonstrate seasonality in total soluble carbohydrates in summer and/or spring from 251.0 ± 41.0 mg g^{-1} DW to 131.0 ± 4.8 mg g^{-1} DW for T-br and from 209.0 ± 35.6 mg g^{-1} DW to 131.3 ± 37.0 mg g^{-1} DW for E-br. Other methodologies, however, identified contents of 53.4 g 100 g^{-1} (T-br) and 51.6 g 100 g^{-1} (E-br) (Masarin et al. 2016), in

addition to 21.1 g 100 g^{-1} (T-br) (Solorzano-Chavez et al. 2019), also in the same cultivation site. Algae synthesize low molecular weight carbohydrates and polysaccharides to maintain cellular osmotic pressure (Karsten et al. 1999). *Kappaphycus alvarezii* strains show greater sensitivity to salinity variations and the study indicates that temperature increases and decreases in salinity can negatively impact polysaccharide contents.

Our study demonstrated that seasonality patterns were related to carrageenan yield, in agreement with other studies carried out with *K. alvarezii* (Othno et al. 1994; Hayashi et al. 2007a).

The seasonality patterns showed a relationship with the carrageenan yields, which reached higher values at the end of winter of 41.6 ± 4.8% although lower when compared with other studies, carried out in the same cultivation site, of 64.5 ± 1.2 % DW (Roldán et al. 2017), 63.5 ± 6.0 % DW (Masarin et al. 2016) and 73.3 ± 1.0 % DW (Solorzano-Chavez et al. 2019).

The differences can be attributed to the extraction methodology used in each study, and comparisons are difficult, because they vary depending on the extraction method, strain, and/or raw material extracted (Hayashi et al. 2007), as well as anthropogenic environmental changes over the 29 years of studies in the same cultivation site.

Carrageenan sulfate levels also showed seasonal variations (29.8 % – 22.7 %), similar to those observed by Hayashi et al. (2007) (T-br: 32.8 – 27.2 %; E-br: 30.1 – 27.9 %).

The levels of 3,6-anhydrogalactose in carrageenans were consistent across all strains, measured at 42.7 ± 3.7%. This finding contrasts with the research conducted by Hayashi et al. (2007), which reported varying levels between tetrasporophytes and gametophytes from the same cultivation site. In their study, they noted values of 8.7 – 7.5 % for T-br and 30.6 – 26.1 % for E-br over 30-day growth cycles.

Presenting results inferior to those of the present study, Solorzano-Chaves et al. (2019), when investigating tetrasporophytes in the same cultivation site, highlighted that alkaline extraction increased sulfate levels (for example, from 12.1 ± 0.8% to 16.1 ± 1.1% in cold extraction), reaching 21.9 ± 1.2% in the residue. The study also highlighted the influence of the methodology applied in carbohydrate quantification.

In conclusion, the data obtained in this study confirm the initial hypotheses about the similarity between the new light green and yellow-green color variants and the light brown lineage of the “Edison de Paula” gametophytes in terms of productivity, total carbohydrates, carrageenan, 3,6-anhydrogalactose, and carrageenan sulfate contents. Multiple interactive factors, exogenous and endogenous, attributed the seasonality patterns, which had a direct impact on productivity, which was higher in summer, and on carrageenan contents, which presented the highest values at the end of winter.

This study reinforces the importance of ploidy in determining the physiological responses of *Kappaphycus alvarezii* strains, showing that diploid strain are more productive and that environmental conditions significantly influence the seasonal productivity patterns and biochemical contents. These results contribute to understanding the interactions between genetic and environmental factors in macroalgae cultivation and highlight the relevance of adequate management to optimize the production of this species.

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Declarations

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Conflict of interest

The authors declare that they have no competing interests. NSY is member of Editorial Board of the Journal of Applied Phycology, and the peer-review process for this article was independently handled by another member of the journal editorial board.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Data availability statement

All data generated or analyzed during this study are included in this published article.

Code availability

“Not applicable”.

Authors' contributions

Leandro Herrera: conceptualization, methodology, investigation, formal analysis, writing – original draft.

Valéria C. Gelli: conceptualization, methodology, investigation, funding acquisition, writing – review & editing.

Nair S. Yokoya: conceptualization, funding acquisition, writing – review & editing.

All authors contributed to the article and approved the submitted version.

Table 4-1: Means of abiotic data (temperature; salinity; water transparency) (n = 24) over 4 growth cycles (cycle 1, Winter: August/15/2022 – September/04/2022, cycle 2, Spring**:** November/17/2022 – December/03/2022, cycle 3, Summer**:** February/16/2023 – March/04/2023, and cycle 4, Autumn**:** May/18/2023 – June/04/2023, (one-way ANOVA, Tukey: p-value < 0.05). SD standard deviation of the means, F statistical analysis of variance (ANOVA) of the ratio between the variation between the groups and the variation within the groups, and p statistically significant difference between groups, at the 5% significance level.

