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RESPOSTAS FISIOLÓGICAS DE MACROALGAS CONTINENTAIS À RADIAÇÃO ULTRAVIOLETA

ANNA ISABEL NASSAR BAUTISTA SARAIVA

Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de doutor em Biologia Vegetal

Junho - 2017

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Rio Claro, 23 de junho de 2017

À minha família amada: Pai, Mãe,
Irmão, Esposo e Filha!
Cheguei e sou o que sou por vocês!

"*Ouça, meu filho, a instrução de seu pai e não despreze o ensino de sua mãe. Eles serão um enfeite para a sua cabeça, um adorno para o seu pescoço" (Provérbios 1:8-9)*


Por trás dos sonhos,
há sacrifícios,
que as pessoas não vêem.

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RESUMO

Nos últimos séculos a história do ser humano no planeta Terra tem sido marcada por intensos impactos ambientais antropogênicos negativos, dentre os quais destaca-se a maior exposição da radiação ultravioleta (UV) na superfície terrestre devido à destruição da Camada de Ozônio ocasionada pela liberação de substâncias tais como os CFCs (clorofluorcarbonetos). A radiação UV tem sido considerada com um dos principais fatores que afetam a distribuição dos organismos fotossintetizantes em ambientes aquáticos, tendo efeitos biológicos diversos como danos no DNA e em moléculas proteicas, alteração dos pigmentos fotossintéticos, modificações nos parâmetros fotossintéticos e crescimento desses organismos. Torna-se evidente, portanto, que as mudanças da incidência de radiação UV na superfície terrestre podem levar a alterações ecofisiológicas nas comunidades algais. Nesse contexto, este trabalho teve como objetivo central analisar as respostas fisiológicas (parâmetros fotossintéticos, concentração de pigmentos fotossintéticos e de substâncias fotoprotetoras) à radiação ultravioleta (UVA e UVAB) em macroalgas continentais (ambientes lóticos e aerofíticos). A idéia geral foi avaliar o nível de resposta (tendência de aumento ou diminuição em maior ou menor grau) na performance em função da exposição à radiação ultravioleta. Duas hipóteses gerais foram formuladas: 1) as respostas das macroalgas consistirão essencialmente de tendência de diminuição de sua performance fisiológica (parâmetros fotossintéticos e concentração de pigmentos) e aumento de substâncias fotoprotetoras sob exposição à radiação UV; 2) o nível de resposta será diferente entre os grupos de algas, sendo que para as algas verdes (tipicamente algas de sol) espera-se que sejam mais tolerantes à radiação UV, enquanto que as rodófitas (tipicamente algas de sombra) tenderão a ser mais sensíveis e as cianobactérias deverão apresentar respostas intermediárias. Hipóteses mais específicas foram formuladas para cada parte do trabalho (capítulos). Experimentos foram realizados em três condições: PAR apenas (400-700nm); PAR + UVA (320-700nm), UVA; PAR + UVA + UVB (280-700nm), UVAB. A tese foi dividida em três capítulos com seus respectivos objetivos e hipóteses: 1) “Respostas fotossintéticas dos estágios gametofítico e esporofítico da macroalga vermelha continental *Kumanoa ambigua* (Rhodophyta, Batrachospermales) sob radiação UV”. O objetivo foi analisar o desempenho fotossintético dos estágios (gametófito e esporófito, 'Chantransia') de *K. ambigua* em cultura sob radiação UV. Hipotetizou-se que os estágios do histórico de vida de *K. ambigua* exibiriam respostas fotossintéticas diferentes à exposição à UV. 2) “Efeitos da radiação UV na fotossíntese e fotoproteção em duas espécies de Cyanobacteria”. O objetivo foi analisar o desempenho fotossintético (parâmetros derivados da fluorescência da clorofila), as concentrações de pigmentos (clorofila *a* e carotenóides) e a presença de compostos absorventes de UV (aminoácidos tipo-micosporinas - MAAs e scitoneminas) em duas cianobactérias aerofíticas, *Leptolyngbya* cf. *henningsii* e *Scytonema* sp. em resposta à exposição à radiação UV em condições de cultura. Hipotetizou-se que ambas espécies teriam desempenho fotossintético semelhantes em resposta à exposição de radiação UV, mas elas difeririam em termos de concentrações de pigmento e formação de compostos absorvedores de UV. 3) Efeitos da radiação UV na fotossíntese de três espécies de macroalgas lóticas tropicais. O objetivo foi analisar o desempenho fotossintético (parâmetros derivados da fluorescência da clorofila), as concentrações de clorofila *a* e a presença de compostos absorventes de UV (MAAs) em *Cladophora glomerata* (Chlorophyta), *Spirogyra* sp. (Streptophyta) e *Sirodotia delicatula* (Rhodophyta) coletadas em condições naturais em resposta à exposição de radiação UV em condições laboratoriais. Hipotetizou-se que *Spirogyra sp*. and *C. glomerata*, típicas algas adaptadas ao sol, apresentariam performances fotossintéticas semelhantes, com uma leve resposta à exposição à radiação UV e que *S. delicatula*, caracterizada por ser uma alga adaptada à sombra, exibiria uma resposta distinta

