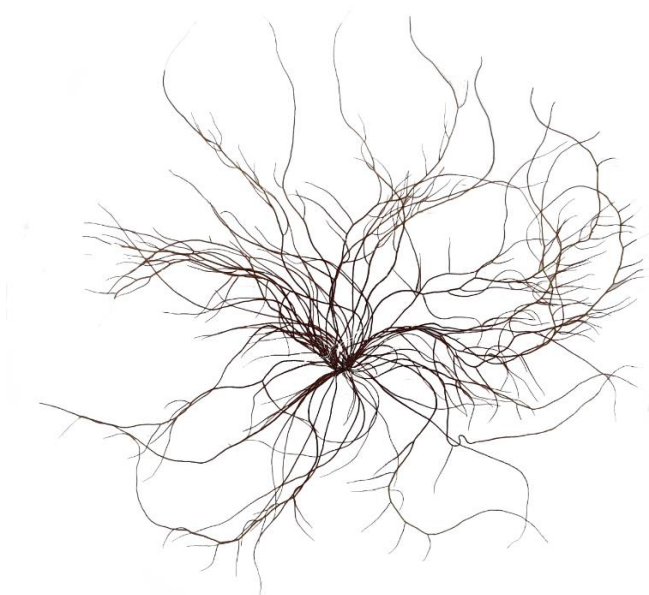


André Vinicius Fonseca de Faria

**“Estudos populacionais em *Gracilariopsis tenuifrons*
(Gracilariales, Rhodophyta): aspectos fisiológicos e
moleculares”**

**“Population studies in *Gracilariopsis tenuifrons*
(Gracilariales, Rhodophyta): physiological and
molecular approaches”**



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André Vinicius Fonseca de Faria

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(Gracilariales, Rhodophyta): aspectos fisiológicos e
moleculares”

“Population studies in *Gracilariopsis tenuifrons*
(Gracilariales, Rhodophyta): physiological and molecular
approaches”

Thesis presented to the Institute
of Biosciences at the University of
São Paulo to obtain PhD degree
in Science, Area Botany.

Advisor: Prof^a. Dr^a. Estela Maria
Plastino

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CAPÍTULO 1 – INTRODUÇÃO GERAL

Diversidade intraespecífica

A diversidade intraespecífica compreende o conjunto de expressões fenotípicas resultantes de processos de aclimação e adaptação. Essas respostas fenotípicas contribuem para o sucesso dos indivíduos e/ou populações, quando submetidos a variações ambientais. A aclimação corresponde às diferentes expressões de ajustamento ao ambiente que um organismo pode sofrer dentro do limite de seu genoma. Já, a adaptação corresponde à expressão de ajustamento ao ambiente decorrente de alteração no genótipo ao longo de gerações (Plastino 2004).

Variações genotípicas intraespecíficas podem ocorrer em uma mesma população ou em populações distintas. Essas variações são denominadas de polimorfismo, quando em um mesmo habitat ocorrem, simultaneamente, duas ou mais formas descontínuas de determinada espécie, em tais frequências que a mais rara delas não pode ser meramente mantida por mutação recorrente ou imigração (Ford 1964). Assim, o polimorfismo é decorrente de processos adaptativos em uma mesma população, e as variantes são denominadas de morfos ou morfotipos (Plastino e Guimarães 2001). No entanto, quando as variações genotípicas estão relacionadas a adaptações dos organismos a ambientes distintos, resultando em populações de uma mesma espécie geneticamente diferentes, utiliza-se o termo ecótipo (Lowry 2012). Ecótipos e morfos podem surgir a partir de variações que ocorrem durante processos meióticos ou mitóticos, podendo resultar, nesse último caso, numa variação intraclonal. A persistência e a transmissão das variações genéticas em descendentes dependem de condições favoráveis para que se estabeleçam nas populações (Plastino 2008).

O fluxo gênico pode ser definido como a migração de alelos que ocorre entre populações próximas. As correntes marinhas, como fator abiótico, muitas vezes não apresentam um papel essencial na sua orientação (Billot et al. 2003). Contudo, a carência na continuidade de habitats, como desembocadura de rios, trechos de substrato impróprio, entre outros, criam barreiras para o fluxo gênico e colaboram com a diferenciação gênica (Kimura e Weiss 1964; Faugeron et al. 2001; Billot et al. 2003; Montecinos et al. 2012). Fragmentos férteis do talo podem ser distribuídos ocasionalmente por dispersão à longa distância, o que contribui para o fluxo gênico (Billot et al. 2003). Estudos realizados com macroalgas apontam que a diferenciação genética pode ocorrer a partir de distâncias

relativamente curtas, maiores que um metro e menores que 10 km, e modos diferentes de dispersão de gametas e/ou esporos resultam em diferentes níveis de estruturas genéticas (Sosa e Lindstrom, 1999; Valero et al. 2001).

A compreensão da diversidade intraespecífica, em particular, a ocorrência de ecótipos é descrita nos diferentes grupos de macroalgas. Em algas vermelhas existem algumas espécies para as quais foram descritos ecótipos com características distintas quanto à morfologia, taxas de crescimento, eficiência fotossintetizante, conteúdo pigmentar e/ou metabólitos (Tabela 1) (Fig. 1).

Tabela 1. Ecótipos descritos para espécies de Rhodophyta e parâmetros avaliados que permitiram a constatação. Fatores abióticos avaliados: temperatura (°C), salinidade (UPS), irradiância (IR) e radiação ultravioleta B (UV). Parâmetros avaliados: taxa de crescimento (TC), fotossíntese (FT), conteúdo pigmentar (CP), antioxidante (AT), coloração (CO), morfologia (MO), metabólitos (ME) e sorbitol (SO). *ecótipos reconhecidos após cultivos em laboratório sem variações nas condições de cultivo.

Espécies	Localização das populações	Fator abiótico	P. avaliados	Ref.
<i>Ahnfeltiopsis glomerata*</i>	Namíbia e África do Sul	-	CO e MO	Anderson e Bolton 1990
<i>Asparagopsis taxiformis</i>	Itália, Austrália e Japão	°C	TC	Ní Chualáin et al. 2004
<i>Asparagopsis taxiformis</i>	Austrália, Espanha	°C	FT	Zanolla et al. 2014
<i>Bostrychia radicans</i>	EUA	UPS	TC	Yarish et al. 1979
<i>Bostrychia radicans</i>	EUA	UPS	TC e SO	Karsten et al. 1994
<i>Caloglossa leprieurii</i>	EUA	UPS	TC	Yarish et al. 1979
<i>Ceramium tenuicorne</i>	Mar Báltico, Estreito da Noruega	UPS	TC	Rueness e Kornfeldt 1992
<i>Ceramium tenuicorne</i>	Mar Báltico	UPS	TC e MO	Bergström e Kautsky 2005
<i>Chondracanthus chamissoi*</i>	Chile	-	FT, CP e AT	Véliz et al. 2018
<i>Delesseria sanguinea</i>	Mar Báltico	UPS	TC e MO	Rietema 1993
<i>Devaleraea ramentacea</i>	Canadá e Europa	°C	TC	Novaczek et al. 1990
<i>Furcellaria lumbricalis</i>	Estreito Dinamarquês	-	MO	Austin 1960
<i>Furcellaria lumbricalis</i>	Canadá e Europa	°C	TC	Novaczek e Breeman 1990
<i>Gracilaria birdiae</i>	Brasil (CE e ES)	IR e °C	FT, MO e ME	Ursi et al. 2003, 2013
<i>Gracilaria caudata</i>	Brasil (CE, BA, ES, SP e SC)	UV, IR e °C	TC, FT e CP	Araújo et al. 2014; Faria et al. 2017; Chiamonte 2022
<i>Gracilaria domingensis</i>	Brasil (ES e SC)	IR e °C	TC e CP	Castro e Yokoya 2018
<i>Gracilaria vermiculophylla</i>	EUA e Coreia do Sul	°C	TC	Gorman et al. 2017
<i>Gracilariopsis tenuifrons</i>	Brasil (MA, RN, BA e SP)	°C	TC, FT e CP	Faria e Plastino 2022
<i>Hypnea pseudomusciformis</i>	Brasil (RN, BA, ES, SP, SC)	IR	TC, FT e CP	Nauer et al. 2021
<i>Mazzaella splendens*</i>	Canadá	-	MO	Shaughnessy 2004
<i>Membranoptera alata</i>	Mar Báltico	UPS	TC e MO	Rietema 1993
<i>Phycodrys rubens</i>	Canadá e França	°C	TC	Novaczek et al. 1990
<i>Phycodrys rubens</i>	Mar Báltico	UPS	TC	Rietema 1991
<i>Polyides rotundus</i>	Canadá e Europa	°C	TC	Novaczek e Breeman 1990
<i>Pterocladia capillacea</i>	Brasil (RJ e SP)	°C	TC	Yokoya e Oliveira 1992
<i>Solieria pacifica</i>	Japão	IR e °C	FT	Borlongan et al. 2017

A diversidade intraespecífica é também observada em algas que apresentam históricos de vida com alternância de gerações. A literatura reporta vantagens e desvantagens dos haplóides e diplóides: i) organismos diplóides possuem duas cópias de

cada gene, o que poderia acelerar o ritmo de adaptação a novos ambientes. Esses indivíduos possuem duas vezes mais probabilidades de ter uma nova mutação benéfica do que os haplóides, conseqüentemente, diplóides evoluíram mais rápido; e ii) diplóides mantêm maior variação genética porque as mutações persistem, e isto pode melhorar a capacidade de diplóides responderem às mudanças ambientais. Porém, indivíduos diplóides estariam em desvantagem em relação aos haplóides devido ao maior gasto energético para replicação de seu DNA, e as células haplóides poderiam ter uma vantagem nutricional em relação a células diplóides, especialmente em fases unicelulares (Lewis 1985; Hughes e Otto 1999). A relação superfície/volume em células haplóides é comumente maior do que em células diplóides, que apresentam uma relação superfície/volume menor (Hughes e Otto 1999). Essa variação na área e no volume entre as fases poderia propiciar melhores taxas de crescimento e/ou sobrevivência aos gametófitos jovens em determinados ambientes, especialmente em locais escassos de nutrientes. Quanto maior a área de superfície da célula, maior a eficiência e capacidade de transportar nutrientes através da membrana plasmática (Lewis 1985; Hughes e Otto 1999). Destombe et al. (1993) observaram que indivíduos haplóides de *Gracilaria gracilis* (como *G. verrucosa*) apresentaram maiores taxas de crescimento que indivíduos diploides, quando cultivados em água do mar escassa em nutrientes; o inverso foi observado quando esses indivíduos foram cultivados em água do mar rica em nutrientes. Assim, indivíduos haplóides, quando comparados a diplóides, poderiam apresentar um desempenho melhor em locais escassos de nutrientes, absorvendo maiores concentrações de nutrientes e sobrevivendo (Lewis 1985).

Além da importância da ploidia, a fertilidade pode também influenciar negativamente o desempenho e o desenvolvimento de gametófitos ou tetrasporófitos. Guillemín et al. (2014) observaram que gametófitos e tetrasporófitos inférteis de *G. chilensis* apresentaram maiores taxas de crescimento e de eficiência fotossintetizante quando comparados a essas mesmas fases na forma reprodutiva. Resultados similares foram observados para *G. caudata*, quando comparados tetrasporófitos férteis e não férteis (Faria e Plastino 2016). Algas no estado reprodutivo reduzem ou cessam o seu crescimento, e os recursos energéticos voltados a esse processo são compartilhados para o desenvolvimento das estruturas reprodutivas (Santelices et al. 1995; Guimarães et al. 1999).

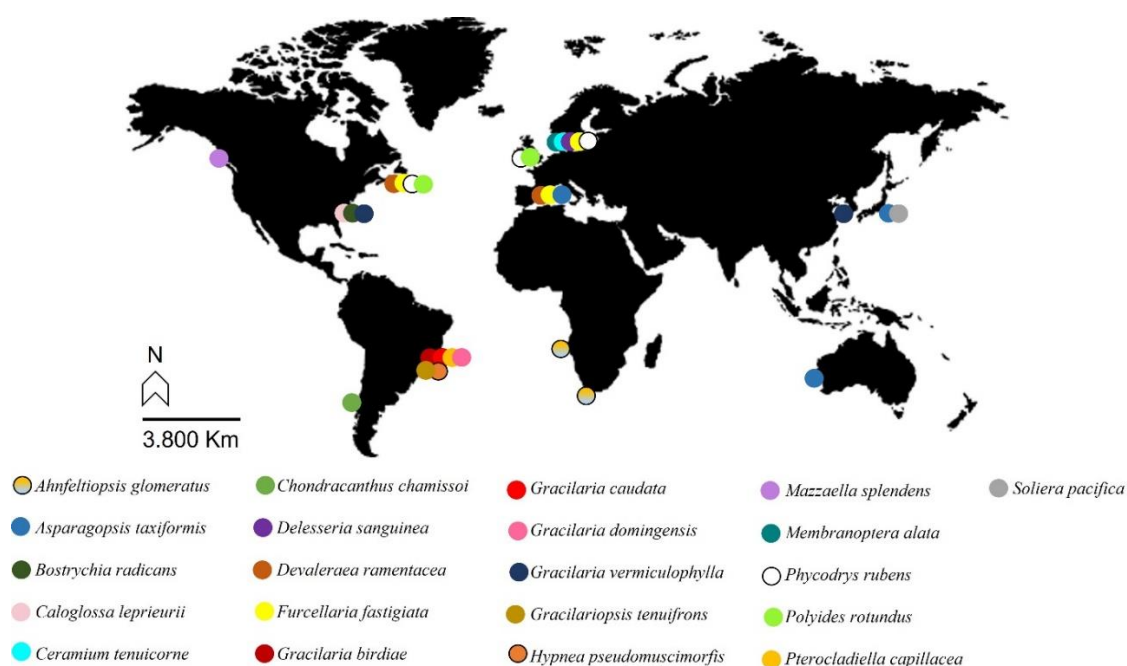


Figura 1. Representação da ocorrência de ecótipos de algas vermelhas referida na literatura. Círculos coloridos representam as espécies. O posicionamento dos círculos no mapa representa os locais onde os estudos foram realizados. Esses estudos constam da Tabela 1.