Paratemeters	Growth cycles	Min	Max	Mean	SD	F	p
Temperature	1	18.0	21.0	19.9	0.8	168.9	**0.0000**
(ºC)	2	21.0	26.0	23.8	1.3		
	3	24.0	27.0	25.7	0.9		
	4	20.0	22.5	21.5	0.7		
Salinity	1	30.0	37.0	34.2	2.1	10.3	**0.0000**
	2	21.0	35.0	31.3	4.2		
	3	26.0	36.0	34.2	2.1		
	4	32.0	37.0	36.0	1.6		
Water Transparency	1	40	290	144	58	15.6	**0.0000**
(cm)	2	70	400	225	101		
	3	170	30	298	53		
	4	100	360	209	74		

bold, significant correlation (p < 0.05).

Table 4-2: Average water content in the thallus of *Kappaphycus alvarezii* strains Brown tetrasporophyte (T-br); Pale-brown Edison de Paula (E-br); Light-green Edison de Paula (E-lg); Greenish-yellow Edison de Paula (E-gy). Winter (cycle 1: August-September/2022), Spring (cycle 2: November-December/2022), Summer (cycle 3: February-March/2023), and Autumn (cycle 4: May-June/2023), (n = 3; Growth cycles = 45 days). Standard deviation of the means (SD).

Growth cycles (Season)	T-br	E-br	E-lg	E-gy
1 (Winter)	92.17 %	90.12 %	90.47 %	91.57 %
2 (Spring)	91.70 %	89.37 %	90.60 %	91.64 %
3 (Summer)	92.86 %	91.89 %	92.24 %	92.26 %
4 (Autumn)	91.91 %	91.67 %	91.36 %	92.26 %
Average (SD)	91.70 ± 0.33 %	89.74 ± 0.53 %	90.53 ± 0.09 %	91.60 ± 0.05 %

Table 4-3: Analyzed by two-way ANOVA to examine the influence of independent variables (Temperature; Salinity; Water transparency; Carbohydrates, Carrageenan, 3,6 anidrogalactose; Total Sulfates) on the dependent variables strains, Brown tetrasporophyte (T-br), Pale-brown Edison de Paula (E-br), Light-green Edison de Paula (E-lg), Greenish-yellow Edison de Paula (E-gy). Growth cycles (cycle 1: August-September/2022, cycle 2: November-December/2022, cycle 3: February-March/2023, cycle 4: May-June/2023 of *K. alvarezii* cultivated in Ubatuba Bay.

Parameters	Source of variatin	df	F	*p*
Productivity	Period	3	75.9	**0.0000**
	Strain	3	140.1	**0.0000**
	Interaction	9	12.1	**0.0000**
Total carbohydrates	Period	3	31.6	**0.0000**
	Strain	3	1.0	0.3796
	Interaction	9	3.0	**0.0071**
Carbohydrates (Meth. extr.)	Period	3	28.5	**0.0000**
	Strain	3	1.6	**0.0379**
	Interaction	9	8.3	**0.0000**
Carbohydrates (Water extr.)	Period	3	30.1	**0.0000**
	Strain	3	1.9	0.6299
	Interaction	9	2.1	**0.0216**
% Carrageenan	Period	3	13.4	**0.0000**
	Strain	3	1.6	0.2024
	Interaction	9	1.3	0.2948
3,6 anidrogalactose	Period	3	2.7	0.0649
	Strain	3	0.9	0.4332
	Interaction	9	0.5	0.8577
Total sulfates	Period	3	5.1	**0.0056**
	Strain	3	0.8	0.4947
	Interaction	9	2.7	**0.0196**

bold, significant correlation ($p < 0.05$).

Table 4-4: Pearson correlation analysis of the parameters (Temperature, Salinity, Water Transparency, Productivity, Total Carbohydrates, and Carrageenan). Brown tetrasporophyte (T-br); Pale-brown Edison de Paula (E-br); Light-green Edison de Paula (E-lg); Greenish-yellow Edison de Paula (E-gy). Growth cycles (cycle 1: August-September/2022, cycle 2: November-December/2022, cycle 3: February-March/2023, cycle 4: May-June/2023 of *K. alvarezii* cultivated in Ubatuba Bay.