com uma maior sensibilidade à exposição à UV. Os principais resultados e conclusões encontrados foram: i) este é o primeiro trabalho que reportou não somente os efeitos da radiação UV sobre o desempenho fotossintético de macroalgas tropicais, bem como descreveu a presença de MAAs em duas espécies de algas vermelhas de água doce; ii) O gametófito de *K. ambigua* respondeu com um aumento da sua performance fotossintética, além da presença de shinorina (MAA) sob UVA, sugerindo que é menos sensível à radiação UV, particularmente UVA, em comparação com o esporófito em condições de cultura, além de indicar que a radiação UVA, quando moderada, tem efeitos benéficos em alguns organismos aquáticos, tais como o gametófito de *K. ambigua*; iii) Espécimes coletados em condições naturais de *S. delicatula* apresentaram dois tipos de MAAs (shinorina e palatina) e foram praticamente insensíveis aos tratamentos com radiação UV, sugerindo que não somente a presença de MAAs é um importante escudo de proteção contra radiação UV, mas também demonstra que apesar de ser uma rodófita tipicamente adaptada à sombra, pode tolerar altas irradiâncias, incluindo-se a radiação UV; iv) *C. glomerata* apresentou uma diminuição mais acentuada da fotossíntese em UVA em relação a UVAB, apesar de menor teor de clorofila *a* ter sido observado em UVAB; v) *Spirogyra* sp. apresentou uma diminuição da performance fotossintética em PAR, sugerindo que a radiação UVAB deve ser importante na indução de fotoreparo e/ou uma fonte energia para fotossíntese; vi) Apesar de serem algas verdes adaptadas ao sol, *C. glomerata* e *Spirogyra* sp., apresentaram respostas distintas no desempenho fotossintético e concentrações de clorofila *a* sob exposição da radiação UV, indicando que elas utilizam estratégias diferentes para lidar com a radiação UV; vii) A ausência de MAAs e de scitoneminas e a pouca variação do desempenho fotossintético indicam que *L.* cf. *henningsii* foi insensível à exposição a UV nas condições testadas, e que o aumento do conteúdo de carotenóides sob UVA e UVAB sugerem que estes pigmentos atuaram como escudo eficaz para a radiação UV; viii) *Scytonema* sp. apresentou um aumento da atividade fotossintética em UVA, sinalizando que também é capaz de usar UVA como fonte de energia para a fotossíntese, e/ou em mecanismos de reparo relacionados ao DNA. Em contrapartida, observou-se uma diminuição do desempenho fotossintético em UVAB, acompanhada de maiores concentrações de MAAs, sugerindo que estes pigmentos não foram suficientemente eficazes para evitar os efeitos negativos da radiação UV. Em síntese, as duas hipóteses iniciais foram refutadas: a primeira, pois nem sempre as algas responderam com tendência à diminuição de sua performance fotossintética ou de produção de substâncias fotoprotetoras à radiação ultravioleta. A segunda, pois as duas espécies de algas verdes, *C. glomerata* e *Spirogyra* sp., típicas algas adaptadas ao sol, não mostraram ser mais tolerantes à radiação UV em relação às algas vermelhas, consideradas adaptadas à sombra. Além disso, as respostas foram espécie-específicas, como exemplificado pelas duas espécies de cianobactérias (*Leptolyngbya* cf. *henningsii* e *Scytonema* sp.) e entre os dois estágios de vida de *K. ambigua*. Estes resultados são muito relevantes por representarem a primeira abordagem para compreensão da adaptação e aclimatação fisiológica de macroalgas tropicais às mudanças globais do clima, notadamente radiação UV.

Palavras-chave: Chlorophyta; Cyanobacteria; fluorescência da clorofila; fotossíntese; macroalgas tropicais; micosporinas; radiação UV; Rhodophyta; substâncias fotoprotetoras; UVA; UVB.