A biologia molecular tem sido empregada como uma nova ferramenta em estudos da diversidade intraespecífica. O fenótipo molecular proveniente de um gene expresso ou de um segmento específico de DNA é definido como marcador molecular (Avise 1994; Ferreira e Grattapaglia 1998). Em algas, os estudos filogenéticos têm utilizado um número limitado de marcadores moleculares, como o gene plastidial da subunidade grande da RuBisco (*rbcL*) e seu espaçador (Freshwater et al. 1994; McIvor et al. 2002; Bellorin et al. 2008), o espaçador mitocondrial *cox2-3* (Zucarello et al. 1999), o DNA nuclear ribossomal (SSU e LSU) e seus espaçadores (Harper e Saunders 2001, 2002; Bellorin et al. 2002), ou o COI-5P, conhecido como o principal marcador proposto para o “DNA barcode” (Saunders 2005), que corresponde à região 5’ do gene mitocondrial que codifica para a enzima citocromo *c* oxidase 1 (COI-5P). Para estudos com variações intraespecíficas, a carência de marcadores moleculares disponíveis tem se tornado um problema (Provan et al. 2004). O COI-5P foi primeiramente empregado na identificação de algas vermelhas por Saunders (2005), demonstrando casos de diversidade intraespecífica em valores que variaram entre 0 e 2 pares de bases. É um trecho curto de DNA (aproximadamente 650 pares de bases) que pode ser prontamente amplificado e sequenciado, tornando-o ideal para detectar espécies proximamente relacionadas e, em

alguns casos, até mesmo diversidade entre populações. O marcador *cox2-3* foi empregado para populações de *G. changii*, evidenciando diversidade populacional na costa da Malásia (Yow et al. 2013); esse mesmo marcador foi utilizado para distinguir espécies proximamente relacionadas, como *G. gracilis* e *G. dura* (Destombe et al. 2010).

É recente o uso de ferramentas moleculares em estudos sobre a diversidade intraespecífica de macroalgas da costa brasileira (Ayres-Ostrock et al. 2019; Nauer et al. 2019; Martins et al. 2022). Análises em *Gracilaria caudata*, empregando-se marcadores como COI-5P e *cox2-3* mostraram que ambos, além de microssatélites, foram eficientes em reconhecer a diversidade intraespecífica de espécies que ocorrem no litoral brasileiro. Em *G. caudata*, ficou evidente a variação de haplótipos ao longo da distribuição geográfica da espécie (Ayres-Ostrock et al. 2019). Essa mesma variação foi relatada para populações de *Hypnea pseudomusciformis* (Nauer et al. 2019), empregando-se somente marcador COI-5P, e para populações de *Colpomenia sinuosa*, empregando-se o marcador *cox3* (Martins et al. 2022). Nesse contexto, torna-se importante avaliar outras espécies de algas marinhas de ampla distribuição na costa brasileira. Esses estudos podem fornecer indícios de possíveis barreiras genéticas influenciadas por fatores ambientais.

Nas últimas décadas, as tecnologias para o sequenciamento do genoma evoluíram rapidamente (Shendure et al. 2017). Consequentemente, houve um aumento significativo de publicações devido ao rendimento, precisão e barateamento nos serviços genômicos (Lathe et al. 2008; Heather e Chain 2016; Levy e Myers 2016; Ardui et al. 2018; Karsch-Mizrachi et al. 2018). Além disso, uma abordagem interessante na biologia molecular é o reconhecimento da expressão dos genes em diferentes espécies, estádios reprodutivos e populações. Compreender a transcrição e a regulação gênica é essencial para o entendimento da manutenção destes organismos. A abordagem “transcriptômica” oferece informações importantes sobre a estrutura, expressão e regulação dos genes e vem sendo empregada em macroalgas marinhas (Fig. 2) (Patwary et al. 2021).

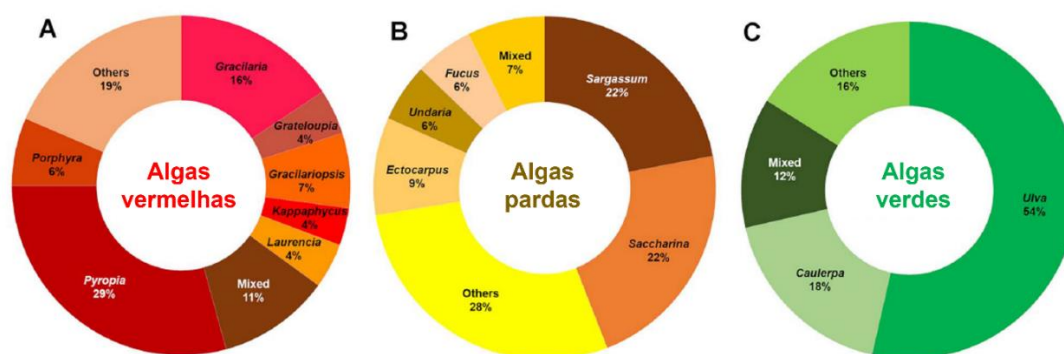


Figura 2. Porcentagem de pesquisas “ômicas” derivado de diferentes gêneros. (A) Gêneros de algas vermelhas. (B) Gêneros de algas pardas. (C) Gêneros de algas verdes. Modificado de Patwary et al. (2021).

O processo de transcrição consiste na conversão de uma sequência de nucleotídeos do DNA em uma cópia dessa sequência sintetizada agora em molécula de RNA (Menck et al. 2017). As moléculas de RNA exercem um papel fundamental como intermediárias entre a informação do genoma ao proteoma (RNA mensageiro ou mRNA) (de Carvalho et al. 2019). O transcriptoma consiste em um conjunto de moléculas de RNA expressas, que podem ser estudadas usando tecnologias de sequenciamento de alto rendimento (RNAseq), microarrays ou tags de sequência expressa (EST) (Wang et al. 2009). Os dados de RNAseq são amplamente utilizados para desvendar e classificar transcritos, anotar uma estrutura gênica e quantificar a mudança de abundância de transcritos ao comparar diferentes amostras biológicas em um momento específico ou condições-alvo (de Carvalho et al. 2019). Entretanto, estudos genômicos e transcriptômicos em algas vermelhas são poucos (Patwary et al. 2021), havendo somente sete estudos abrangendo o sequenciamento completo do genoma nuclear, i.e., em *Chondrus crispus* (Cólleen et al. 2013), *Gracilaria changii* (Ho et al. 2018), *G. domingensis* (Nakamura-Gouvea et al. 2022), *Gracilariopsis chorda* (Lee et al. 2018), *Gp. lemaneiformis* (Sun et al. 2018), *Kappaphycus alvarezii* (Jia et al. 2020), e *Porphyra umbilicalis* (Brawley et al. 2017).

Além do sequenciamento do genoma em algas vermelhas, surgiram estudos de transcriptoma para compreender o perfil dos genes expressos. Em *Gracilariopsis lemaneiformis* foram abordados mecanismos moleculares que estão envolvidos na síntese de ficoeritrina (Huang et al. 2017). Em *Gracilaria chilensis* foram identificadas proteínas de ligação que fazem parte da construção do ficobilissomo (Vorphal et al. 2017). Em *G. changii* e *G. salicornia* foi avaliado o efeito de um ambiente privado de sulfato em

resposta à qualidade do ágar (Wang et al. 2015). Em *G. vermiculophylla* avaliou-se mecanismos moleculares à tolerância de calor (Fu et al. 2020). Por fim, em *G. dura* foi elucidada a expressão de diferentes genes, quando comparados gametófitos femininos e tetrasporófitos (Sambhwani et al. 2022).

As pesquisas ômicas direcionadas para algas marinhas, utilizando várias estruturas computacionais, têm o potencial de desenvolver rapidamente nossa compreensão dos processos biológicos em nível molecular e contribuir nas soluções dos problemas ambientais e sociais mais urgentes do nosso tempo (Patwary et al. 2021).

Temperatura

O principal fator abiótico que atua na distribuição das algas marinhas é a temperatura (McLachlan & Bird 1984). Segundo o relatório do Painel Intergovernamental sobre Mudanças Climáticas (IPCC 2021), há evidências do aumento mais rápido em mudanças ambientais nas últimas décadas do que em qualquer outro momento nos últimos 25 milhões de anos. Dentre estas, destaca-se o aquecimento global, que vem aumentando devido ao desmatamento, aumento das emissões de gases do efeito estufa, industrialização global e queima de combustíveis fósseis (IPCC 2021). Além disso, dados de temperaturas do oceano, registrados nos últimos anos, evidenciam também esse aquecimento. Especula-se para os próximos 60 anos, um aumento de 1,5-2°C num cenário otimista, um aumento entre 2,7-3,6°C num cenário intermediário, ou um aumento de 4°C num cenário drástico (Fig. 3).

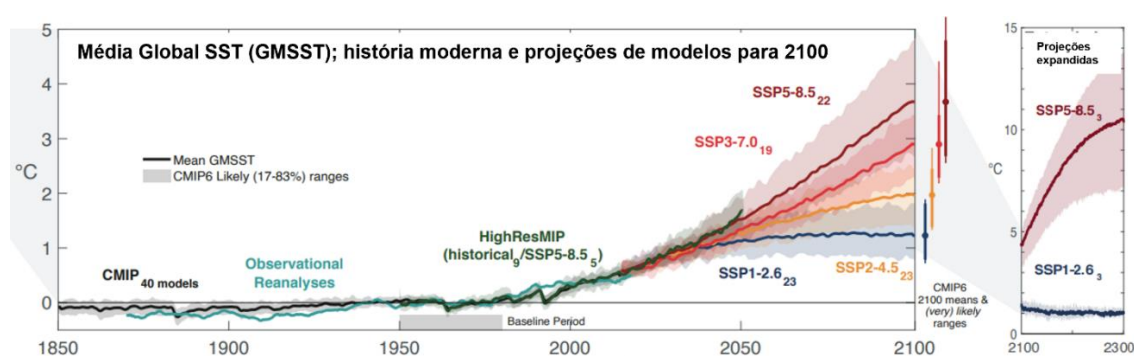


Figura 3. Temperatura superficial da água do mar (SST) e suas mudanças com o tempo. Série temporal da anomalia média global da SST em relação à climatologia entre 1850-2100 e projeções expandidas entre 2100-2300, segundo o relatório do IPCC (2022). SSP, Trajetória Socioeconômica Compartilhada ou “Shared Socioeconomic Pathways (SSP)” são cenários de mudanças socioeconômicas globais projetadas até 2100. Eles são usados

para prever cenários de emissões de gases de efeito estufa com diferentes políticas climáticas. Modificado de IPCC (2022).

Considerando os diferentes ambientes de “hotspots” para a biodiversidade, há evidências de que a velocidade das mudanças climáticas nos ambientes marinhos está sendo maior do que nos ambientes terrestre e de água doce (IPCC 2022). Essas mudanças climáticas, além de influenciarem locais específicos de biodiversidade, estão afetando 54% de espécies endêmicas marinhas com ameaça de extinção. As espécies endêmicas sofrem até 10 vezes mais com as mudanças climáticas, quando comparadas com espécies introduzidas ou invasoras, estas que podem se beneficiar com o ambiente (IPCC 2022). Para reduzirmos o risco de extinção de diversas espécies, precisaríamos adotar o acordo de Paris 2015, reduzindo o impacto antropogênico, e assim mesmo, haveria um aumento de temperatura de 1,5°C e redução de 2% de espécies marinhas endêmicas. Caso isso não seja possível, há previsão de um aumento de temperatura de 3°C, que poderá causar uma redução de 32% de espécies marinhas (Fig. 4) (IPCC 2022).

Devido às mudanças rápidas no clima, as espécies podem não estar mais adaptadas às condições ambientais de uma determinada região e, portanto, poderiam estar fora do seu nicho climático (Bellard et al. 2012). Vários estudos indagam sobre a capacidade das espécies em se adaptar com rapidez para acompanhar o ritmo acelerado das mudanças climáticas (Lavergne et al. 2010; Salamin et al. 2010). Independentemente do tipo de resposta adaptativa, os mecanismos fundamentais são devido à microevolução (ou seja, as espécies podem se adaptar a novas condições por meio de mutações ou seleção de genótipos existentes) ou plasticidade, que fornece um meio de resposta de curto prazo (Salamin et al. 2010; Charmantier et al. 2008).

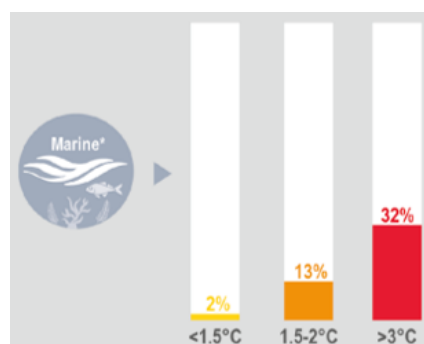


Figura 4 Risco projetado de extinção de espécies em níveis de aquecimento global de <1,5°C, 1,5-2,0°C e >3°C em “hotspots” de biodiversidade marinha. Modificado de IPCC (2022).

A temperatura pode determinar o desempenho de algas marinhas, desde os níveis fundamentais de processos enzimáticos até funções metabólicas (Raven e Geider 1988; Lobban e Harrison 1997). As algas desenvolveram processos bioquímicos e fisiológicos, variações e concentrações de proteínas e propriedades das membranas celulares, permitindo otimizar o desempenho a variações de temperaturas (Eggert 2012). Embora algas marinhas sejam geralmente bem adaptadas ao seu ambiente de temperatura, elas podem ficar suscetíveis a mudanças ambientais (baixa ou alta temperatura), resultando em estresse e causando danos celulares e subcelulares (Davison e Pearson 1996; Eggert et al. 2012). Danos celulares podem retardar o crescimento, atrasar o desenvolvimento e levar à morte (Davison e Pearson 1996).

Ainda há muito a ser conhecido com relação à dependência térmica dos principais processos fisiológicos que controlam o crescimento, a reprodução e a sobrevivência em toda a faixa de temperaturas experimentada por um indivíduo durante seu período de vida (Harley et al. 2012). Em algas vermelhas, sendo mais específico, para o gênero *Gracilaria*, existem algumas espécies para as quais foram descritos o ótimo de temperatura e a faixa de tolerância térmica (em geral, entre 18-30°C) (Castro e Yokoya 2018).