Parameters	Salinity	Transparency	Productivity	Carbohydrates	Carrageenan
Temperature	-0.25 (0.354)	0.96 **(0.0000)**	0.55 **(0.0283)**	-0.24 (0.3618)	-0.65 **(0.0065)**
Salinity		0.02 (0.9499)	-0.15 (0.5876)	-0.67 **(0.0047)**	-0.02 (0.9429)
Transparency			0.53 **(0.0369)**	-0.44 (0.0862)	-0.72 **(0.0018)**
Productivity				0.00 (0.9797)	-0.33 (0.2173)
Carbohydrates					0.36 (0.1681)

bold, significant correlation ($p < 0.05$).

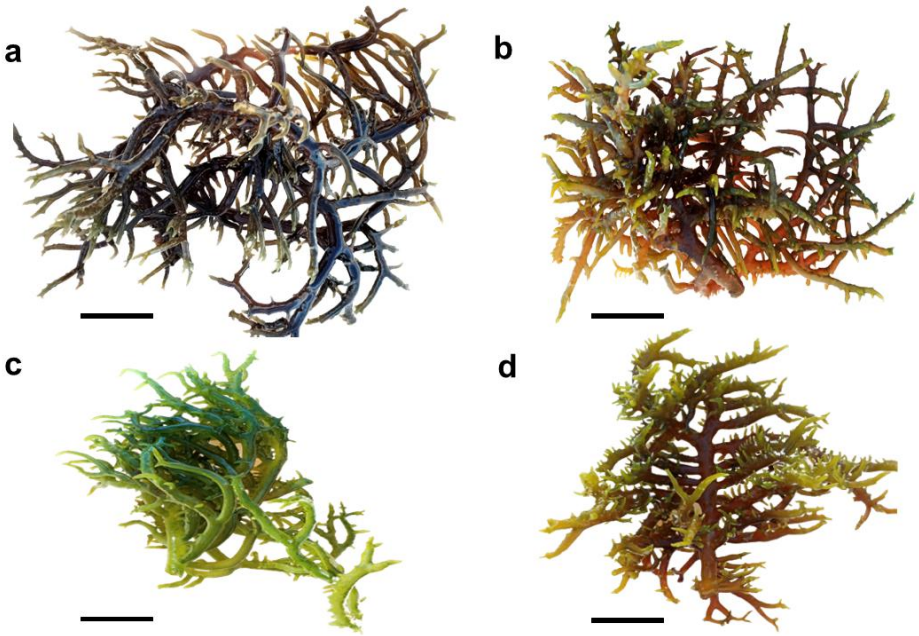

Figure 4-1: Macroscopic morphology of four color strains of *Kappaphycus alvarezii* cultivated in the Experimental Marine Farm of the Fisheries Institute, Ubatuba bay, southeastern Brazil. (a) Brown tetrasporophyte; (b) Pale-brown Edison de Paula gametophyte; (c) Light-green Edison de Paula gametophyte; (d) Greenish-yellow Edison de Paula gametophyte. Scale bar = 5 cm.