‘

ABSTRACT

The history of human on planet Earth has been marked by intense negative anthropogenic environmental impacts, which enhanced the exposure to ultraviolet (UV) radiation the Earth's surface due to depletion of the ozone layer caused by the release of substances, such as, CFCs (chlorofluorocarbons). UV radiation has been considered as one of the main factors that affect the distribution of photosynthetic organisms in aquatic environments, with varied biological effects, such as, damage to DNA and protein molecules, alteration of photosynthetic pigments, changes in photosynthetic parameters and growth of these organisms. Changes in the incidence of UV radiation on the Earth's surface can lead to ecophysiological alterations in algal communities. In this context, the objective of this investigation was to analyze the physiological responses (photosynthetic parameters, concentration of photosynthetic pigments and photoprotective substances) to ultraviolet radiation (UVA and UVAB) in inland macroalgae (lotic and aerophytic habitats). The general idea was to evaluate the level of response (trend of increase or decrease to greater or lesser degree) in photosynthetic performance to ultraviolet exposure. Two general hypotheses were formulated: 1) the responses of macroalgae will essentially consist of a trend to decrease in their physiological performance (photosynthetic parameters and pigment concentration) and increase photoprotective substances under UV exposure; 2) the response level will be different among algal groups, with green algae (typically sun-adapted algae) expected to be more tolerant to UV radiation, whereas red algae (typically shaded-adapted algae) to be more sensitive and the cyanobacteria having intermediate responses. Specific hypotheses were formulated for each part of the work (chapters). Experiments were performed under three conditions: PAR only (400-700nm); PAR + UVA (320-700nm), UVA; PAR + UVA + UVB (280-700nm), UVAB. The thesis was divided into three chapters with their respective objectives and hypotheses: 1) “UV-radiation effects on photosynthesis and photoprotection in gametophytic and sporophytic stages of the freshwater red alga *Kumanoa ambigua* (Rhodophyta, Batrachospermales)”. The aim was to analyze the photosynthetic performance of gametophytic and sporophytic (‘Chantransia’) stages of *Kumanoa ambigua* in culture under UV radiation. We hypothesized that the life history stages of *K. ambigua* would exhibit different photosynthetic responses to UV exposure; 2) “UV-radiation effects on photosynthesis and photoprotection of two species of Cyanobacteria”. The aim was to analyze the photosynthetic performance (parameters derived of chlorophyll fluorescence), pigment concentrations (chlorophyll *a* and carotenoids) and the presence of UV-absorbing compounds (mycosporine-like aminoacids - MAAs and scytonemin) in two terrestrial cyanobacteria, *Leptolyngbya* cf. *henningsii* and *Scytonema* sp. in response to UV radiation exposure under culture conditions. It was hypothesized that the responses to UV exposure would be similar in both species for photosynthetic performance but they would differ in terms of pigment concentrations and UV-absorbing compounds; 3) “UV-radiation effects on photosynthesis in three species of tropical lotic macroalgae”. The aim was to analyze the photosynthetic performance (parameters derived of chlorophyll fluorescence), chlorophyll *a* concentrations and the presence of UV-absorbing compounds (mycosporine-like aminoacids - MAAs) in three tropical lotic macroalgae, *Cladophora glomerata* (Chlorophyta), *Spirogyra* sp. (Streptophyta) and *Sirodotia delicatula* (Rhodophyta) collected in natural conditions in response to UV radiation exposure under laboratory conditions. We hypothesized that *Spirogyra sp*. and *C. glomerata*, typical sun-adapted algae, would present similar photosynthetic performance with slight responses to UV exposure, while *S. delicatula*, a typical shaded-adapted alga, would exhibit a distinct strategy with higher sensitivity to UV exposure. The main results and conclusions were: i) this is the first work that reported not only the effects of UV radiation on the photosynthetic performance of tropical macroalgae, but also described the presence of MAAs in two species of freshwater red algae; ii) the increase of photosynthetic performance and the presence of shinorine (MAA) under UVA, suggests that gametophyte of *K. ambigua* is less sensitive to UV radiation, particularly UV in comparison to the sporophyte under culture conditions. In addition, UVA radiation, in moderate levels, could have beneficial effects in some aquatic organisms, such as the gametophyte of *K. ambigua*; iii) specimens collected under natural conditions of *S. delicatula* had two types of MAAs (shinorine and palythine)

and were practically insensitive to treatments with UV radiation. These results suggest that not only the presence of MAAs is an important protection shield against UV radiation, but also demonstrates that some Rhodophyta, typically shaded-adapted algae, can tolerate high irradiances, including UV radiation; iv) *C. glomerata* presented a more pronounced decrease of photosynthesis under UVA in relation to UVAB, although a lower chlorophyll *a* content has been observed in UVAB; v) *Spirogyra* sp. had a decrease in photosynthetic performance in PAR, suggesting that UVAB radiation should be important in the induction of photorepair and/or as energy source to photosynthesis; vi) *C. glomerata* and *Spirogyra* sp., although considered typical sun-adapted algae, presented distinct responses on photosynthetic performance and chlorophyll concentrations under UV exposure, indicating that they use different strategies to deal with UV radiation; vii) the lack of MAAs and scitonemins and the weak responses of photosynthetic performances indicate that *L.* cf. *henningsii* was insensitive to UV exposure under the conditions tested. In addition, the increased carotenoid content under UVA and UVAB suggests that these pigments act as effective shield for UV radiation; viii) *Scytonema* sp. showed an increase in photosynthetic activity under UVA, signaling that it is also capable to use UVA as a source of energy for photosynthesis, and/or in repair mechanisms of DNA. In contrast, a decrease in the photosynthetic performance in UVAB was observed, although associated to higher concentrations of MAAs, suggested that these pigments were not effective enough to avoid the negative effects of UV radiation. In summary, the two initial hypotheses were rejected: the first, because the algal species not always responded with a trend to decrease of their physiological performances or to produce photoprotective substances under UV radiation. The second, because the two green algae, *C. glomerata* and *Spirogyra* sp., typical sun-adapted, were not shown to be more tolerant to UV radiation in comparison to the red algae, considered as shade-adapted. In addition, the responses were species-specific as clearly demonstrated by the two cyanobacterial species (*Leptolyngbya* cf. *henningsii* e *Scytonema* sp.) and between the two stages of *K. ambigua*. These results are relevant to be a first approach to a understanding of the adaptation and physiological acclimation of tropical macroalgae to global climate changes, notably UV radiation.

Keywords: chlorophyll fluorescence; Chlorophyta; Cyanobacteria; mycosporine-like amino acids; photosynthesis; Rodophyta; screening substances; tropical macroalgae; UVA; UVB; UV radiation.