Importância econômica

As macroalgas vêm sendo cultivadas há séculos, com registros dessas atividades por volta de 1650 (Ohno e Largo 1998). Inicialmente, os cultivos foram desenvolvidos empiricamente e utilizados para a produção de alimento humano em países com histórico no consumo de macroalgas marinhas, como Japão, Coreia e China (Oohusa 1993).

Algumas macroalgas são importantes produtoras de hidrocolóides, e o mercado de algas para essa produção continua a crescer, impulsionado pelos mercados emergentes da China, Leste Europeu, Brasil, entre outros (Bixler e Porse 2011).

Várias espécies da família Gracilariaceae apresentam potencial para maricultura (Dawes et al. 1999). São consideradas fontes de ágar, sendo este ficocolóide também utilizado em indústrias alimentícias (Bixler e Porse 2011). Além disso, suas espécies vêm sendo testadas e empregadas na biorremediação de efluentes provenientes da maricultura (Jones et al. 2001; Msuya e Neori 2002; Yang et al. 2006; Marinho-Soriano et al. 2002, 2006; Carneiro et al. 2021).

O gênero *Gracilariopsis* E.Y. Dawson, juntamente com *Gracilaria*, produz 91% do valor total do ágar, e outros 9% são produzidos por outras algas vermelhas (Lim et al. 2017; Porse e Rudolph 2017). Indústrias de ficocolóides utilizam o ágar como principal recurso para a produção de ração animal (Qi et al. 2010; Johnson et al. 2014), biomaterial para embalagem, espessante para alimentos ou produtos para a pele (Leandro et al. 2020). Segundo FAO (2021), *Gracilariopsis* e *Gracilaria* tornaram-se as principais algas vermelhas cultivadas no mundo com 3,6 milhões de toneladas de produção anual. A China (cerca de 95%) seguida pela Indonésia (cerca de 3%) são os principais cultivadores de ambos os gêneros no mundo (FAO 2021).

No Brasil, o cultivo das algas no ambiente marinho foi proposto como uma alternativa ao extrativismo para a obtenção de matéria-prima e produção de ficocolóides (Oliveira 1998). A costa brasileira, banhada por águas tropicais e subtropicais, propicia locais adequados ao crescimento de algas e à riqueza de espécies, principalmente espécies com alto valor econômico (Oliveira e Miranda 1998). Tentativas de cultivos de algas no Brasil são escassas. Alguns dos motivos pelo insucesso desses cultivos são: i) a falta de tradição dessa atividade econômica no país; ii) a falta de tradição no consumo dessas algas na alimentação da população brasileira; e iii) as escassas informações sobre a fisiologia das espécies com potencial para cultivo (Oliveira 1981, 1998; Reis et al. 2017).

Mesmo apresentando um histórico comercial sem grandes sucessos, o extrativismo de algas gracilarióides para a obtenção de ágar no Brasil vem crescendo desde a década de 60 (Câmara-Neto 1987; Simioni et al. 2019), focado especialmente em *Gracilaria birdiae*. Outro gênero, produtor de ágar, ocorre na costa brasileira, *Gracilariopsis*, porém, não existem tentativas de seu cultivo no mar no país. Entretanto, relatos com base em estudos publicados em periódicos indicaram que existiu cultivo comercial de *Gp. tenuifrons* (C. J. Bird & E. C. Oliveira) Fredericq e Hommersand na Venezuela até 2000, e esses estudos comprovaram altos valores de rendimento e qualidade do ágar para a espécie (Zecchin et al. 2000).

Gracilariopsis tenuifrons

O gênero *Gracilariopsis* (Rhodophyta, Gracilariales) inclui cerca de 24 espécies (Guiry & Guiry 2022). O estabelecimento desse gênero ocorreu com base em três características diferenciais em comparação aos demais gêneros da família Gracilariaceae: i) cavidade cistocárpica não completamente preenchida por gonimoblastos; ii) ausência

de células tubulares nutritivas no cistocarpo; e iii) presença de espermatângios superficiais (Gurgel et al. 2003).

Gracilariopsis tenuifrons (C.J. Bird & E.C. Oliveira) Fredericq & Hommersand possui uma ampla distribuição geográfica, ocorrendo no México, Cuba, Venezuela (local onde a espécie foi explorada como uma importante agarófita, Zecchin et al. 2000) e na costa brasileira (Lyra et al. 2021). No Brasil, a espécie é referida desde o Estado do Maranhão (2.4°S) até o Estado de São Paulo (24°S, limite sudeste) (Lyra et al. 2021). Ocorre em região costeira marinha, em ambientes estuarinos, bem como em lagos salinos, o que indica grande tolerância a variações de salinidade (Plastino et al. 1998). A espécie apresenta um talo cilíndrico, de coloração marrom avermelhada escura ou marrom clara, dependendo da exposição luminosa, e pode atingir até 40 cm de comprimento e 1 mm de diâmetro. O talo é pouco ramificado, porém, podem ocorrer ramos de até quarta ordem (Bird e Oliveira 1986) (Fig. 5). O histórico de vida trifásico, do tipo “*Polysiphonia*”, compreende a fase carposporofítica (diplóide), tetrasporofítica (diplóide) e gametofítica (haplóide), sendo as duas últimas isomórficas e independentes, enquanto o carposporófito é dependente do gametófito feminino (Brito e Silva 2005).

Gracilariopsis tenuifrons apresenta potencial para exploração no Brasil, já que possui ótimos de crescimento entre 24-32°C e 15-40µps (Plastino et al. 1998). Outros experimentos de curta duração em laboratório mostraram que a espécie é tolerante a irradiâncias de 60 e 1000 µmol de fótons.m⁻².s⁻¹ (Torres et al. 2015). Estudos populacionais realizados na costa brasileira (Plastino 1985) e na Venezuela (Brito e Silva 2005) mostraram que tetrasporófitos de *Gp. tenuifrons* foram encontrados em maior porcentagem quando comparados a plantas cistocárpicas. O mesmo é descrito para algumas espécies de *Gracilaria* que ocorrem na costa brasileira (Plastino 1985; Carneiro et al. 2011; Ayres-Ostrock 2014). Além disso, *Gp. tenuifrons* tem sido considerada economicamente importante como um biofiltro eficiente para absorção de nutrientes na aquicultura multitrófica integrada, auxiliando na redução do uso de água e na remoção de metabólitos produzidos por peixes marinhos (Hernández et al. 2006) e camarões (Carneiro et al. 2021).

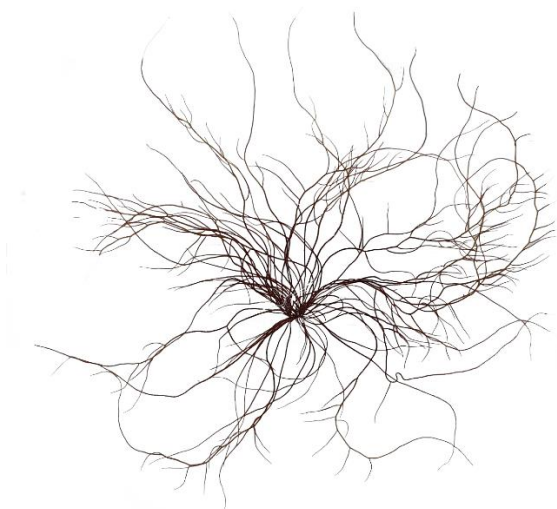


Figura 5. Aspecto geral de *Gracilariopsis tenuifrons* cultivado em laboratório (procedência: Praia Dura, Ubatuba, estado de São Paulo). Gametófito feminino.

Devido à ampla distribuição de *Gracilariopsis tenuifrons*, há interesse em melhor conhecer aspectos fisiológicos e também se suas populações são geneticamente diversas ao longo da costa. Esses estudos podem contribuir para uma melhor compreensão sobre a distribuição de outras espécies que também apresentem ampla distribuição na costa brasileira. Além de possibilitar um melhor conhecimento sobre o perfil fisiológico de populações de *Gp. tenuifrons*, contribuindo para seleção de linhagens para maricultura, estudos com essa abordagem são de grande relevância para futuros projetos de conservação que poderão estar associados com atividades educacionais, de recreação e ecoturismo nas comunidades marinhas costeiras (Vásquez et al. 2014).

Objetivo e hipóteses

O objetivo deste trabalho foi verificar e compreender os aspectos fisiológicos e moleculares da diversidade intraespecífica de *Gracilariopsis tenuifrons*. Esses estudos incluíram abordagens sobre os eventuais efeitos da temperatura (18, 20, 23, 25, 28, 30, 33 e 35°C) em gametófitos femininos não fertilizados procedentes de cinco populações distintas, três delas do Nordeste (Estado do Maranhão (MA), Rio Grande do Norte (RN) e Bahia (BA)) e outras duas do Sudeste brasileiro (Estado de São Paulo, região de Ubatuba, SP1, e Itanhém, SP2). Foi também avaliada a diversidade genética de seis populações de *Gp. tenuifrons* (as mesmas citadas anteriormente e mais uma população do Estado de Alagoas, AL), acessando os marcadores genéticos como *rbcL*, *COI-5P* e *cox2-3*. Além disso, foi avaliado o efeito da temperatura (20, 25 e 30°C) em diferentes estádios

reprodutivos (gametófitos femininos não fertilizados, tetrasporófitos férteis, gametófitos masculinos férteis e plantas cistocárpicas) de indivíduos de uma mesma população. Ao final, utilizando-se ferramentas de transcriptoma, foi montada a ontologia biológica dos genes expressos em temperatura ótima (25°C) e de estresse (33°C) para a espécie.

Essas abordagens permitiram testar as seguintes hipóteses:

- i, Todas as cinco populações (MA, RN, BA, SP1 e SP2) de *Gracilariopsis tenuifrons* selecionadas para este trabalho são ecotípicas à temperatura (Cap.2);
- ii, Temperaturas de 20, 25 e 30°C influenciam diferentemente os estádios reprodutivos (gametófitos femininos não fertilizados, tetrasporófitos férteis, gametófitos masculinos férteis e plantas cistocárpicas) (Cap.3);
- iii, indivíduos das populações da região nordeste (MA, RN, AL e BA) são distintos geneticamente dos indivíduos da região sudeste (SP1 e SP2) (Cap.4); e
- iv, temperatura de estresse (33°C) induz expressão diferencial dos genes referentes à manutenção biológica (Cap.5);

Material e métodos

Material biológico

Ápices de um total de 25 indivíduos de *Gracilariopsis tenuifrons* (plantas cistocárpicas, gametófitos masculinos ou tetrasporófitos) foram coletados em cada uma das seis populações ao longo da costa brasileira (n = 150). Ápices de cinco indivíduos (tetrasporófitos) de cada uma das populações foram destinados para experimento de fisiologia (Capítulo 2, 3 e 5). Ápices de outros 20 indivíduos de cada uma das populações foram limpos com papel absorvente e preservados com dessecante de sílica gel até a extração do DNA (Capítulo 4).

A amostragem nas seis populações foram realizadas em (i) outubro de 2018 na Praia de São Marcos (2,4°S 44,28°W), São Luís, Estado do Maranhão (MA), uma população marginal do litoral nordeste brasileiro; (ii) agosto de 2018 no manguezal em Diogo Lopes (5,4°S 36,26°W), Macau, Rio Grande do Norte (RN); (iii) maio de 2016 na Praia do Sonho Verde (9°S 35°W), Maceió, Alagoas (AL); (iv) agosto de 2016 na Praia Me Ache (14,48°S 39,01°W), Ilhéus, Estado da Bahia (BA); (v) agosto de 2017 na Praia Dura (23,5°S 45,1°W), Ubatuba, Estado de São Paulo (SP1); e (vi) junho de 2018 na Praia

Cibratel (24,2°S 46,8°W), Itanhaém, Estado de São Paulo (SP2), limite e distribuição marginal sudeste da espécie. Os espécimes voucher foram depositados no herbário do Instituto de Biociências da Universidade de São Paulo (MA, SPF-58489; RN, SPF-58286; AL, SPF-24001; BA, SPF-58287; SP1, SPF-58491; SP2, SPF-58940).

Análises fisiológicas

Os métodos empregados para as análises fisiológicas, como (i) condições gerais de cultivo, (ii) desenho experimental, (iii) avaliação da taxa de crescimento, fotossíntese e conteúdo pigmentar, (iv) comprimento do talo e diferenciação dos ramos, (v) curva de performance térmica e (vi) estatística estão descritos detalhadamente no capítulo 2 e 3, na seção “Material and methods”.

Análises para filogeografia

Os métodos empregados para as análises moleculares como (i) extração de DNA, amplificação por PCR, sequenciamento e alinhamento de DNA, (ii) análises filogenéticas, (iii) filogeografia e (iv) inferências coalescentes da história demográfica estão descritos detalhadamente no capítulo 4, na seção “Material and methods”.

Análise para transcriptoma

Os métodos empregados para as análises de expressão dos genes como (i) extração de RNA, construção de biblioteca cDNA e sequenciamento de RNA, e (ii) processamento e análise dos dados de transcriptoma estão descritos detalhadamente no capítulo 5, na seção de “Material and methods”.

CAPÍTULO 2

Ecotypic differentiation in populations of Brazilian coast: recognizing adaptation to temperature in *Gracilariopsis tenuifrons* (Gracilariales, Rhodophyta)

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Keywords: Marginal populations, ecotypes, Brazilian coast, climate change, sea surface temperature, thermal adaptation.

Abstract

The Brazilian coast presents diverse abiotic characteristics, such as temperature, and we know that surface seawater temperatures are rising as a result of climate change. Therefore, to better interpret the effects of such changes, we chose to investigate *Gracilariopsis tenuifrons*, a species of red algae widely occurring on the Brazilian coast. We specifically evaluated the effect of different temperatures (18-35°C) on growth rates (GRs), photosynthetic parameters, and pigment contents of female gametophytes from five different populations distributed along 7,450km of coastline, including Maranhão State (MA, 2.4°S), Rio Grande do Norte State (RN, 5.4°S), Bahia State (BA, 14.48°S), and São Paulo State (SP1, 23.5°S and SP2, 24.2°S). All sample tolerated temperatures between 18-33°C, but none of them could tolerate temperatures of 35°C, irrespective of the populations. However, the highest GRs varied among populations (MA, 23-30°C; RN, 28-33°C; BA, 23-28°C; SP1, 25-28°C; SP2, 28°C). MA and BA populations showed the highest maximal measurement (μ_{max}) of thermal performance curve when compared to SP1 and SP2 populations. The RN population showed the worst μ_{max} values, 5.6% day⁻¹; however, it was more resilient to stressful temperatures (33°C) than the others. The BA population presented some physiological characteristics similar to populations from the northeastern and southeastern regions. Our data showed that *Gp. tenuifrons* presents

latitudinal ecotypes associated with temperature. Northeastern populations could be more vulnerable in a drastic scenario of climate change (up to 4°C); otherwise, this species could occupy regions further to the south of its current distribution.