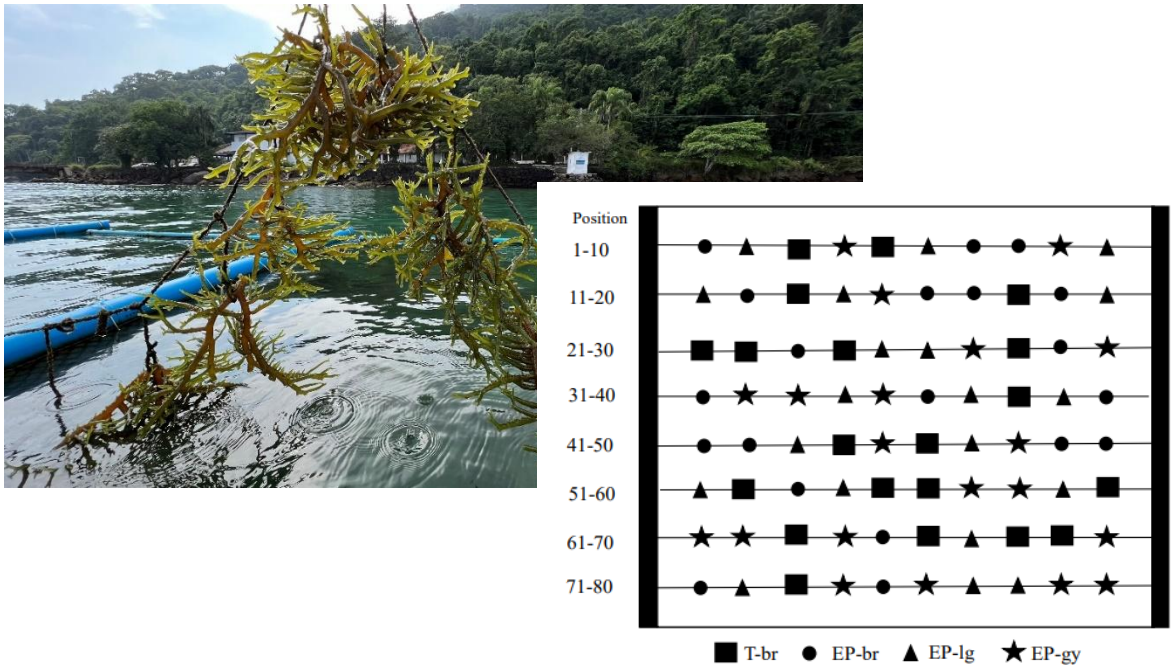

Figure 4-2: General aspect of cultivation site with a floating raft system, and experimental design of distribution of *Kappaphycus alvarezii* strains Tetrasporophyte brown (T-br); Edison de Paula light brown gametophyte (E-br); Edison de Paula light green gametophyte (E-lg); Edison de Paula yellow-green gametophyte (E-gy), (n = 20).

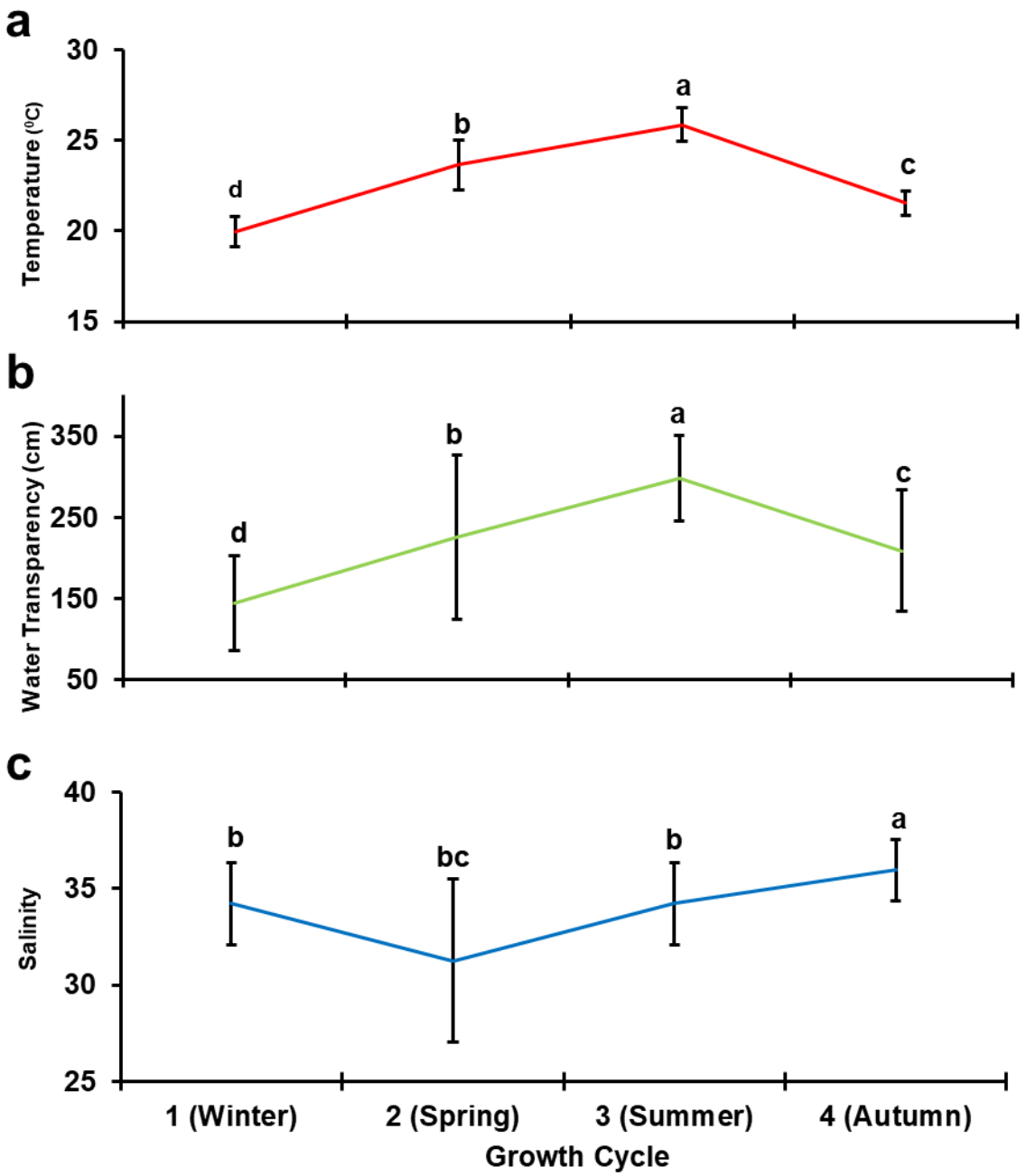

Figure 4-3: Variation of Temperature (a), Water Transparency (b), and Salinity (c) at the *Kappaphycus alvarezii* cultivation site in Ubatuba Bay. Data represent means ± standard deviation (n = 24) recorded over 45 days during the four climatic seasons throughout the year. 1 (Winter): August-September/2022, 2 (Spring): November-December/2022, 3 (Summer): February-March/2023, and 4 (Autumn): May-June/2023. Different letters represent significant differences among growth cycles or months (one-way ANOVA, Tukey´s multiple comparison test, $p < 0.05$).