INTRODUÇÃO GERAL

A destruição da camada de ozônio, causada pela liberação de poluentes atmosféricos antropogênicos, aumentou a radiação solar ultravioleta (UV), particularmente UVB, na superfície da Terra, incluindo os sistemas aquáticos (HÄDER *et al.* 2007; MCKENZIE *et al.* 2011). Apesar do sucesso do Protocolo de Montreal, há incertezas sobre os níveis futuros de radiação UVB até o final do século XXI. Hegglin & Shepherd (2009) previram, usando modelos físicos, que os índices de UV poderiam aumentar cerca de 4% nos trópicos. Watanabe *et al.* (2011) relataram aumento de UVB apesar da recuperação da camada de ozônio em diferentes latitudes. Sabe-se atualmente que a quantidade de radiação UV é influenciada pelas mudanças climáticas globais, tornando a diminuição da radiação UV na superfície da Terra mais duvidosa e enfatizando a necessidade de estudar seus efeitos nos sistemas biológicos (RASTOGI *et al.*, 2014)

Segundo a *Commission Internationale de L'Eclairage* (ou "Comissão Internacional de Iluminação"), as subdivisões da radiação UV foram definidas conforme as propriedades de transmissão de três filtros de cristal. Assim, a radiação UVC compreende os comprimentos de onda entre 100 a 280 nm (cristal Pirex). Essa radiação é completamente filtrada ou absorvida por átomos de O_2 e O_3 na estratosfera. A radiação UVB inclui comprimentos de onda entre 280 e 315 nm (cristais de Bário, Sílex e Pirex) é parcialmente filtrada pela camada de ozônio. Por fim, a radiação UVA, constituída por ondas de comprimento entre 315 e 400 nm (cristal de Bário e Sílex), atravessa a atmosfera e atinge a superfície terrestre. Entretanto, de acordo com a efetividade biológica dos comprimentos de onda da radiação UV, pesquisadores em fotobiologia separam a radiação UV da seguinte maneira (BARUFI, 2010): UVC, entre 100 e 280 nm; UVB, entre 280 e 320 nm; e UVA,

entre 320 e 400nm. A radiação UV corresponde a cerca de 7% da radiação solar total que atinge a superfície terrestre.

A radiação UV tem sido considerada com um dos principais fatores que afetam a distribuição dos organismos fotossintetizantes em ambientes aquáticos (SINHA & HÄDER, 2002; BJÖRN, 2007), tendo efeitos biológicos diversos, os quais são predominantemente negativos. De acordo com Häder *et al.* (2007), as radiações UVA e UVB diminuem a taxa de crescimento, o rendimento quântico da fotossíntese e causam acúmulo de danos no DNA em macroalgas marinhas. Por outro lado, há trabalhos esparsos sinalizando efeitos positivos da radiação UVA e/ou UVB nesses mesmos organismos. A radiação UVA, em níveis moderados, mostrou ser fonte de energia para a fotossíntese, ou estar relacionada em mecanismos de reparo ao DNA (HÄDER *et al.*, 2007; ROLEDA *et al.*, 2012), enquanto a radiação UVB pode ser usada como um sinal para induzir processos fotoprotetores e/ou de fotoreparo (XU & GAO, 2010). E mais, mesmo espécies estreitamente relacionadas do mesmo gênero podem ter sensibilidade UV significativamente diferente (BISCHOF *et al.*, 2002).

A fotossíntese é uma das principais funções metabólicas das algas e é responsável pela transferência de energia para sua manutenção e crescimento (SABATER *et al.*, 2016) e, entre vários processos fisiológicos, é potencialmente o alvo principal da radiação UV, não só em plantas superiores, mas também em algas (SCHREIBER *et al.*, 1994). Um bioindicador eficaz para avaliar o estado fisiológico de algas sob radiação UV é fluorescência “in vivo” de clorofila *a*. Nesse sentido, o rendimento quântico máximo (F_v/F_m) e a taxa de transporte de elétrons (ETR) têm sido utilizados como indicadores de estresse fisiológico em algas (FIGUEROA *et al.*, 1997, 2003).

Os organismos se diferenciam na maneira de lidar com a radiação UV, tanto na proteção contra a mesma, quanto nos mecanismos para reparar os danos que venham a ocorrer (BJÖRN, 2007). Segundo Sinha & Häder (2002), a influência mais visível da radiação UV em macroalgas marinhas é o dano no DNA (PAKKER & BREEMAN, 1997), na composição de pigmentos e no aparato fotossintético (AGUILERA *et al.*, 1999). No entanto, outras estratégias que os organismos fotossintetizantes podem apresentar a fim de lidar com a exposição à radiação UV consistem na síntese de compostos filtradores de radiação UV, como os aminoácidos tipo micosporina (MAAs), observados, principalmente em Rhodophyta (SINHA & HÄDER, 2002; HÄDER *et al.*, 2015; NAVARRO *et al.*, 2016) e Cyanobacteria (EHLING-SCHULZ & SCHERER, 1999; CASTENHOLZ & GARCIA-PICHEL, 2012), as scitoneminas em Cyanobacteria (EHLING-SCHULZ & SCHERER, 1999; CASTENHOLZ & GARCIA-PICHEL, 2012) e os compostos fenólicos em Chlorophyta (FIGUEROA *et al.*, 2009).