Introduction

The concept of ecotype is a precursor to speciation, and it is related to populations of species that have different physiological adaptations owing to environmental conditions or geographic barriers (Lowry 2012). The occurrence of ecotypes along a latitudinal distribution can bring advantages for the permanence of a species, even in the face of climate change, since some of them may present characteristics that are more adaptable to the changes imposed. Among the red algae, ecotypes of some species have already been characterized relative to different abiotic factors, including temperature (Zanolla et al. 2014; Borlongan et al. 2017).

Marine heatwaves and increased sea surface temperatures (SST) have occurred with more frequency in the last 30 years compared to the early 20th century (Turkington et al. 2019; Smale et al. 2019). This climatic change is related to anthropogenic activities, and it causes the redistribution and reorganization of species in natural habitats (Chen et al. 2011; Burrows et al. 2011). Therefore, range-edge populations are likely to be most vulnerable and may be affected by increase of SST (Smale et al. 2019). The extinction of a species can be avoided if (i) individuals are able to physiologically acclimate, (ii) populations undergo evolutionary adaptations, or (iii) populations move to more favorable habitats (Hoffmam and Sgro 2011; Jueterbock et al. 2013). Thus, if we know how marine species populations respond to changes in temperature, we can better predict changes in coastal ecosystems, according to IPCC forecast scenarios (optimistic 1.5-2°C, intermediate 2.7-3.6°C, and drastic 4°C), related to gas emissions, such as aerosol, methane, nitrous oxide, carbon dioxide, and others (IPCC 2021).

Along the Brazilian coast, an extensive shore with wide latitudinal variation of irradiance, temperature, and nutrients, ecotypic populations have been recognized in four species of algae, including *Gracilaria birdiae* Plastino & E.C. Oliveira (Ursi et al. 2013), *G. caudata* J. Agardh (Araujo et al. 2014; Faria et al. 2017), *G. domingensis* (Kützinger) Sonder ex Dickie (Castro and Yokoya 2018), and *Hypnea pseudomusciformis* (Nauer et al. 2020). These ecotypic populations could allow us to gain a better understanding of adaptation induced by different environmental conditions, as well as a perspective of how

individual populations respond to environmental variations. Moreover, knowing the thermal tolerance of species can contribute to a better interpretation of their geographical distribution, as shown by McLachlan and Bird (1984) for different species of *Gracilaria*.

The genus *Gracilariopsis*, together with *Gracilaria*, produces 91% of the total agar value, and another 9% is produced by other red algae (Lim et al. 2017; Porse and Rudolph 2017). Phycocolloid industries use agar as a main resource for producing animal feed (Qi et al. 2010, Johnson et al. 2014), biomaterial for packing, thickener for foods, or skin care products (Leandro et al. 2019). According to FAO (2021), *Gracilariopsis* and *Gracilaria* have become the principal red algae cultivated in the world with 3.6 million tons of annual production. China (around 95%) followed by Indonesia (around 3%) are the main cultivators for both genera in the world (FAO 2021).

Gracilariopsis tenuifrons (Fig.1C) occurs from Mexico, Cuba, and Venezuela (Hernandez et al. 2020) to the Brazilian coast (Lyra et al. 2021) where it is found between Maranhão (2.4°S) and São Paulo States (24°S, southern limit). In Brazil, the distance between limit populations is around 7,450km. This species has a “*Polysiphonia*”-type life history (Brito and Silva 2005), and individuals of a single studied population from Alagoas State (9°S) tolerates a wide range of salinity (15-40) and temperature (17-32°C) under laboratory conditions (Plastino et al. 1998). Furthermore, *Gp. tenuifrons* has been considered economically important as an efficient biofilter for absorbing nutrients in an integrated multi-trophic aquaculture, helping in the reduction of water usage and removal of metabolites produced by marine fishes (Hernandez et al. 2006) and shrimps (Carneiro et al. 2021).

As noted, *Gp. tenuifrons* is economically important and has a wide geographical distribution along the Brazilian coast with a wide range of sea surface temperature (27-29°C, northeastern Brazil; and 20-26°C, southeastern Brazil) (Fig.1B). Nonetheless, few studies have reported on the putative thermal adaptations, considering different populations. Therefore, we aimed to elucidate and characterize putative ecotypes by evaluating the effects of temperatures (18, 20, 23, 25, 28, 30, 33, and 35°C) on five populations, including those that occur at the extremes of their distribution (2.4°S and 24°S). At the higher range of SST, these results are expected to help in the interpretation of the effects of higher temperatures owing to climate change on these algal populations. This study should also advance a preliminary analysis of the performance of these populations for future mariculture in Brazil and for the development of effective

conservation strategies in an environment subject to abiotic and anthropogenic factors, such as habitat fragmentation, degradation, and overharvesting.

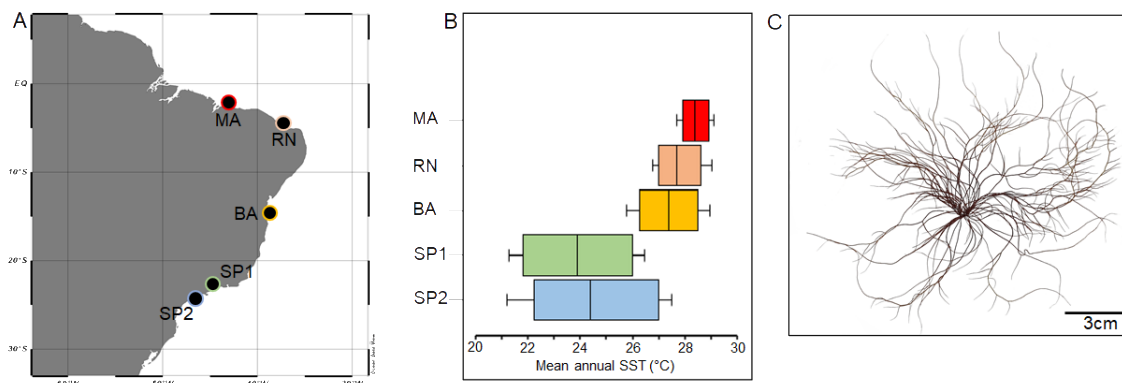


Fig.1 (A) Map of the Brazilian coast showing five site places where *Gp. tenuifrons* was collected: São Luis-Maranhão State (MA), Macau-Rio Grande do Norte State (RN), Ilhéus-Bahia State (BA), Ubatuba-São Paulo State (SP1), and Itanhaém-São Paulo State (SP2); (B) Mean annual (2015-2019) of sea surface temperature at each sampling location (MA, RN, BA, SP1, and SP2) based on satellite-obtained mean monthly SST datasets (<http://podaac-tools.jpl.nasa.gov/soto>); and (C) Habit of *Gp. tenuifrons*, scale bar = 3 cm.

Material and methods

Biological material

Five tetrasporophytes of *Gracilariopsis tenuifrons* were collected from each of five localities along the Brazilian coastline (Fig.1A). Collections were made (i) in October, 2018 at São Marcos Beach (2.4°S 44.28°W), São Luis, Maranhão State (MA), a marginal population of the northeastern Brazilian coast; (ii) in August, 2018 at Diogo Lopes Mangrove (5.4°S 36.26°W), Macau, Rio Grande do Norte State (RN); (iii) in August, 2016 at Me Ache Beach (14.48°S 39.01°W), Ilhéus, Bahia State (BA) (hereinafter considered an intermediate population between MA/RN and the following sites); (iv) in August, 2017 at Dura Beach (23.5°S 45.1°W), Ubatuba, São Paulo State (SP1); and (v) in June, 2018 at Cibratel Beach (24.2°S 46.8°W), Itanhaém, São Paulo State (SP2), the limit and marginal southeastern distribution of the species. Samples were collected in two different environments, i.e., rocky shores (i, iii, iv, and v) and mangrove (ii). Unialgal non-axenic cultures of unfertilized female gametophytes were established from tetraspores released and cultivated in control conditions (Plastino and Oliveira 1990). Tetraspores produced male and cystocarpic plants. Small apical branches of the latter

were cultivated at the same culture conditions for at least 2 months, assuring absence of fertilized carpogonia prior to the start of experimentation. Experimental plants are hereinafter designated as female gametophytes from Maranhão State (MA); from Rio Grande do Norte State (RN); from Bahia State (BA); from Ubatuba, São Paulo State (SP1); and from Itanhaém, São Paulo State (SP2). Plants derived from spores were kept in the Gracilariaceae Germplasm Bank of the University of São Paulo (Costa et al. 2012). Voucher specimens were deposited in the herbarium of the Bioscience Institute at the University of São Paulo (MA, SPF-58489; RN, SPF-58286; BA, SPF-58287; SP1, SPF-58491; SP2, SPF-58940).

General culture conditions

Apical segments of strains were maintained in von Stosch-enriched seawater with modifications (Ursi and Plastino 2001) and diluted to 50% with sterile seawater (salinity at 32). The algae were kept in a temperature-controlled room at $25\pm 1^\circ\text{C}$ with a photoperiod of 14 (14:10, light:dark). Photosynthetically active radiation (PAR) was $70\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$ provided by Osram 40 W Daylight fluorescent tubes and was measured by a quantameter (Li-COR model L1-185). Cultures were aerated for $30\ \text{min h}^{-1}$, and the medium was renewed weekly.

Experimental design

One week before the start of experimentation (pretreatment in each temperature), 32 apical segments of *Gp. tenuifrons* 20 mm in length were obtained from one individual (total: five individuals and 160 tips) of unfertile female gametophytes derived from each of the five populations (MA, RN, BA, SP1, and SP2; total 800 apical segments). These tips of each individual (total of 32) were grouped into clusters of four and cultivated in eight Erlenmeyer flasks (total: 40 flasks per population), containing 400 mL of enriched seawater (four apical segments, and about 12 mg of algae per flask), under general culture conditions. According to our experimental design, plants received eight treatments: 18 and 20°C (low temperatures); 23, 25, and 28°C (intermediate temperatures); and 30, 33, and 35°C (high temperatures) provided by BOD Incubator (Bio-Oxygen Demand) with accurate temperature control (EL141/3, Eletrolab). Five replicates per treatment were made for each population with cultivation over a period of 28 days. Each replicate was represented by one distinct individual. Growth was assessed weekly; *in vivo* chlorophyll fluorescence and pigment content were assessed at the end of the experiment.

Growth rates

Growth was assessed by measuring fresh mass. Growth rates were estimated as $GR = [(Fm_t/Fm_0)^{1/t} - 1] \times 100\%$, where Fm_t is final fresh mass, Fm_0 is initial fresh mass, and t is time (Yong et al. 2013).

In vivo chlorophyll fluorescence

Fluorescence measurements were performed after 28 days for all individuals (n=5). The measurements were made from 11:00 to 14:00 after 4h of exposure to light in the culture chamber. *In vivo* chlorophyll fluorescence was measured using an underwater Diving-PAM fluorometer (Walz, Effeltrich, Germany). Apical segments were arranged on a magnetic sample holder to avoid overlap and acclimated in the dark for 10 minutes for PSII exposure. We used 8 levels of irradiance to construct electron transport rate (ETR) \times irradiance (PAR) curves: 8, 25, 50, 90, 132, 184, 276, and 378 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The exposure time of the apical segments in each irradiance was 20s, interspersed with saturation pulse of 0.8s, approximately 6,100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The maximum quantum yield (F_v/F_m) was provided by the Diving-PAM after an initial saturation pulse when the samples were at a low intensity pulse of blue light (approximately 1.5 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). ETR was calculated as $ETR = \Delta F/F_m' \times EPAR \times A \times 0.15$, where $\Delta F/F_m'$ is the effective quantum yield measured; EPAR is the incident irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$); A, absorptance, is the fraction of incident light absorbed by algae; and 0.15 is the fraction of incident light absorbed by PSII, equivalent to red algae (Figueroa et al. 2003). Absorptance was calculated as $A = 1 - (E\lambda_0/E\lambda)$, where $E\lambda_0/E\lambda$ is the transmittance (Suggett et al. 2011). ETR \times PAR curves were fitted using the Kaleida Graph software, according to the models of Webb et al. (1974), respectively: $ETR = ETR_{\max} \times [1 - \exp(-\alpha ETR \times EPAR / ETR_{\max})]$, where ETR is the electron transport rate, ETR_{\max} is the maximum electron transport rate, αETR is the photosynthetic efficiency based on ETR data, and EPAR is the irradiance. The following parameters were calculated and analyzed (Schreiber 2004): αETR , ETR_{\max} , and saturating irradiance from ETR data ($E_k - ETR = ETR_{\max} / \alpha ETR$).

Pigment analyses

Phycobiliprotein extractions were carried out at 4°C, according to Kursar et al. (1983), with modifications (Plastino and Guimarães 2001). Briefly, 100 mg FW of apical segments from each replicate (n=5) were ground in liquid nitrogen, followed by addition

of 1 mL of phosphate buffer 50 mM, pH 5.5. Crude extracts were centrifuged at $44,000 \times g$ for 20 min. The supernatant containing phycobiliproteins, including phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC), was removed, separated and kept in sealed vials at 4°C until read by spectrophotometry (HP 8452A). Chlorophyll *a* (Chlo-*a*) was extracted after dissolving the pellet from the previous procedure in methanol (addition of 1 mL) and then centrifuging at $12,000 \times g$ for 15 min. PE, PC, and APC concentration was calculated according to Kursar et al. (1983), and Chlo-*a* concentration was calculated according to Torres et al. (2014).