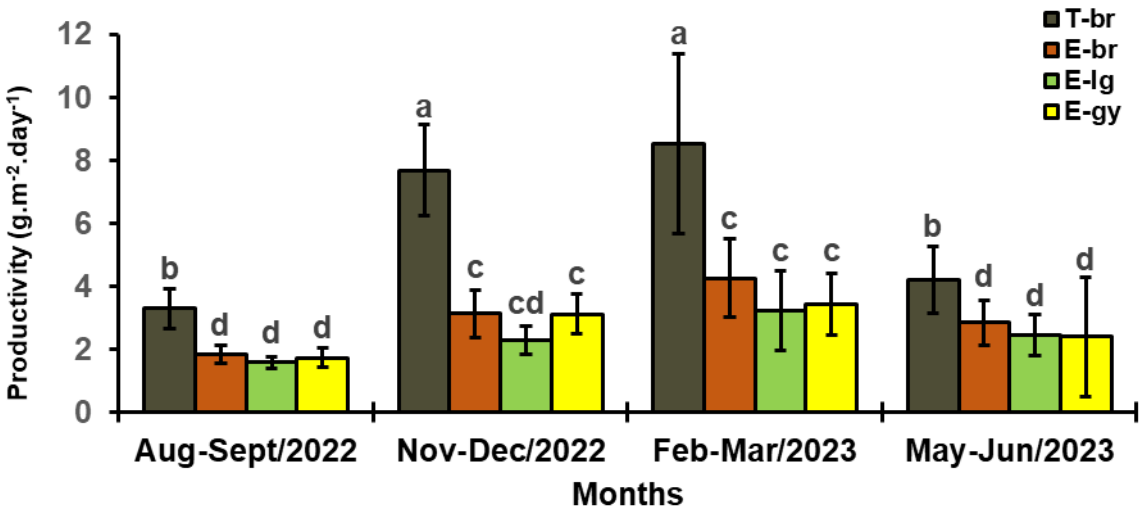

Figure 4-4: Productivity of *Kappaphycus alvarezii* color strains cultivated in Ubatuba bay, from August/2022 to August/2023, comprising four 45-day growth cycles interspersed with sample collections every two cycles (Mean ± SD, n = 20). Brown tetrasporophyte (T-br); Pale-brown Edison de Paula gametophyte (E-br); Light-green Edison de Paula gametophyte (E-lg); Greenish-yellow Edison de Paula gametophyte (E-gy). Growth cycle 1: August-September/2022 (Winter), cycle 2: November-December/2022 (Spring), cycle 3: February-March/2023 (Summer), cycle 4: May-June/2023 (Autumn). Bars, standard deviation of the means (SD). Different letters represent significant differences among seasons (Two-way ANOVA, Tukey´s multiple comparison test, $p < 0.05$).