Em comparação com macroalgas marinhas, a informação sobre os mecanismos de fotoproteção contra radiação UV em macroalgas de água doce são escassos (ROLEDA *et al.*, 2010). A maioria dos estudos sobre a relação entre radiação e algas continentais bentônicas inclui análise de irradiância, e foca em grupos específicos ou espécies individuais de macroalgas continentais, tais como as algas vermelhas (NECCHI & ZUCCHI, 2001; NECCHI, 2005; BAUTISTA & NECCHI, 2007) e algas verdes (GRAHAM *et al.*, 1995; ENSMINGER *et al.*, 2000, 2001; BAUTISTA & NECCHI, 2008). Necchi (2004) é um dos raros trabalhos que trata de macroalgas continentais em geral, que concluiu que Rhodophyta são algas adaptadas à sombra, contrastando com a maioria das espécies de Chlorophyta testadas, que mostraram características de algas de sol.

Cyanophyta e Xantophyta foram considerados grupos intermediários em relação às adaptações à luz.

Para Roleda *et al.* (2010), informações sobre os mecanismos de proteção à radiação UV em macroalgas continentais são raras, quando comparadas à macroalgas marinhas. Para rodófitas marinhas, vários estudos investigaram a resposta desses organismos à radiação UV em relação aos parâmetros fotossintéticos e compostos absorventes de UV (FIGUEROA *et al.*, 1997, ROLEDA *et al.*, 2004; SIMIONI *et al.*, 2014), enquanto há apenas um estudo que trata dessas respostas para algas vermelhas de água doce (AIGNER *et al.*, 2017). No mesmo sentido, vários estudos de macroalgas verdes marinhas têm reportado resultados sobre performance fotossintética e radiação UV (ALTAMIRANO *et al.*, 2000; ROLEDA *et al.*, 2010; FIGUEROA *et al.*, 2009), enquanto que, segundo nosso conhecimento, não há trabalhos publicados que tratem desse assunto em macroalgas verdes continentais e mais especificamente de regiões tropicais. Estudos sobre respostas à radiação UV em cianobactérias focam na síntese de substâncias fotoprotetoras de UV (EHLING-SCHULZ & SCHERER, 1999; GAO & GARCIA-PICHEL, 2011; RASTOGI & INCHAROENSAKDI, 2014). Apenas algumas investigações relataram os efeitos da radiação UV sobre o desempenho fotossintético de cianobactérias usando a fluorescência "in vivo" de clorofila *a* (GEORGE *et al.*, 2001, GAO *et al.*, 2007, LESSER, 2008).

Dessa forma, estudos que avaliem os efeitos da radiação UV em ambientes continentais e tropicais são relevantes, pois podem indicar distintas estratégias de proteção e adaptação frente às alterações ambientais. Dada a importância dos problemas ambientais atuais, como a destruição da camada de ozônio e as incertezas frente aos níveis de radiação UVB para as próximas décadas, o presente trabalho visou comparar as respostas fisiológicas de macroalgas continentais e aerofíticas de três grupos taxonômicos: vermelhas

(Rhodophyta), verdes (Chlorophyta/Streptophyta) e azuis (Cyanobacteria) à exposição de radiação UV (UVA e UVAB).

Este trabalho teve como objetivo central analisar as respostas fisiológicas (parâmetros fotossintéticos, concentração de pigmentos e de substâncias fotoprotetoras) à radiação ultravioleta (UVA e UVAB) em macroalgas continentais de ambientes lóticos e aerofíticos. A ideia foi avaliar o nível de resposta (tendência de aumento ou diminuição em maior ou menor grau) na performance em função da exposição à radiação ultravioleta.

Considerando-se a grande carência de estudos ecofisiológicos com espécies de macroalgas continentais relacionados à exposição à radiação UV em ecossistemas lóticos tropicais, e no Brasil em particular, este estudo destaca-se pelos seguintes aspectos relevantes: gerar informações para o conhecimento das respostas de algumas espécies ecologicamente importantes frente às alterações climáticas, particularmente o aumento da exposição à radiação ultravioleta na superfície terrestre. Estas informações poderão, em última análise, servir de base para melhor entendimento das estratégias de aclimatação dessas espécies de macroalgas continentais às mudanças ambientais.

Duas hipóteses robustas foram testadas neste projeto: 1) Espera-se que as respostas das macroalgas consistirão essencialmente de tendência de diminuição de sua performance fisiológica (parâmetros fotossintéticos e concentração de pigmentos) e aumento de substâncias fotoprotetoras sob exposição à radiação ultravioleta; 2) Espera-se encontrar distintas estratégias de aclimatação à radiação UV entre espécies dos diferentes grupos taxonômicos tratados, ou seja, o nível de resposta deverá ser diferente entre os grupos de algas, sendo que as algas verdes (tipicamente algas de sol) deverão ser, em regra, mais tolerantes à radiação UV, enquanto que as rodófitas (tipicamente algas de sombra) tenderão

a ser mais sensíveis à essas duas variáveis e as cianobactérias apresentar respostas intermediárias. Hipóteses mais específicas foram apresentadas em cada capítulo.

A presente tese foi dividida em três capítulos, a saber:

1. UV-radiation effects on photosynthesis and photoprotection in gametophytic and sporophytic stages of the freshwater red alga *Kumanoa ambigua* (Rhodophyta, Batrachospermales);
2. UV-radiation effects on photosynthesis and photoprotection of two species of Cyanobacteria;
3. UV-radiation effects on photosynthesis in three species of tropical lotic macroalgae.