Sea surface temperature dataset

Sea surface temperature (SST) data were obtained from the PODAAC GHRSSST database (O'Carroll et al. 2019), which incorporates corrected estimates of temperature obtained from a high-resolution radiometer (AVHRR) at a temporal and spatial resolution of one day. Local SST estimates were taken at the same coordinates as the collection sites, namely MA, BA, SP1, and SP2. For the RN population, the SST estimate was performed closer to the mangrove's mouth owing to a lack of data at the coordinates of collection sites. SST datasets were plotted from January 2015 to December 2019. The highest SST values were observed from January to April, and the lowest SST values were observed from May to October. During summer, January to March, temperature ranges around $28 \pm 0.3^{\circ}\text{C}$ in the northeastern region and around $26 \pm 0.6^{\circ}\text{C}$ in the southeastern region. In the middle of autumn to the end of winter, May to September, temperature ranges around $27 \pm 1^{\circ}\text{C}$ in the northeastern region and around $22 \pm 1^{\circ}\text{C}$ in the southeastern region (Fig. 1B).

Quantifying thermal performance curve

The non-linear relationship between each of five populations (MA, RN, BA, SP1, and SP2) and the range of temperatures noted above was analyzed according to Fernández et al. (2020). Briefly, a growth rate performance curve was constructed using a model-fitting approach (Gaitán-Espitia et al. 2014). The data were fitted for several mathematical models, e.g. Gaussian, modified Gaussian, Flinn, LRF, and Quadratic, using the *rTPC* and *nls.multstart* R package (Padfield et al. 2021). Thermal performance curve (TPC) parameters, such as maximal measurement (μ_{max}), thermal optimum (T_{opt}), thermal tolerance ($T_{\text{tolerance}}$), and critical thermal minimum and maximum at which population growth decreases (CT_{min} and CT_{max}) were derived from the best-fitted models. Overall, the Quadratic_2008 model (Montagnes et al. 2008) was best-fitting according to the

Akaike Information Criterion (AIC) (Angilletta 2006). Parameters of the curves were compared through confidence intervals (CI) computed from the likelihood profile and using AIC and the extra sum-of-square F test.

Statistics

The assumptions of normality and homogeneity of variances were tested using the Kolmogorov-Smirnov and Cochran test, respectively. When necessary, logarithmic transformation was employed [$x=\log(x+1)$] and retested (Zar 1999). Growth rates, *in vivo* chlorophyll fluorescence (F_v/F_m , ETR_{max} , E_k , and α), and pigment concentrations (PE, PC, APC, and Chlo-a) were analyzed by two-way factorial ANOVA (independent variables: temperature and population). Thermal performance curve (TPC) parameters related to GRs were analyzed by one-way ANOVA (μ_{max} , T_{opt} , $T_{tolerance}$, CT_{min} , and CT_{max}) (Gaitán-Espitia et al. 2013). In all cases, the *a posteriori* Newman-Keuls test was used to establish statistical differences. Statistical analyses were done using the Statistica 12 program, considering $p<0.05$. Raw data of GRs, photosynthesis, and pigment concentration are available as supplementary information (Tab.S2)

Results

Gracilariopsis tenuifrons cultivated at 35°C did not survive after one week of treatment, regardless of population. In addition, all populations, excepted by the RN, showed the worst physiological performance at 33°C. The populations showed different thermal adaptation and the best GRs for each population varied according to the temperature (MA, 23-30°C; RN, 28-33°C; BA, 23-28°C; SP1, 25-28°C; SP2, 28°C) (Fig.S1, Fig.S2).

Thermal performance curves (TPCs)

Gracilariopsis tenuifrons from distinct populations exhibited the typical non-linear relationship between temperatures and growth rates (Fig.2, Table 1), accordingly to the typical bell shape curve observed for ectothermic organisms (Angilletta Jr et al. 2002). However, photosynthetic and pigment parameters exhibited negative and linear relationship, respectively, and it is making impossible to evaluate the main traits of the TPCs.

MA and BA populations showed the highest μ_{max} , around 12.2-12.7% day⁻¹, when compared to SP1 and SP2 populations that showed μ_{max} around 9.6-10.6% day⁻¹.

Furthermore, the RN population showed the worst μ_{\max} values, 5.6% day⁻¹ (Tab.1). However, mangrove population (RN), showed the highest T_{opt} (27.4°C), CT_{max} (37.4°C), and $T_{\text{tolerance}}$ (20.0°C), when compared to rocky shore populations (MA, BA, SP1, and SP2) (Tab.1). CT_{max} values were about the same for rocky shore populations (34.7-35.1°C). However, MA and SP2, as the species distribution limit, showed higher CT_{min} (17.2-17.4°C), when compared to BA and SP1 (15.9-16.3°C) (Tab.1). However, BA and SP1 showed higher $T_{\text{tolerance}}$ (18.6-18.8°C), when compared to MA and SP2 (17.5-17.7°C). For T_{opt} , MA showed higher optimum temperature (26.2°C), when compared to BA and SP1 (25.3 and 25.6°C, respectively). T_{opt} for SP2 (25.7°C) was similar to that for MA (26.2°C) and SP1 (25.6°C) (Tab.1).

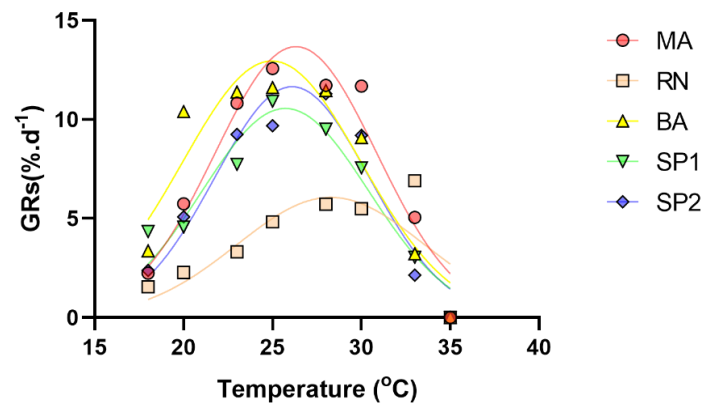


Fig.2 Growth rate curves of *Gracilariopsis tenuifrons* from distinct populations (red circle = Maranhão State, MA; pink square = Rio Grande do Norte State, RN; yellow triangle = Bahia State, BA; green upside triangle = Ubatuba-São Paulo State, SP1; and blue diamond = Itanhaém-São Paulo State, SP2). Each point represents the mean growth (n=5) at each temperature treatment (18, 20, 23, 25, 28, 30, 33, and 35°C).

Table 1. TPC traits (i.e. modelled data) for the growth rate of *Gracilariopsis tenuifrons* cultivated at different temperatures (18-35°C) – maximal measurement (μ_{\max}), thermal optimum (T_{opt}), thermal tolerance ($T_{\text{tolerance}}$), and critical thermal maximum and minimum (CT_{min} and CT_{max}). Each emphasis used (regular, bold, italic, and underlined) on the different letters indicates a different statistical treatment.

Population	μ_{\max}	CT_{min} (°C)	T_{opt} (°C)	CT_{max} (°C)	$T_{\text{tolerance}}$
MA	12.7 ^a	17.4 ^a	26.2 ^b	35.1 ^b	17.7 ^c
RN	5.6 ^c	17.4 ^a	27.4 ^a	37.4 ^a	20.0 ^a
BA	12.2 ^a	15.9 ^b	25.3 ^d	34.7 ^b	18.8 ^b
SP1	9.6 ^b	16.3 ^b	25.6 ^{cd}	34.9 ^b	18.6 ^b
SP2	10.6 ^b	17.2 ^a	25.7 ^{bc}	34.7 ^b	17.5 ^c

***In vivo* chlorophyll fluorescence**

Maximum quantum yield (F_v/F_m), maximum electron transport rate (ETR_{max}), saturating irradiance (E_k), and photosynthetic efficiency (α) of female gametophytes of *Gp. tenuifrons* cultivated for 28 days differed according to population (MA, RN, BA, SP1, and SP2) and temperature (18, 20, 23, 25, 28, 30, and 33°C) (isolated and interactive effects) (Table S1).

Comparing the same population in different tested temperatures

Rocky shore (MA, BA, SP1, and SP2) and mangrove (RN) populations showed similar F_v/F_m (around 0.47) between 18 and 30°C, whereas the populations occupying the highest latitudes of the Brazilian coast (SP1 and SP2) showed the lowest F_v/F_m at 33°C (around 0.33) (Fig.3a). In general, the intermediate population (BA) and populations occupying the lowest latitudes of the Brazilian coast (MA and RN) showed the highest ETR_{max} at 18-23°C ($1.61 \mu\text{mol e}^-\text{m}^{-2} \text{s}^{-1}$), but the lowest ETR_{max} between 25 and 33°C ($0.79 \mu\text{mol e}^-\text{m}^{-2} \text{s}^{-1}$) (Fig.3b). In contrast, southeastern populations (SP1 and SP2) showed the lowest ETR_{max} at 33°C compared to other temperatures. Moreover, southeastern populations showed the lowest E_k values at 33°C ($\pm 13 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and the highest values at 18-23°C ($\pm 75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Fig.3c).

Comparing different populations in each tested temperature

In general, temperatures promoted similar photosynthetic values as observed among the different populations tested between 20 and 30°C for F_v/F_m , at 28°C for E_k , and between 25 and 30°C for α (Fig.3a, b, d).

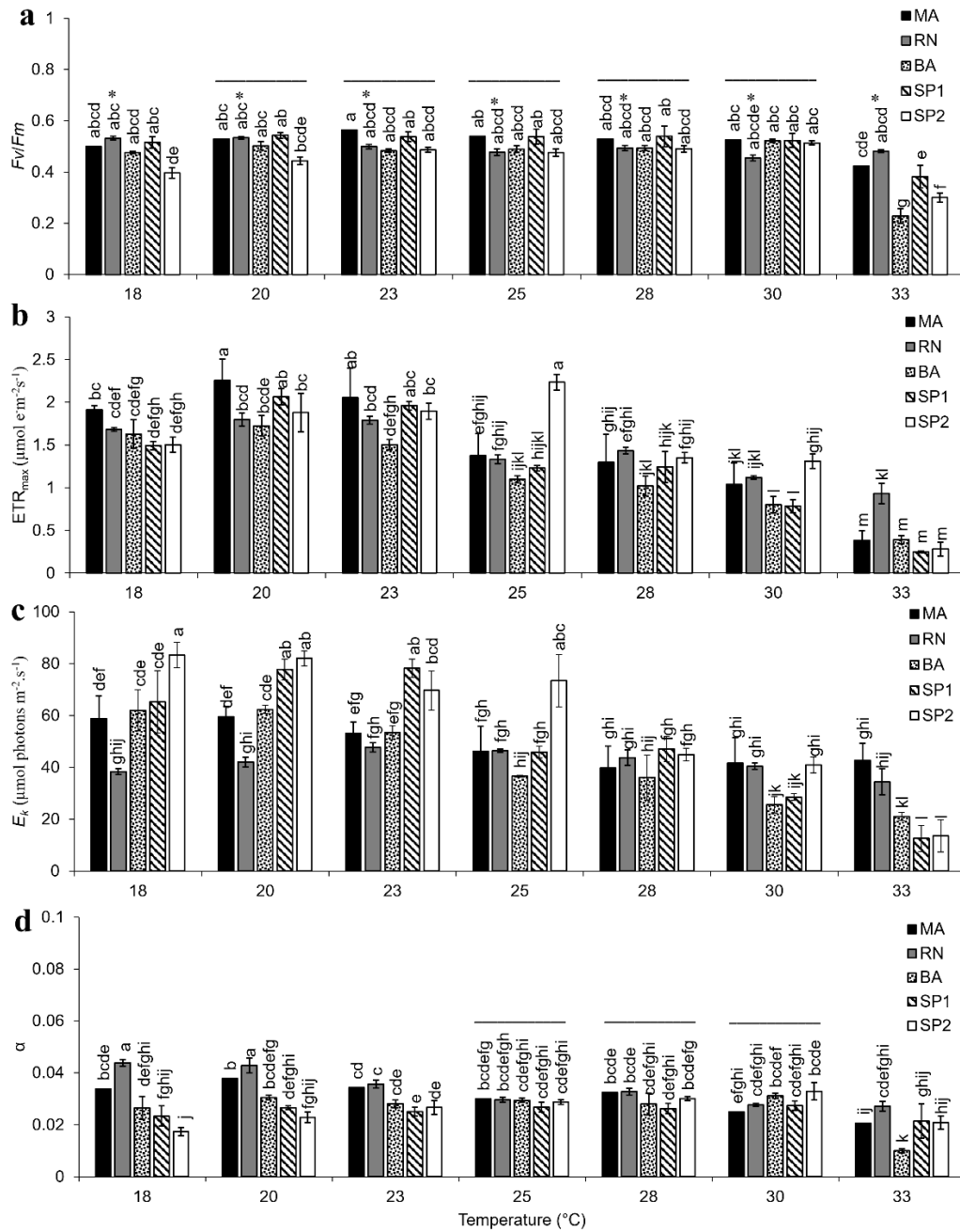


Fig.3 *In vivo* chlorophyll fluorescence of *Gracilariopsis tenuifrons* from distinct populations (Maranhão State, MA; Rio Grande do Norte State, RN; Bahia State, BA; Ubatuba-São Paulo State, SP1; and Itanhaém-São Paulo State, SP2), cultivated at different temperatures (18, 20, 23, 25, 28, 30, and 33°C): (a) F_v/F_m , maximum quantum yield; (b) ETR_{max} , maximum electron transport rate; (c) E_k , saturating irradiance; and (d) α , photosynthetic efficiency. Data presented as mean and standard deviation (n=5). Different letters represent significant differences among temperatures and populations (p<0.05). Extended line means similar result among populations, and asterisk means similar result among temperatures in a single population.

Low temperatures: At 18°C, SP2 showed the highest E_k value (83 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), MA, BA, and SP1 showed intermediate E_k values ($\pm 62 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), and RN showed the lowest E_k value (42 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Fig.3c). At 20°C, SP1 and SP2 showed the highest E_k values (79 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), RN showed the lowest E_k value (83 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), and MA and BA showed intermediate E_k values (60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), among all populations (Fig.3c).