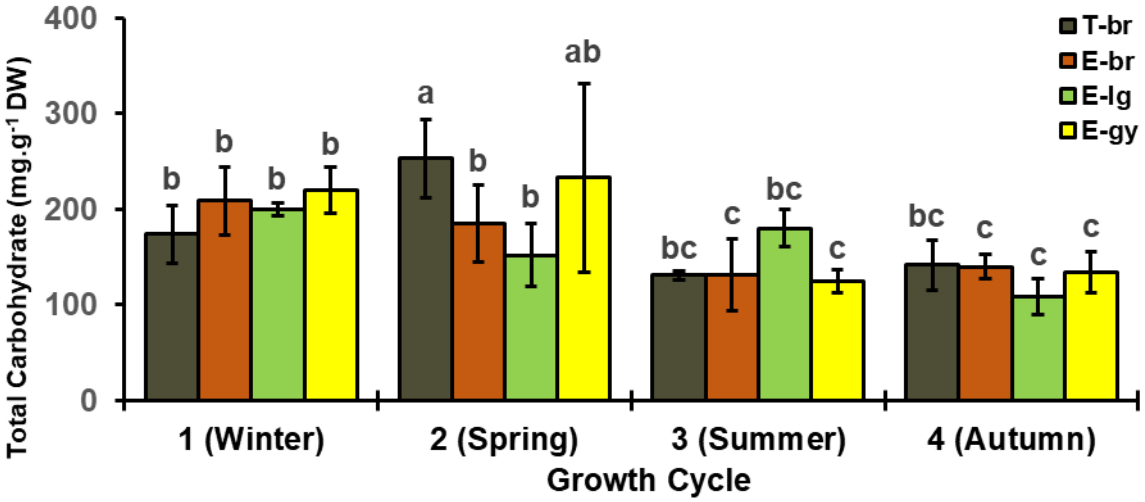

Figure 4-5: Soluble carbohydrate contents (mean ± SD, n=4) of *Kappaphycus alvarezii* strains cultivated in Ubatuba bay for 45 day-growth cycle in each season. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula gametophyte (E-br); Light-green Edison de Paula gametophyte (E-lg); Greenish-yellow Edison de Paula gametophyte (E-gy). 1 (Winter): August-September/2022, 2 (Spring): November-December/2022, 3 (Summer): February-March/2023, 4 (Autumn): May-June/2023. Bars, standard deviation of the means (SD). Different letters represent significant differences among seasons (Two-way ANOVA, Tukey´s multiple comparison test, $p < 0.05$).