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CAPÍTULO 1

UV-radiation effects on photosynthesis and photoprotection in gametophytic and sporophytic stages of the freshwater red alga *Kumanoa ambigua* (Rhodophyta, Batrachospermales)

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Running title: UV effects on photosynthesis of *Kumanoa*

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SUMMARY

The aim of this investigation was to analyze the photosynthetic performance of gametophytic and sporophytic ('Chantransia') stages of *Kumanoa ambigua* in culture under UV radiation. We hypothesized that both life history stages of *K. ambigua* would exhibit different photosynthetic responses to UVR exposure. Experiments were performed under three conditions: (1) PAR only (400-700nm), control, P treatment; (2) PAR + UVA (320-700nm), PA treatment; (3) PAR + UVA + UVB (280-700nm), PAB treatment. The photosynthetic parameters were measured as *in vivo* chlorophyll a fluorescence. Differences were found between life stages, with gametophyte presenting higher values of *NPQ*, maximal (F_v/F_m) and effective ($\Delta F/F_{m'}$) quantum yield under UVA and PAR in comparison to sporophyte. One type of mycosporine-like amino acid (MAA) – shinorine - was detected in the gametophyte in all treatments, but not in the 'Chantransia' stage. The increased photosynthetic performance for some parameters and the presence of MAA in gametophyte suggest that it is less sensitive to UV radiation, particularly UVA, in comparison to sporophyte under culture conditions. This approach is relevant for a better understanding of the adaptation and physiological acclimation of freshwater Rhodophyta to varying light climates in terms of global changes.

Keywords chlorophyll fluorescence; mycosporine-like amino acids; UVA; UVB; lotic macroalgae

INTRODUCTION

Effects of ultraviolet (UV) radiation on algal ecophysiological responses become an important issue, ever since the first reports of man-made changes in the stratospheric ozone layer (Simioni *et al.* 2014). There is uncertainty about future levels of UVB radiation (280-315nm) by the end of the 21st century. Hegglin and Shepherd (2009), using physical models, predicted that UV indices may increase by about 4% in the tropics. Bais *et al.* (2011) estimated that the UV changes have been very small so far in the tropics and UV-Ery (erythermal solar irradiance) will continue to be roughly at the 1980 levels or even higher by a factor of 2–3 %.

UV radiation has been considered as one of the main factors that affect the distribution of photosynthetic organisms in aquatic environments (Sinha & Häder 2002). Häder *et al.* (2007) stated that exposure to solar UV radiation can reduce productivity, affect reproduction and development, and increase the mutation rate in organisms, including macroalgae.

Several studies (Bischof *et al.* 2006; Hanelt *et al.* 2006; Häder *et al.* 2007; Barufi *et al.* 2011) have shown that UVB effects on photosynthetic organisms are negative and highly variable, involving processes from molecular to ecophysiological scales. UVA radiation (315-400nm), in spite of being deleterious, was also shown to have some positive effects, including source of energy for photosynthesis, or in DNA-related repair mechanisms (Häder *et al.* 2007).

An effective bioindicator to evaluate the physiological status of algae under UV radiation is *in vivo* chlorophyll *a* fluorescence. In this sense, maximal quantum yield and

electron transport rate have been used as indicators of physiological stress in algae (Figueroa *et al.* 1997, 2003). In addition, dynamic photoinhibition as a mechanism of acclimation to increased photosynthetic irradiance can be estimated by chlorophyll fluorescence (Figueroa *et al.* 2003; Necchi 2005; Bischof *et al.* 2006; Bautista & Necchi 2007).

According to Häder *et al.* (2007), both UVA and UVB radiations decrease growth rate, quantum yield of photosynthesis and cause accumulation of DNA damage. However, even closely related species of the same genus may have significantly different UV sensitivity (Bischof *et al.* 2002). The elimination of total UV or UVB alone reduces the severity of photoinhibition and shortens recovery time in many species (Hanelt *et al.* 2006).

Rautenberg *et al.* (2009) reported that photoacclimation and inhibition of photosynthesis have been identified as alternative ways to avoid impairments induced by UV radiation in macroalgae. Macroalgae have different photoprotection strategies. According to Sinha and Häder (2002), the main influences of UV radiation on macroalgae are the DNA damage (Pakker *et al.* 2000), the changing of pigments composition and the functioning of photosynthetic apparatus (Häder *et al.* 2015). An important mechanism to reduce the damaging impact of UVR on these organisms is the synthesis and accumulation of UV-absorbing compounds (Sinha & Häder 2002), such as mycosporine-like amino acids (MAAs) and high energy dissipation as high non photochemical photoquenching related to xanthophyll cycle (Demmig–Adams & Adams 2006). Among the various groups of macroalgae, Rhodophyta has the highest percentage of species that synthesize MAAs (Barufi *et al.* 2011).

Freshwater red algae are important primary producers in running waters (Sheath & Hambrook 1990) and have a typical response to irradiance, most being considered as shade-

adapted algae (Necchi 2005; Bautista & Necchi 2007). However, they are typically found in shallow parts of streams and rivers and, thus, are exposed to high solar radiation during some periods (Sheath & Hambrook 1990; Necchi 2005; Bautista & Necchi 2007). Thus, they are expected to have mechanisms to cope with such high irradiances, as shown in some previous studies (Necchi 2005; Bautista & Necchi 2007; Aigner *et al.* 2017). Members of the freshwater red algal order Batrachospermales have the exclusive *Lemanea* type of life history (Sheath & Hambrook 1990), with two alternating stages: a large and more complex gelatinous gametophyte and a small and simple filamentous sporophyte (called the 'Chantransia' stage).