Middle temperatures: At 23°C, southeastern populations (SP1 and SP2) showed the highest E_k values ($\pm 73 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) in contrast to northeastern populations (MA, RN, and BA) ($\pm 51 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Fig.3c). At 25°C, SP2 showed the highest ETR_{max} and E_k values ($\pm 2.23 \mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$, ETR_{max} ; $\pm 73 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, E_k) in comparison to all other populations ($\pm 1.25 \mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$, ETR_{max} ; $\pm 44 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, E_k) (Fig.3b, c). At 28°C, all populations showed similar ETR_{max} values (1.26 $\mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$), except for BA, which showed lower values than RN (1.43 $\mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$) (Fig.3b).

High temperatures: At 33°C, the worst photosynthetic performance was observed for SP2, followed by SP1, when compared to the others. Evaluating F_v/F_m , MA and RN showed higher values (± 0.45) when compared to SP2 (± 0.38), SP1 (± 0.29), and BA (± 0.22). Evaluating ETR_{max} , RN showed the highest value (0.93 $\mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$) in contrast to all other populations ($\pm 0.32 \mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$) (Fig.3b). Evaluating E_k , MA and RN showed higher values ($\pm 39 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) than BA, SP1, and SP2 ($\pm 16 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Fig.3c).

Pigments content

Phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC), and chlorophyll-*a* (Chlo-*a*) contents of female gametophytes of *Gp. tenuifrons* cultivated for 28 days differed by population (MA, RN, BA, SP1, and SP2) and temperature (18, 20, 23, 25, 28, 30, 33, and 35°C) (isolated and interactive effects) (Table S1).

Comparing the same population in different tested temperatures

For the mangrove population (RN), middle temperature (25°C) promoted 1.88 times more PE and PC contents than all other temperatures. Otherwise, the lowest temperature (18°C) promoted the lowest PE and PC contents (Fig.4a, b). Low, middle, and high

temperatures (20-30°C) promoted 2.10 times more Chlo-a contents than extreme temperatures (18 and 33°C) (Fig.4d).

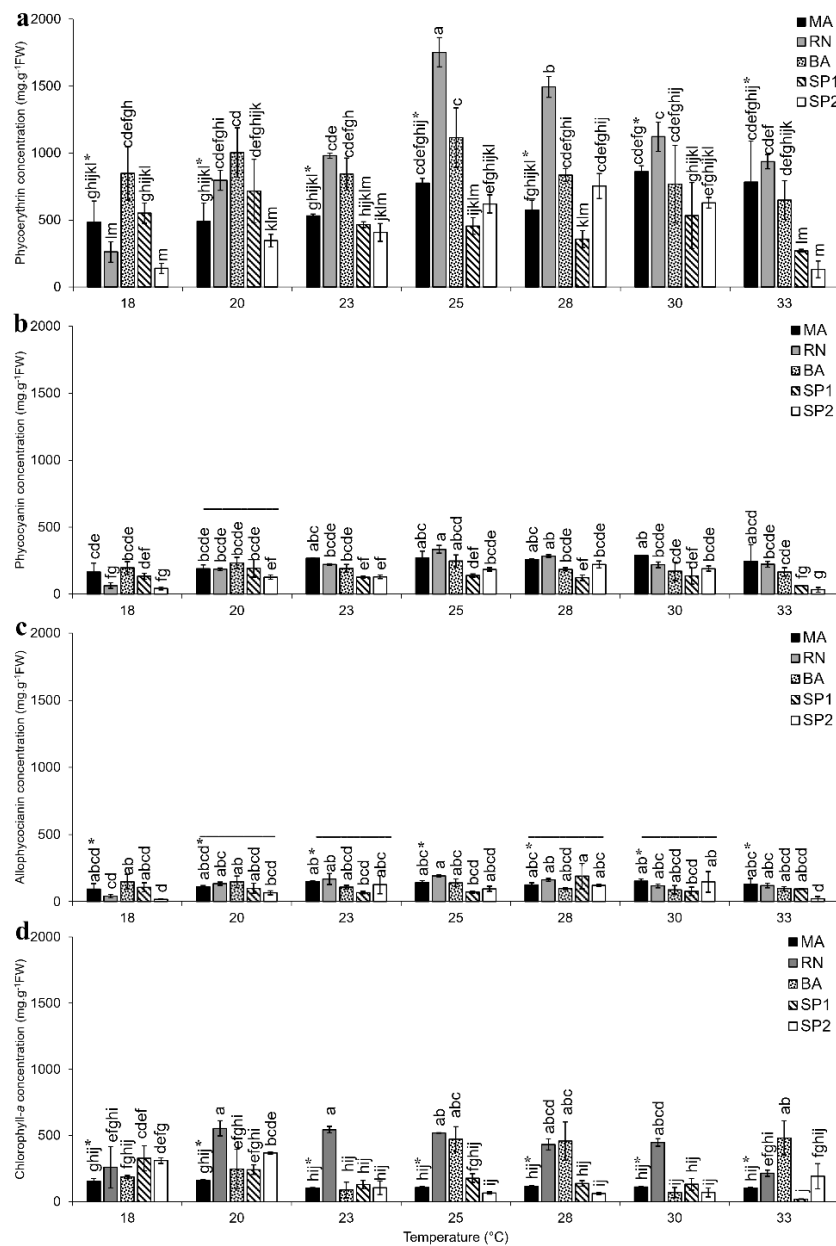


Fig.4 Pigment concentration of *Gracilariopsis tenuifrons* from distinct populations (Maranhão State, MA; Rio Grande do Norte State, RN; Bahia State, BA; Ubatuba-São Paulo State, SP1; and Itanhaém-São Paulo State, SP2), cultivated at different temperatures (18, 20, 23, 25, 28, 30, and 33°C): (a) phycoerythrin (PE); (b) phycocyanin (PC); (c) allophycocyanin (PC); and (d) chlorophyll *a* (Chlo-a). Data presented as mean and standard deviation (n=5). Different letters represent significant differences among temperatures and populations (p<0.05). Extended line means similar result among populations, and asterisk means similar result among temperatures in a single population.

For the intermediate population (BA), middle temperature (25°C) promoted 1.71 times more PE content than high temperature (33°C) (Fig.4a). Furthermore, middle (25-28°C) and high temperatures (only 33°C) promoted 3.18 times more Chlo-a content than 18-23 and 30°C (Fig.4d).

Considering the southeastern populations, SP1 exposed to low temperature (20°C) showed 2.64 times more PE content than when exposed to high temperature (33°C) (Fig.4a). Furthermore, SP1 exposed to low temperature (18°C) promoted 3.13 times more Chlo-a content than when exposed to 23, 28, 30, and 33°C (Fig.4d).

For the southeastern marginal population (SP2), middle temperature (28°C) promoted 3.65 times more PE content than low and high temperatures (18-20, and 33°C) which showed the lowest PE contents (Fig.4a). Furthermore, SP2 exposed to 20-30°C showed 5.17 times more PC content than when exposed to 33°C (Fig.4b). SP2 exposed to low temperatures promoted 4.48 times more Chlo-a content than when exposed to middle and high temperatures (Fig.4d).

Comparing different populations in each tested temperature

In general, the mangrove population (RN) presented higher chlorophyll-*a* content when compared to rocky shore populations (MA, BA, SP1, and SP2), as observed at 20, 23, and 30°C (Fig.4d). Furthermore, RN presented higher phycoerythrin content at middle temperatures (23-28°C) than that presented by other populations.

Low temperatures: At 18°C, MA, BA, and SP1 showed higher PE and PC contents than the southeastern marginal population (SP2) (Fig.4a,b). RN, however, showed content similar to that among all populations (Fig.4a, d); BA population showed higher APC content than RN and SP2 (Fig.4c). At 20°C, BA population showed higher phycoerythrin content than both marginal populations (MA and SP2) (Fig.4a).

Middle temperatures: At 23°C, the northeastern marginal population (MA) showed the highest PC content when compared to the southeastern populations (SP1 and SP2) (Fig.4b). At 25-28°C, the mangrove population (RN) showed the highest PE content when compared to all other populations (Fig.4a). Considering chlorophyll-*a*, RN and BA showed the highest contents at 25-28°C, when compared to all other populations (Fig.4d).

High temperatures: At 30°C, all populations showed similar PE contents, except the southeastern populations (SP1 and SP2) that showed lower contents than RN (Fig.4a). At

33°C, the northeastern populations (MA, RN, and BA) showed the highest PE and PC contents when compared to the southeastern populations (SP1 and SP2) (Fig.4a, b).

Discussion

Female gametophytes of *Gracilariopsis tenuifrons* from five distinct populations (MA, RN, BA, SP1, and SP2), distributed along 7,450km of the Brazilian coast, tolerated temperatures between 18 and 33°C, but less tolerance at 35°C, which was considered deleterious, irrespective of the analyzed population. However, populations showed different thermal adaptation as revealed by their physiological performance. Therefore, our data support the hypothesis that *Gp. tenuifrons* presents latitudinal ecotypes and that such ecotypes differ according to the temperature range and populations in which they occur.

The occurrence of ecotypes in a species of wide distribution on the Brazilian coast, such as *Gp. tenuifrons*, evidences the potential to house other species with adaptations already established according to the temperature gradient presented along this coast. Indeed, ecotypes related to temperature were reported in other red algae that occurs along this same coastline, such as *Gracilaria birdiae* (Ursi et al. 2003) and *G. domingensis* (Castro and Yokoya 2018). However, other parameters were also used to recognize ecotypes along that coast, such as: (i) photosynthetically active radiation in *Gracilaria caudata* (Faria et al. 2017) and *Hypnea pseudomusiformis* (Nauer et al. 2020); and (ii) ultraviolet radiation B in *G. caudata* (Araujo et al. 2014).

The intermediate population (BA) of *Gp. tenuifrons* and those populations occupying the lowest latitudes of the Brazilian coast (MA and RN) present some unique physiological characteristics. However, we further showed that they are better able to physiologically develop over higher (23-28°C) and stressful (30-33°C) temperatures when compared to southeastern populations (SP1 and SP2). Indeed, for most of populations analyzed, it was possible to find a statistical significance, either at the highest range, e.g., T_{opt} (MA at 26.2°C; RN at 27.4°C; BA and SP1 at 25.3-25.6°C; SP1 and SP2 at 25.6-25.7°C), or the lowest range, e.g., μ_{max} (RN, 5.6% day⁻¹; SP1 and SP2, 9.6-10.6% day⁻¹). BA and SP1 populations differed each other based on their μ_{max} (BA, 12.2% day⁻¹; SP1, 9.6% day⁻¹), highest growth temperature range (BA, 23-28°C; SP1, 28-30°C), GRs (at 20-23°C and 28-30°C), and some photosynthetic parameters. SP1 and SP2 populations differed each other based on their CT_{min} , $T_{tolerance}$, highest growth temperature range (SP1,

28-30°C; SP2, 28°C), GRs (at 18°C and 30°C), and some photosynthetic parameters. The physiological differences observed between the two southern populations (SP1 and SP2) showed that different adaptation patterns can also occur between close populations, even in the presence of putative gene flow, as already observed in other red algae (Ayres-Ostrock et al. 2019; Nauer et al. 2019).

Physiological differences between populations of *Gp. tenuifrons* were also evident in the analysis of the maximum quantum yield (F_v/F_m). Specifically, temperatures of 20-30°C promoted higher F_v/F_m , but stressful temperature (33°C) promoted lower F_v/F_m . Both MA and RN populations were exceptions as they showed similar values at 33°C. It was difficult to compare our photosynthetic parameter results to other studies since different species adapt differently to different temperatures according to environmental conditions. However, the thermal influence on photosynthetic performance in other red algae was also evident, e.g., *Gp. lemaneiformis* (Liu et al. 2017), *Gelidiella acerosa* (Fujimoto et al. 2015), *Hydropuntia edulis* and *H. eucheumatoides* (Vo et al. 2015), *Kappaphycus alvarezii* (Terada et al. 2016), *Kappaphycus* sp. (Borlongan et al. 2017), *Pyropia tenera* (Watanabe et al. 2014), and *P. yezoensis* (Watanabe et al. 2016).

Particularly, the decreases in photosynthesis of *Gp. tenuifrons* may be related to changes in the state of PSII or in the proportion of energy transferred during the process of the ATP production. Our study showed a significant negative effect of stressful temperature, 33°C, on BA, SP1, and SP2 populations for which F_v/F_m , ETR_{max} , and E_k presented the lowest values, together with minimal physiological performance. Furthermore, lower PE and PC contents were observed (only southern populations). Thermal stress can rearrange the structures of the thylakoid membranes, deactivate PSII (Roleda 2009), or inhibit synthesis *de novo* of protein D1 in PSII owing to the accumulation of hydrogen peroxide (Allakhverdiev and Murata 2004; Allakhverdiev et al. 2008; Takahashi and Murata 2008).

Rocky shore x Mangrove populations

Our results also highlighted the differences between populations of *Gp. tenuifrons* that occur in rocky shore (MA, BA, SP1, and SP2) and mangrove (RN). At 33°C, RN showed the highest values of GRs and ETR_{max} when compared to the others. Otherwise, rocky shore populations showed better μ_{max} on TPCs and higher GRs between 20°C and 30°C. However, even though RN showed the lowest growth than the other populations,

it showed the highest T_{opt} , CT_{max} , and $T_{\text{tolerance}}$ on TPCs, and it produced the highest pigment contents (PE, PC, and Chlo-a) when compared to some, not all, rocky shore populations (SP1 and SP2). Although the mangrove population developed unique adaptive characteristics that allowed these individuals to establish themselves and survive in this environment, they also adapted to this site at the cost of metabolic losses, which ended up compromising growth. Comparing both environments, mangrove (RN) has salinity that varies between 38 (near the mouth) to 50 (near the end of the system upstream) and temperature between 28 and 37°C (Santiago et al. 2005; Lucena 2012). In contrast, rocky shore populations (MA, BA, SP1, and SP2) have stable salinity of 35 and temperatures between 20 and 26°C at the southern coast and between 27 and 29°C at the northeastern coast. These distinct environmental characteristics may explain the differences observed in terms of growth and other physiological parameters between the mangrove population and those of rocky shore. However, it is important to consider the laboratory conditions used in our experimental design to evaluate physiological responses, since these may have favored rocky shore populations over mangrove population.