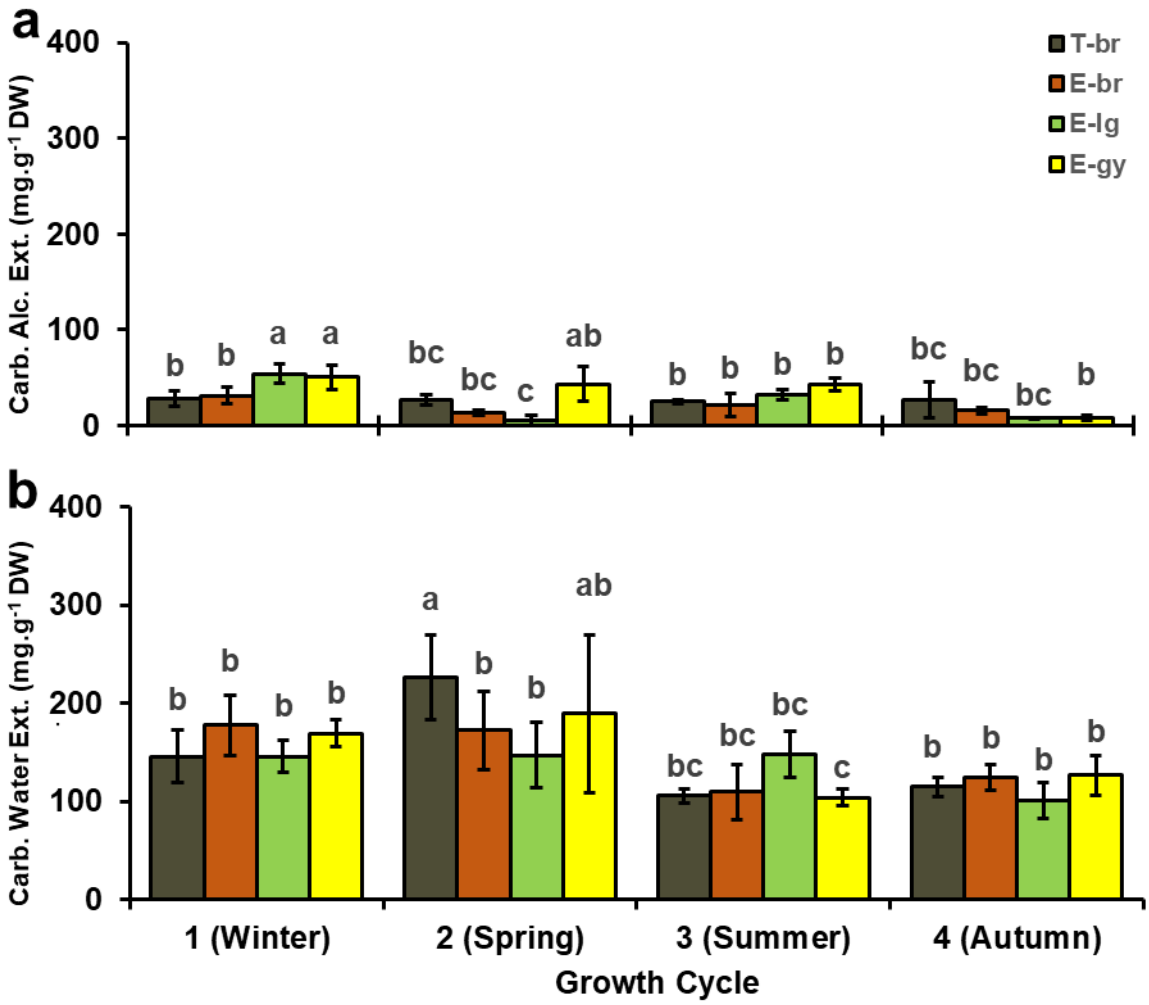

Figure 4-6: Soluble carbohydrate contents (mean ± SD, n=4) of *Kappaphycus alvarezii* strains cultivated in Ubatuba bay for 45 day-growth cycle in each season. a ethanolic extraction, b aqueous extraction. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula gametophyte (E-br); Light-green Edison de Paula gametophyte (E-lg); Greenish-yellow Edison de Paula gametophyte (E-gy). 1 (Winter): August-September/2022, 2 (Spring): November-December/2022, 3 (Summer): February-March/2023, 4 (Autumn): May-June/2023. Bars, standard deviation of the means (SD). Different letters represent significant differences among seasons (Two-way ANOVA, Tukey´s multiple comparison test, $p < 0.05$).

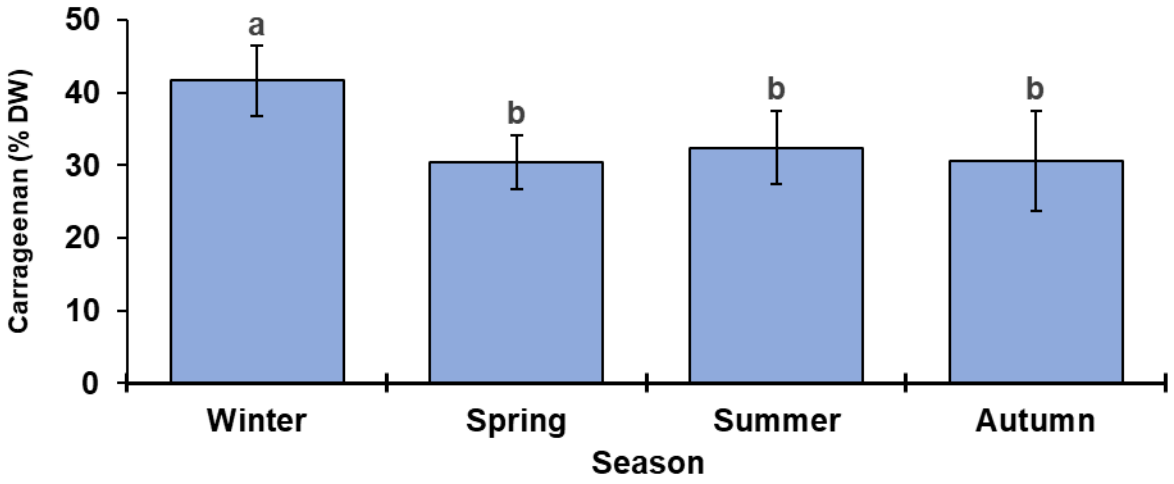

Figure 4-7: Carrageenan contents (mean ± SD, n=3) of *Kappaphycus alvarezii* strains cultivated in Ubatuba bay for 45 day-growth cycle in each season. The blue columns represent the averages of the four strains together since there were no significant differences among them. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula gametophyte (E-br); Light-green Edison de Paula gametophyte (E-lg); Greenish-yellow Edison de Paula gametophyte (E-gy). Winter (August-September/2022), Spring (November-December/2022), Summer (February-March/2023), Autumn (May-June/2023). Bars, standard deviation of the means (SD). Different letters represent significant differences among seasons (Two-way ANOVA, Tukey´s multiple comparison test, $p < 0.05$).