Although various studies have investigated the response of marine macroalgae to UV radiation in relation to photosynthetic apparatus and UV-absorbing compounds (Hanelt *et al.* 2006; Barufi *et al.* 2011), to our knowledge, there is only one study dealing with the responses to UV radiation for freshwater red algae (Aigner *et al.* 2017). They reported that exposure to either UV-A or UV-AB led to a strong transient drop in effective quantum yield in *Batrachospermum turfosum* Bory, but the alga was capable of recovering it after UVR treatment. In addition, studies comparing the effects of UV radiation on different life history stages in Rhodophyta are relatively scarce (Roleda *et al.* 2004; Araújo *et al.* 2014; Navarro *et al.* 2010, 2016). Necchi and Alves (2005) is the only study that evaluated the photosynthetic performance of the two life history stages in response to temperature and irradiance in populations of the freshwater red alga *Sirodotia delicatula* (cited as *Batrachospermum delicatulum*). A comparative analysis of field and culture populations revealed no consistent pattern in a same population or stage, suggesting high capacity to adjust their photosynthetic apparatus to variations in PAR and temperature. However, little is known on the responses of freshwater red algae to UV radiation.

The main objective of this investigation was the evaluation of the photosynthetic performance of gametophytic and 'Chantransia' sporophytic stages of *Kumanoa ambigua* under UV radiation. The specific aims were to compare the photosynthetic responses, and production of MAAs, between the life stages of *K. ambigua* after UV exposure. Based on previous findings: Necchi and Alves (2005) that reported distinct photosynthetic characteristics between gametophytes and 'Chantransia' stages under field and culture conditions. We hypothesized that both life history stages of *K. ambigua* would exhibit different photosynthetic responses to UVR exposure.

MATERIAL AND METHODS

Biological material

Kumanoa ambigua was chosen because it is one of the few species available in culture with both life history stages. Gametophytic stage of *K. ambigua* (Montagne) Entwisle, M.L. Vis, W.B. Chiasson, Necchi & A.R. Sherwood was collected in Brazil, Santa Catarina state, Salto (27º34'S, 48º50'W) on March 12, 1996. It was isolated in culture under irradiance level of 50 µmol photons m^{-2} s^{-1}, 20.0 $\pm$ 1.0ºC and photoperiod of 12 hours and kept in Bold's Basal Medium (Watanabe 2005) in Revco RI 12-555 incubators (Asheville, NC, USA). 'Chantransia' stage was obtained from the fertile gametophyte after six months in culture from germination of carpospores. This assured that it belongs to the respective gametophyte. Identification was made based on current morphological characters and molecular analyses using two molecular markers (Necchi *et al.* 2010).

Experimental design

Three treatments (with five replicates) were performed (1) PAR only (400-700nm), control, P treatment; (2) PAR + UVA (320-700nm), PA treatment; (3) PAR + UVA + UVB (280-700nm), PAB treatment. All treatments were provided by two cool white fluorescent lamps Osram L 15W "cool daylight" (Osram, Hildesheim, Germany), covered with neutral black mesh and by one Ultra-Vitalux 300W lamp, 230V E27/ES (Osram, Hildesheim, Germany). Lamps were positioned in front of the vessels with the alga. Radiation treatments were obtained by placing cut off filters in front of the samples (Figueroa *et al.* 2003; Roleda *et al.* 2012). PAR treatment (1) used filter Lee n. 226, blocking UVA (320-400nm) + UVB radiation (280-320nm). PA treatment (2) used filter Lee n.130, blocking UVB radiation. PAB treatment (3) did not use a filter. PAR and UVR were measured inside the vessel.

Average irradiance level in each treatment was 179±18 µmol photons m^{-2} s^{-1} in order to adjust the irradiance described in the literature for freshwater macroalgae (Necchi, 2004). Measurements in µmol photons $m^{-2}s^{-1}$ were made with a LI-COR LI-189 quantameter (Li-Cor, Lincoln, NE, USA) coupled to a spherical quantum sensor LI-193 SA. Absolute irradiance ($W.m^{-2}$) was measured by the spectroradiometer UV-Visible USB2000 + RAD (Ocean Optics, Dunedin, USA). The UVB/UVA ratio was 0.25 in laboratory, whereas in the field it was 0.22 (Table S1).

Incubations were performed at a temperature of 22.0±2.0ºC in refrigerated incubator Marconi, MA mod 830/A, with orbital agitation of 100±5 rpm. To ensure uniform irradiation of all samples, replicates positions were changed every two days during the experiment. Thalli were incubated in UV-transparent 300mL metacrylate plastic vessels. Incubations were done in Bold's Basal Medium and changed every three days in order to avoid nutrients and CO_2 limitation and water evaporation. The initial fresh weights

for the gametophytic and 'Chantransia' stages were 140.0±5.0 mg and 50.0±5.0 mg wet weight, respectively, for each replicate. Before experiments, all samples were acclimated for 24 hours under the control conditions. The incubation time in each treatment was ten days with a photoperiod of 12h.

Photosynthetic performance as in vivo chlorophyll a fluorescence

The photosynthetic activity, measured as *in vivo* chlorophyll a fluorescence, of both gametophyte and 'Chantransia' stages was evaluated before (PAR only) and after UV exposures (UVA and UVAB) using a Diving-PAM underwater fluorometer (Walz, Effeltrich, Germany). Algal thalli were placed directly on the tip of the fluorometer optic fiber using the supplied magnet sample holder. The biomass was high enough to cover the area of the fluorometer optic fibertip.