Northeastern (MA) x Southeastern populations (SP1 and SP2)

The population located further toward the northeast (MA) of the country and those in the southeast (SP1 and SP2) have been influenced by different temperatures throughout the years, enabling *Gp. tenuifrons* to make a distinct thermal adaptation. As was expected, at stressful temperature, 33°C, SP1 and SP2 populations showed worst physiological developments, when compared to MA that showed a slightly better physiological development (F_v/F_m , E_k , PE, and PC). MA population also presented higher μ_{max} (12.7% day⁻¹) on TPCs when compared to southeastern populations. Moreover, SP1 and SP2 showed the lowest values of CT_{min} , according to the lowest temperatures that they are submitted. It is possible that populations of *Gp. tenuifrons* of the southeast would benefit from an increase of sea surface temperature, as predicted by IPCC (1.5-2.0°C, until 2050 in an optimistic scenario; 2021) since their physiological performance was not so bad between 28 and 30°C, as well as by the higher CT_{max} observed. Moreover, it is not expected for populations located closer to the equator (MA and RN) any change in an optimist scenario since they are already subjected to more stable and higher temperatures throughout the year. However, a synergy among local abiotic factors is expected, including radiation and nutrients, which could lead to different responses. Indeed, a study

on the southern marginal population of *Laurencia catarinensis* (27.6°S, Santa Catarina State) showed the synergic effect of temperature and nutrients on its physiology (Gouvêa et al. 2018).

Intermediate population (BA)

The BA population showed some physiological characteristics similar to those observed in populations located further to the northeast and others more similar to those observed for populations located further to the southeast. According to some studies on red algae (Ayres-Ostrock et al. 2019; Nauer et al. 2019), Bahia State is considered an intermediate region on the Brazilian coast owing to the Vitória-Trindade Seamount Chain between northern Espírito Santo and southern Bahia States. This chain was recognized as a barrier to gene flow, and it was responsible for the current genetic and floristic structure (Bard et al. 1990; Corrêa 1996; Lambeck et al. 2002). Thus, it was expected that BA would show some similar physiological characteristics in comparison to southeastern individuals, regardless of the temperature. Our results corroborate other studies that tested different irradiances on *Gracilaria caudata*. The BA population of this species showed some physiological characteristics similar to northeastern (Ceará State, closer to RN) and southeastern (SP1, same site) populations (Faria et al. 2017). Besides, molecular studies on other red algae showed that the BA region may correspond to a refugium and a contact zone between the highly differentiated northeastern and southeastern regions (Ayres-Ostrock et al. 2019; Nauer et al. 2019). Accordingly, a comparison of genetic diversity in *Gp. tenuifrons* will be necessary to better characterize the populations along the Brazilian coast.

Stressful temperature (33°C)

Stressful temperature of 33°C differentially affected the populations located closer to the equator (MA and RN) compared to the southeastern region (SP1 and SP2). Populations closer from the equator presented higher photosynthesis (F_v/F_m ; E_k) and pigment contents (PE and PC) than SP1 and SP2 populations. It is possible that MA and RN populations produce more accessory pigments like PE and PC because they are already adapted to regions with higher irradiances and temperatures. Additional pigment content would contribute to increase electron uptake, as well as ATP production, necessary for the acclimation of individuals. On the contrary, southeastern populations, in order to maintain their basic physiological functions at 33°C, use their pigments, like

PE, as a source of nitrogen, as already demonstrated in previous studies on other red algae (Lapointe 1981; Talarico 1996; Faria and Plastino 2016).

Conclusion

Our data support the hypothesis that the five populations of *Gp. tenuifrons* are ecotypes. This result can be attributed to the wide distribution of the species on the Brazilian coast (2.4°S-24°S), which presents quite diverse environmental characteristics, for example, temperature. Rocky shore populations (MA, BA, SP1, and SP2) proved to be more adapted to low-high (18-30°C) temperatures than the mangrove (RN) population. The latter is also more resilient to stressful temperature (33°C) than the others, although it showed the lowest GR in other temperatures. Furthermore, BA population presented some physiological characteristics similar to populations located further northeast and southeast. None of the populations survived at 35°C.

If the SST should rise to around 1.5-2.0°C (optimistic scenario) in the next 30 years (IPCC 2021), *Gp. tenuifrons* populations could not be affected, especially the southeastern populations (SP1 and SP2). However, in a drastic scenario (up to 4°C), especially MA and BA populations could be affected and may suffer a decline. In the present study, individuals did not show satisfactory physiological performances with temperatures above 28-30°C (rocky shore) and 33°C (mangrove). RN population showed the lowest growth in all temperatures, with exception of 33°C, in which the other populations showed worst physiological performances. Timing of growth are considered important responses to global climate changes, and they could alter the composition of communities and the nature of species interactions (Hoffman and Sgro 2011). Based on physiological characteristic observed in our experiment, especially the lowest growth at almost all temperatures, we believe RN would also be affected in a future drastic scenario. However, the southeastern populations (SP1 and SP2) could benefit from such rise, including in a drastic scenario, and this would allow the expansion of the species to regions further to the south of their current distribution.

Finally, since some northeastern states are now developing artisanal cultivation techniques of Gracilariales (Simioni et al. 2019), it is plausible to suggest that experimental mariculture tests of *Gp. tenuifrons* as a new species be exploited. Among studied populations, the MA and BA populations are highlighted, because of mariculture already exists in the northeast region of the country.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Supplementary material



Fig.S1 *Gracilariopsis tenuifrons* from distinct populations (Maranhão State; Rio Grande do Norte State; Bahia State; Ubatuba-São Paulo State; and Itanhaém-São Paulo State), cultivated at different temperatures (18, 20, 23, 25, 28, 30, and 33°C) for a period of 28 days. Scale bar = 1 cm.

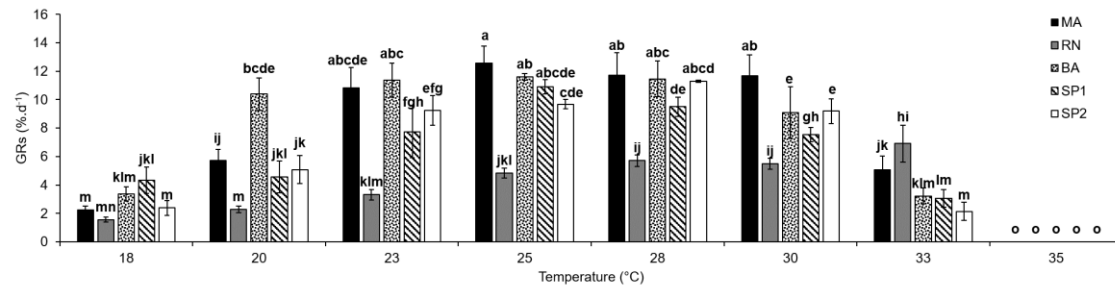


Fig.S2 Growth rates of *Gracilariopsis tenuifrons* from distinct populations (Maranhão State, MA; Rio Grande do Norte State, RN; Bahia State, BA; Ubatuba-São Paulo State, SP1; and Itanhaém-São Paulo State, SP2), cultivated at different temperatures (18, 20, 23, 25, 28, 30, and 33°C). Data presented as mean and standard deviation (n=5). Different letters represent significant differences among populations and temperatures ($p < 0.05$).

TableS1. Summary of one and two-way analysis of variance of *Gracilariopsis tenuifrons* from different populations (1) (Maranhão State, Rio Grande do Norte State, Bahia State, Ubatuba-São Paulo State, and Itanhaém-São Paulo State) cultivated at distinct temperatures (2) (18, 20, 23, 25, 28, 30, 33, and 35°C) considering, maximum electron transport rate (ETR_{max}), saturating irradiance (E_k), maximum quantum yield (F_v/F_m), photosynthetic efficiency (α), pigment content (PE, phycoerythrin; PC, phycocyanin; APC, allophycocyanin; Chl-a, chlorophyll *a*), and thermal performance curve parameters (maximal measurement- μ_{max} ; thermal optimum- T_{opt} ; thermal tolerance- $T_{tolerance}$; critical thermal maximum and minimum- CT_{min} and CT_{max}). Bold numbers mean interaction between factors.

Source of Variation	GRs				ETR_{max}			E_k			F_v/F_m			α				
	<i>df</i>	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P		
Population (1)	5	76.08	98.63	0	0.41	21.43	0.00	948.8	30.49	0.00	0.02	17.56	0.00	0.000319	36.32	0.00		
Temperature (2)	7	268.15	347.61	0	4.05	209.15	0.00	3360.2	107.97	0.00	0.04	34.92	0.00	0.000223	25.40	0.00		
(1)*(2)	35	14.76	19.13	0	0.16	8.62	0.00	404.1	12.98	0.00	0.00	4.20	0.00	0.000073	8.26	0.00		
Residual		0.77			0.01			31.1			0.00			0.00				
Source of Variation	PE	PC			APC			Chlo-a										
	MS	F	P	MS	F	P	MS	F	P	MS	F	P						
Population (1)	1432	84.68	0.00	5343	37.96	0.00	8602	7.91	0.00	3175	83.03	0.00						
Temperature (2)	4020	23.76	0.00	2331	16.56	0.00	6217	5.71	0.00	3780	9.88	0.00						
(1)*(2)	1711	10.11	0.00	6891	4.89	0.00	4440	4.08	0.00	4899	12.81	0.00						
Residual	16917			1408			1087			3825								
Source of Variation	<i>df</i>	MS	F	P														
μ_{max}	5	32.2	59	0.00														
T_{opt}	5	2.6	36.4	0.00														
$T_{tolerance}$	5	4.05	14.2	0.00														
CT_{min}	5	2.62	36.4	0.00														
CT_{max}	5	5.37	36	0.00														

CAPÍTULO 6 – CONCLUSÃO GERAL

O objeto de estudo do presente trabalho foi a alga vermelha *Gracilariopsis tenuifrons*, uma espécie com ampla distribuição geográfica, que ocorre desde o México até a região sudeste do Brasil (estado de São Paulo, Município Itanhaém, 24,2°S). A espécie é considerada uma das principais agarófitas do nordeste brasileiro, dentre *Gracilaria caudata* e *G. birdiae*.

Este trabalho teve como objetivo verificar e compreender os aspectos fisiológicos e moleculares da diversidade intraespecífica de *Gracilariopsis tenuifrons*. Nós focamos em elucidar e caracterizar possíveis ecótipos associados à temperatura. Para isso, avaliamos os efeitos das temperaturas (18, 20, 23, 25, 28, 30, 33 e 35°C) em diferentes indivíduos procedentes de cinco populações distintas, localizadas nos estados do Maranhão (MA, 2.4°S), Rio Grande do Norte (RN, 5.4°S), Bahia (BA, 14.48°S) e São Paulo (Municípios de Ubatuba, 23.5°S, SP1; e Itanhaém, 24.2°S, SP2). Os parâmetros analisados foram crescimento (curva de performance térmica), fotossíntese e conteúdo pigmentar. Além disso, considerando indivíduos procedente do Estado de São Paulo (Município de Itanhaém), distribuição limite para *Gp. tenuifrons*, avaliamos os efeitos das temperaturas (20, 25 e 30°C) em diferentes estádios reprodutivos do histórico de vida (tetrasporófitos férteis, gametófitos femininos não fertilizados, plantas cistocárpicas e gametófitos masculinos férteis) para compreender vantagens e desvantagens de organismos de ploidia e estádios reprodutivos diferentes. Os parâmetros analisados foram comprimento do talo, número de ramos diferenciados, crescimento, fotossíntese e conteúdo pigmentar.

À parte, para compreender a diversidade genética e filogeográfica em *Gp. tenuifrons*, avaliamos seis populações distribuídas ao longo da costa brasileira (as mesmas populações citadas anteriormente, mais indivíduos procedentes do Estado de Alagoas, 9°S, AL), utilizando uma combinação de marcadores de DNA, mitocondriais (COI-5P e *cox2-3* concatenados) e cloroplastidial (*rbcL*). Ao fim, para elucidar os efeitos da temperatura a nível molecular, nós avaliamos a expressão diferencial dos genes (DEGs), utilizando sequenciamentos de RNA (RNAseq) de diferentes indivíduos cultivados em temperaturas ótima (25°C) e de estresse (33°C, limite térmico de sobrevivência). Para compreensão dos DEGs, utilizamos a ontologia dos genes (GOs), abrangendo processos biológicos (BP), funções moleculares (MF) e componentes celulares (CC).

As cinco populações de *Gp. tenuifrons* foram caracterizadas como ecótipos associados à temperatura. Esse resultado pode ser atribuído à ampla distribuição da espécie no litoral brasileiro, que apresenta características ambientais diversas, como, por exemplo, temperatura. As populações que ocorrem em costão rochoso (MA, BA, SP1 e SP2) são adaptadas a maior amplitude de temperatura (18-30°C) do que a população de manguezal (RN). Entretanto, RN é mais resistente à temperatura de estresse (33°C) do que as demais, embora tenha apresentado, comparativamente, as menores taxas de crescimento nas outras temperaturas testadas. Nenhuma das populações sobreviveu a 35°C. Além disso, a temperatura de estresse (33°C) afetou diferentemente populações localizadas mais próximas ao equador (MA e RN) em relação à região sudeste (SP1 e SP2), já que populações nordestinas apresentaram maior fotossíntese (F_v/F_m ; E_k) e conteúdo pigmentar (PE e PC) do que as populações do sudeste. É possível que populações do MA e RN produzam mais pigmentos acessórios como PE e PC pelo fato de já estarem adaptadas a regiões com maiores irradiâncias e temperaturas. Maior conteúdo pigmentar contribuiria para aumentar a captação de elétrons, bem como a produção de ATP, necessários para a aclimação dos indivíduos.