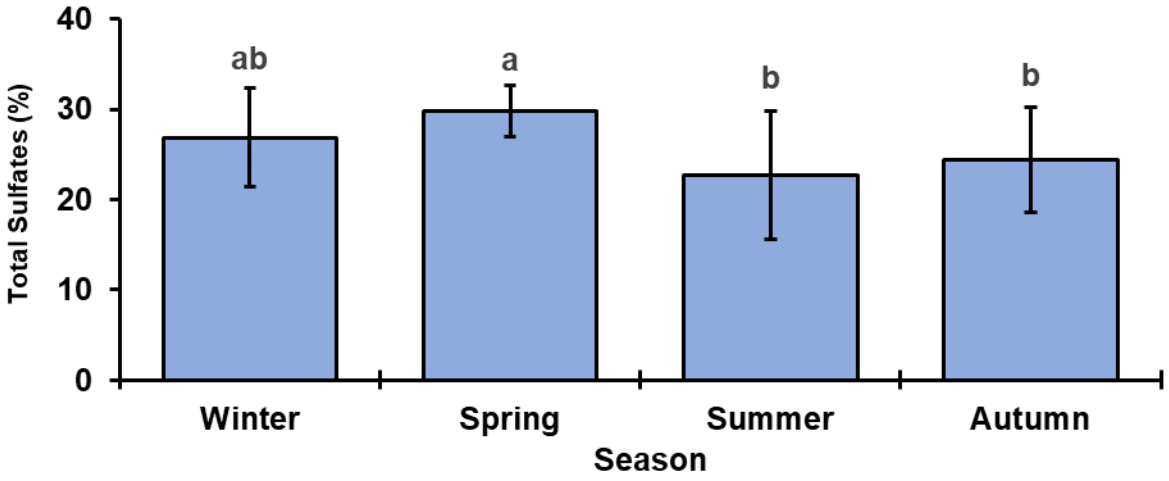

Figure 4-8: Total Sulfates contents (mean ± SD, n=4) of *Kappaphycus alvarezii* strains cultivated in Ubatuba bay for 45 day-growth cycle in each season. The blue columns represent the averages of the four strains together since there were no significant differences among them. Brown tetrasporophyte (T-br); Pale-brown Edison de Paula gametophyte (E-br); Light-green Edison de Paula gametophyte (E-lg); Greenish-yellow Edison de Paula gametophyte (E-gy). Winter (August-September/2022), Spring (November-December/2022), Summer (February-March/2023), Autumn (May-June/2023). Bars, standard deviation of the means (SD). Different letters represent significant differences among seasons (Two-way ANOVA, Tukey´s multiple comparison test, $p < 0.05$).

5. Conclusões e considerações finais

Com base nos resultados apresentados, é possível identificar as linhagens de *Kappaphycus alvarezii* com desempenho superior e maior potencial biotecnológico para cultivos comerciais, considerando os parâmetros de crescimento, eficiência fotossintética e composição bioquímica:

1. O "efeito de borda" no sistema de cultivo de *Kappaphycus alvarezii* foi identificado como um fator que influenciou negativamente as taxas de crescimento no delineamento experimental inicial;

2. Linhagem tetrasporofítica marrom como destaque principal: Esta linhagem apresentou as maiores taxas de crescimento e produtividade, especialmente no verão, período em que há maior incidência de luz e temperatura mais elevada. Esse desempenho pode estar relacionado à sua maior concentração de ficobiliproteínas, importantes pigmentos envolvidos na fotossíntese e no aproveitamento da luz. Essa característica confere à linhagem tetrasporofítica maior resiliência fisiológica e potencial produtivo, tornando-a altamente promissora para cultivos comerciais em larga escala, principalmente na região de Ubatuba;

3. Linhagem gametofítica verde-amarelo (E-gy): Apesar de não apresentar os maiores índices de crescimento, a E-gy demonstrou maior eficiência fotossintetizante entre as gametofíticas, sugerindo um bom desempenho metabólico sob condições ambientais favoráveis. Essa linhagem pode ser vantajosa em sistemas de cultivo que priorizam

eficiência energética e estabilidade fisiológica, mesmo que sua taxa de crescimento absoluta seja inferior à tetrasporofítica;

4. Linhagens gametofíticas verde-claro (E-lg) e marrom-claro (E-br): A linhagem E-lg apresentou menores concentrações de ficoeritrina e taxas de crescimento reduzidas sob altas temperaturas, indicando baixa adaptação a condições estivais. Já a E-br, embora mais próxima das outras em termos de pigmentação, não se destacou significativamente nos parâmetros avaliados. Essas linhagens, portanto, apresentam menor potencial biotecnológico imediato, especialmente para regiões de cultivo com alta temperatura média;

5. Considerações bioquímicas complementares: A queda nas concentrações de proteínas solúveis no verão e o aumento de carotenoides com função fotoprotetora demonstram a importância da resiliência metabólica sob estresse térmico, característica mais evidenciada na linhagem tetrasporofítica. Além disso, embora os teores de carragenana e sulfatos não tenham variado entre as linhagens, a estabilidade sazonal desses compostos reforça a viabilidade do cultivo comercial;

Conclusão:

A linhagem tetrasporofítica marrom é a que reúne maior desempenho na produção de biomassa, com potencial superior para cultivos comerciais, especialmente na região de Ubatuba e durante o verão. A linhagem gametofítica E-gy representa uma alternativa complementar, com destaque para sua eficiência fotossintética, sendo útil em condições controladas ou como parte de estratégias de cultivo misto.

Diante do exposto, algumas questões ainda permanecem em aberto e poderiam ser aprofundadas. Este estudo suscitou reflexões sobre o comportamento fisiológico de *Kappaphycus alvarezii* frente às variações cromáticas das diferentes linhagens analisadas, bem como sobre seus parâmetros fotossintetizantes ao longo das oscilações ocorridas no ciclo diário.