Comparou-se pela primeira vez em *Gp. tenuifrons* tetrasporófitos férteis, gametófitos femininos não fertilizados, plantas cistocárpicas e gametófitos masculinos férteis, levando-se em consideração os efeitos da temperatura. Foi possível testar e confirmar a hipótese de que fases háploides e diplóide, apesar de isomórficas, respondem diferentemente, independentemente da temperatura. Diferenças fisiológicas entre estádios reprodutivos permitem que a espécie se aclimate a variações térmicas no ambiente, ampliando as possibilidades de sobrevivência. Além disso, pudemos testar a hipótese de que plantas cistocárpicas e gametófitos masculinos férteis (ambos haplóides) são mais suscetíveis a temperaturas extremas (20 e 30°C), do que gametófitos femininos não fertilizados (haplóide) e tetrasporófitos (diplóide), devido ao resultado do particionamento de energia para produzir estruturas reprodutivas. Estudos anteriores para algumas espécies de *Gracilaria* demonstram que independentemente das fases serem isomórficas, tetrasporófitos e gametófitos, essas podem apresentar respostas fisiológicas distintas, dependendo das condições abióticas (Guimarães 2000; Ursi et al. 2001; Costa 2005; Ferreira 2008; Barufi et al. 2015; Faria et al. 2017). Vantagens e desvantagens de organismos haplóides e diplóides vêm sendo relatadas para espécies que apresentam alternância de gerações. Uma dessas fases teria vantagem sobre a outra, dependendo do

ambiente a que são expostas (Lewis 1985; Hughes e Otto 1999), trazendo, portanto, benefícios para a permanência da espécie em locais onde ocorrem variações abióticas.

Considerando-se a ampla distribuição geográfica de *Gp. tenuifrons*, pudemos confirmar nossas duas hipóteses, de que existem barreiras oceânicas ao fluxo gênico ao longo da costa brasileira, e que houve refúgio no passado com forte isolamento entre regiões, moldando geneticamente as populações em três filogrupos principais, NE (MA, RN, AL e BA), filogrupo I (SP1) e filogrupo II (SP2). A principal barreira geográfica para o fluxo gênico entre populações de *Gp. tenuifrons* está relacionada aos montes submarinos da Cadeia Vitória-Trindade, localizada entre os estados da Bahia e Espírito Santo. Em estudos anteriores essa barreira geográfica tem sido proposta para explicar discontinuidades genéticas de vários táxons marinhos com distribuição contínua ao longo da costa brasileira, como moluscos (Lazoski et al., 2011), crustáceos (Hurtado et al. 2016), poliquetas (Paiva et al., 2019), e outras espécies de macroalgas (Ayres-Ostrock et al. 2019; Nauer et al. 2019; Martins et al. 2022). Durante os máximos glaciais do Quaternário, o desnível do mar emergiu grandes áreas da plataforma continental, incluindo o topo dos montes submarinos de Vitória-Trindade, dividindo o litoral do continente brasileiro em uma região norte mais quente, possivelmente tropical, e uma subtropical mais fria, possivelmente quente-temperada, região Sul.

Pela primeira vez, em *Gp. tenuifrons*, foi identificada a expressão diferencial dos genes (DEGs) em função biológica, celular e molecular, levando-se em consideração o efeito de duas temperaturas, ótima (25°C) e de estresse (33°C). Foi possível avaliar como a espécie responde nessas diferentes temperaturas. De maneira geral, identificamos um elevado número de DEGs em indivíduos cultivados em 25 e 33°C. Entretanto, esse valor representa uma pequena porcentagem (5,25-5,75%) do total de genes compreendidos (17.556). Em 33°C, observou-se DEGs envolvidos em vários passos na transcrição do DNA (replicação, reparo e recombinação), além de DEGs relacionados à segregação cromossômica e organização dos telômeros. Portanto, a expressão desses genes em *Gp. tenuifrons* pode estar relacionada a maiores taxas de divisão celular, colaborando no reparo dos componentes biológicos danificados por alta temperatura. Entretanto, em 25°C, observou-se DEGs relacionados à fotossíntese, atividades de agente transportador através da membrana e à estrutura das células (e.g. tilacóide, parede celular e organização de membranas). Portanto, a expressão desses genes pode estar relacionada ao funcionamento ativo das células, o que propicia, em última análise, altas taxas de

crescimento. Possivelmente, estariam também ativos mecanismos para evitar a fotoinibição e danos celulares, assim como, outras condições de estresse.

Concluindo, foi possível confirmar as hipóteses iniciais propostas neste trabalho. Esses resultados permitem afirmar que *Gp. tenuifrons* apresenta ecótipos relacionados à temperatura ao longo da costa brasileira. A distinção genética entre populações da espécie foi confirmada pelos estudos de filogeografia, que evidenciaram nítida distinção entre as populações que ocorrem na região nordeste e sudeste. Em nível de população, a presença de alternância de gerações, mesmo sendo isomórficas, contribui para a manutenção da espécie, já que os estádios apresentam respostas fisiológicas distintas dependendo das condições abióticas, como a temperatura. Por fim, por processo de termorregulação, *Gp. tenuifrons* apresentou diferentes expressões gênicas relacionadas à prevenção de danos causados pela temperatura e manutenção do desempenho fisiológico.

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Resumo

Gracilariopsis tenuifrons (C.J. Bird e E.C. Oliveira) Fredericq & Hommersand é considerada uma agarófita de importância econômica amplamente distribuída ao longo da costa brasileira (7.450 km). Apresenta histórico de vida trifásico, com duas gerações isomórficas (gametófito e tetrasporófito). Devido à sua ampla distribuição geográfica, as populações de *Gp. tenuifrons* estão sujeitas a diferentes fatores ambientais, como temperatura. A temperatura superficial da água do mar poderá aumentar entre 1,5-2°C até 2050, de acordo com o melhor cenário de previsões do IPCC, e ainda conhecemos muito pouco sobre a influência das mudanças climáticas nas algas tropicais. Portanto, é necessário ampliar o conhecimento sobre essas algas, incluindo estudos sobre filogeografia, adaptação térmica fisiológica de populações e fases do histórico de vida e caracterização da expressão gênica frente à temperatura. Para melhor abordar esses assuntos, avaliamos em *Gp. tenuifrons*: i, a diversidade genética de seis populações da costa brasileira (estados do Maranhão-MA, 2,4°S; Rio Grande do Norte-RN, 5,4°S; Alagoas-AL, 9°S, Bahia-BA, 14,48°S, e São Paulo-SP1 23,5°S, SP2 24,2°S), usando marcadores de DNA mitocondrial (COI-5P e *cox2-3* concatenado) e cloroplastidial (*rbcL*); ii, o efeito de oito diferentes temperaturas (18-35°C) em cinco populações da costa brasileira (citadas anteriormente, com exceção de AL), usando curva de performance térmica (taxas de crescimento), parâmetros da fotossíntese e conteúdo pigmentar; iii, o efeito de três diferentes temperaturas (20-30°C) em estádios reprodutivos (tetrasporófitos férteis-T, gametófitos femininos não fertilizados-FG, plantas cistocárpicas-CP e gametófitos masculinos férteis-MG), usando comprimento do talo, número de ramos diferenciados, taxas de crescimento, parâmetros da fotossíntese e conteúdo pigmentar; e iv, a expressão gênica em indivíduos do estado de São Paulo (SP2) cultivados em duas temperaturas (25 e 33°C) usando sequenciamento de RNA (RNAseq). A análise concatenada (COI-5P+*cox2-3*) evidenciou uma nítida separação entre três filogrupos, um deles para a região nordeste (NE, de 2,4°S a 14,48°S; 17 haplótipos) e outros dois para sudeste (SP1, 23,5°S, três haplótipos; SP2, 24,2°S, seis haplótipos) com quatro etapas mutacionais entre indivíduos das duas regiões. Duas etapas mutacionais foram observadas separando os dois filogrupos da região sudeste. Essa distinção é atribuída à presença de barreira geográfica para o fluxo gênico, neste caso, a Cadeia Vitória-Trindade (NE≠SP1+SP2) e o estuário de Santos (SP1≠SP2). Observou-se ainda adaptação térmica fisiológica em diferentes populações. Dentre essas, MA e BA

apresentaram as maiores medidas máximas (μ_{\max} ; 12,2-12,7% dia⁻¹) na curva de performance térmica, quando comparadas às populações SP1 (9,6% dia⁻¹) e SP2 (10,6% dia⁻¹). A população do RN apresentou os piores valores de μ_{\max} (5,6% dia⁻¹), entretanto, foi mais resistente em 33°C do que todas as outras populações. A população da BA apresentou algumas características fisiológicas semelhantes às populações das regiões nordeste e sudeste. Nenhuma população sobreviveu em 35°C. A avaliação da adaptação térmica em diferentes estádios reprodutivos mostrou que T apresentaram maiores taxas de crescimento e conteúdo de ficoeritrina, quando comparados aos FG, cultivados em temperaturas extremas (20°C: T, 6,8% dia⁻¹, FG, 5% dia⁻¹; 30°C: T, 10% dia⁻¹, FG, 8% dia⁻¹). No entanto, 25°C promoveu maior desempenho fisiológico em FG (12% dia⁻¹, 14,64 cm), quando comparados aos T (10% dia⁻¹, 9 cm). Plantas haplóides, como MG e CP, apresentaram desempenho fisiológico reduzido, evidenciando sua fragilidade quando comparadas às demais fases, T e FG. Por fim, pudemos identificar genes diferencialmente expressos em temperaturas ótima e de estresse. Em 25°C, observaram-se genes diferencialmente expressos relacionados à fotossíntese, atividades de agente transportador através da membrana e estrutura das células. Entretanto, em 33°C, observaram-se genes diferencialmente expressos envolvidos em vários passos na transcrição do DNA (replicação, reparo e recombinação) e também na segregação cromossômica e organização dos telômeros. Portanto, o processo de termorregulação resultou em expressões gênicas distintas, relacionadas à prevenção de danos causados pela temperatura (33°C) ou manutenção do desempenho fisiológico (25°C). Em conclusão, *Gp. tenuifrons* apresenta uma ampla diversidade genética ao longo da costa brasileira, que também pôde ser observada quanto às respostas fisiológicas das populações analisadas frente a diferentes temperaturas. Em nível de população, a presença da alternância de gerações isomórficas contribui para manutenção da espécie, já que os estádios apresentam respostas fisiológicas distintas dependendo das condições abióticas, como a temperatura. Nosso estudo abre perspectivas para novas abordagens em genética que visem compreender os efeitos de outros fatores abióticos tanto nas populações, quanto nas diferentes fases do histórico de vida da espécie.

Abstract

Gracilariopsis tenuifrons (C.J. Bird and E.C. Oliveira) Fredericq & Hommersand is an agarophyte of economic importance widely distributed along the Brazilian coast (7,450 km). Owing to its wide distribution, populations of *Gp. tenuifrons* are subject to different environmental factors, such as temperature. According to optimistic IPCC forecasts, the SST may increase by 1.5-2°C until 2050, and our knowledge is incipient to know how tropical seaweeds can manage this change. Therefore, knowledge about phylogeographic diversity, physiological thermal adaptations in populations and isomorphic life cycles, and gene expression characterization is necessary. To better approach these subjects, we evaluated the: i, genetic diversity and structure of six populations of *Gp. tenuifrons* from Brazilian coast (Maranhão-MA, 2.4°S; Rio Grande do Norte-RN, 5.4°S; Alagoas-AL, 9°S, Bahia-BA, 14.48°S, and São Paulo-SP1 23.5°S, SP2 24.2°S States), using a combination of mitochondrial (COI-5P and *cox2-3* concatenated) and chloroplastial (*rbcL*) DNA markers; ii, effect of eight different temperatures (18-35°C) among five populations from Brazilian coast (cited before, less AL), using thermal performance curve (growth rates), photosynthetic parameters, and pigment contents; iii, effect of three different temperatures (20-30°C) in distinct reproductive phases (fertile tetrasporophytes-T, unfertilized female gametophytes-FG, cystocarpic plants-CP, and fertile male gametophytes-MG), using thallus length, number of differentiated branches, growth rates, photosynthetic parameters, and pigment contents; and iv, gene expression in individuals from São Paulo State (SP2) cultivated at different temperatures (25 and 33°C) by RNAseq. Therefore, by COI-5P+*cox2-3* concatenated, *Gp. tenuifrons* populations showed an evident clear separation among three phylogroups, such as northeast (NE, from 2.4°S to 14.48°S; 17 haplotypes), and other two southeast (SP1, 23.5°S, three haplotypes; SP2, 24.2°S, six haplotypes) regions by four mutational steps between individuals from the two regions, owing to geographic barrier to the gene flow, such as Vitoria-Trindade seamount chain (NE≠SP1+SP2). Furthermore, between Southern phylogroups (SP1 and SP2), two mutational steps were observed, owing to the Santos Bay (an estuary between SP1 and SP2). Moreover, by physiological thermal adaptations among populations, MA and BA populations showed the highest maximal measurement (μ_{max} ; 12.2-12.7% day⁻¹) of thermal performance curve when compared to SP1 (9.6% day⁻¹) and SP2 (10.6% day⁻¹) populations. The RN population showed the worst μ_{max} values (5.6% day⁻¹); however, it was more resilient to stressful temperatures (33°C) than the others. The BA

population presented some physiological characteristics similar to populations from the northeastern and southeastern regions, but none of them could tolerate temperatures of 35 °C, irrespective of the populations. Besides, by physiological thermal adaptations among reproductive phases, T showed higher growth rates and phycoerythrin, when compared to FG, under extreme tested temperatures (20°C: T, 6.8% day⁻¹, FG, 5% day⁻¹; 30°C: T, 10% day⁻¹, FG, 8% day⁻¹). However, 25°C promoted higher physiological performance in FG (12% day⁻¹, 14.64 cm) when compared to T (10% dia⁻¹, 9 cm). Haploid plants, such as MG and CP, showed reduced physiological performance evidencing their fragility when compared to the others. Finally, by gene expression characterization, we could identify differentially expressed genes between optimum and stressed temperatures. Thus, 25°C promoted genes enriched in photosynthesis and cellular treat, and 33°C promoted genes enriched within DNA transcripts (replication, repair, and recombination). Therefore, the thermoregulation process resulted in distinct gene expressions related to the prevention of temperature damage (33°C) or maintenance of physiological performance (25°C). In conclusion, *Gp. tenuifrons* has a wide genetic diversity along the Brazilian coast, which could also be observed regarding the physiological responses of the populations analyzed in relation to different temperatures. At the populations, the alternation of isomorphic generations contributes to the maintenance of the species, since the different stages present distinct physiological responses depending on abiotic conditions, such as temperature. Our study opens perspectives for new approaches in genetics that aim to understand the effects of other abiotic factors both in populations and in the different stages of the life history of the species.