Photosynthesis-irradiance (P-E) curves, as rapid light curves (White & Critchley 1999), consisted of the fluorescence responses to eight increasing irradiances from 0 to 792 μmol m^{-2} s^{-1}, using the "light curve" option of the Diving-PAM. The exposure time at each irradiance was 15 s, each separated by a saturating flash (0.8 s, ~6,000 μmol m^{-2} s^{-1}). The calculations and terminology followed Schreiber *et al.* (1994) and van Kooten and Snel (1990), respectively. The following parameters were determined from each sample: 1) Effective quantum yield of PSII, $\Delta F/F_m' = (F_m' - F_t)/F_m'$, being F_m' maximal flurescence of light adapted thalli and F_t the transient fluorescence of light-acclimated thalli; 2) Non-photochemical quenching (*NPQ*) was determined as $NPQ = (F_m - F_m')/F_m'$. 3) Electron transport rate (ETR), calculated as $ETR = \Delta F/F_m' \times E_{PAR}$ (μmol photons m^{-2} s^{-1})xAxF_{II} being E_{PAR} the incident irradiance of PAR in μmol m^{-2} s^{-1}. A absorption, or fraction of light absorbed by PSII in an optical cross section. A was estimated by each alga tested (0.59 to

gametophyte and 0.98 to 'Chantransia' stage), based on measurements made with and without the alga within a circle of 0.5 cm in diameter, similar to the area of the fluorometer optic fibertip (Necchi 2004; Bautista & Necchi 2007). F_{II} is the fraction of chlorophyll *a* associated to photosystem II. F_{II} in red macroalgae and cyanobacteria present a value of 0.15 (Figueroa *et al.* 1997; Johnsen & Sakshaug 2007). Based on the ETR versus irradiance curves measured for each treatment, maximum electron transport rate (ETR_{max}), photosynthetic efficiency (α_{ETR}), saturation parameter (E_k) and the slope of photoinhibition (β_{ETR}) were obtained by fitting these curves to the tangential fitting reported by Platt *et al.* (1980).

Dark/light induction-recovery (Kautsky) curves were performed on thalli dark-acclimated. For dark acclimation, apices of algal thalli were placed for 10 min directly on the tip of the fiberoptic by using the supplied dark leaf clip determining the basal fluorescence (F_o) and maximal fluorescence (F_m) after a saturating light pulse (0.8 s, ~6,000 μmol m^{-2} s^{-1}). First, a saturation pulse was applied for determination of maximal quantum yield of PSII, F_v/F_m, being $F_v=F_m-F_o$ (Schreiber *et al.* 1994). Then, a constant actinic irradiance (221 μmol photons m^{-2} s^{-1}, close to the incubation irradiance) was applied by using the halogen light source of the Diving PAM, separated by eight saturating light pulses at 15 s intervals, and initiated 30 s after the first saturation pulse. After recording of dark/light induction, six saturation pulses were applied at successive intervals (10, 30, 60 seconds and 2, 5 and 10 minutes) to assess the dark recovery of F_v/F_m, by comparing the initial and final values.

Mycosporine-like amino acids

After experimental periods, samples of 20.0±5.0 mg DW (dry weight procedure according to Necchi *et al.* 2010) were used for the extraction and identification of photoprotective substances, mycosporine-like amino acids (MAAs). They were extracted in 1 ml of 20% aqueous methanol (v/v) for 2 h at 45 ° C.

Aliquots of 600 µL were transferred to new tubes and the liquid contents of the tubes were removed by a vacuum microcentrifuge. To this solid residue, 600 µL of 100% chromatographic methanol was added to remove salts and the extract proteins, mixed by vortex, and centrifuged at 13000 g, 4 ° C for 10 min. A total of 100 µL of the supernatant of this extract was filtered and transferred to glass tubes in the Waters system (Barcelona, Spain) HPLC.

The MAAs were detected by using an isocratic run containing 2.5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in distilled water as the mobile phase. The flow rate was 0.5 mL min^{-1} and each run took 20 min; 30 µL of the each sample was injected into a Sphereclone C8 column (Phenomenex, Germany) with a pre-column attached (5-mm packing; 250×4 mm I.D.).

Mycosporine-like amino acids were detected with a Waters Photodiode Array Detector 996. For each second, absorption spectra were recorded between 290 and 400 nm. Finally, a chromatogram selected for absorbance at 330 nm was obtained, and the observed peaks were identified according to the spectrum and retention time, compared with a secondary standard of *Porphyra leucosticta*. MAAs were quantified according to Korbee-Peinado *et al.* (2004). Data were collected and analyzed with the Millennium 3.2 software.

Statistics

Values are represented as the mean ± standard deviation. Parametric statistical tests were applied considering that the variables followed a normal distribution according to graphical assessment of normality based on the regression curves. A multifactorial repeated-measures analysis of variance (ANOVA) was performed for photosynthetic parameters (α_{ETR}, ETR_{max}, E_k, β_{ETR}, *NPQ*, $\Delta F/F_m'$, F_v/F_m and recovery capacity of F_v/F_m) considering the interactions among treatments (P, PA and PAB), life stage (gametophyte and sporophyte) and time (pre and post-exposure). In these analyses, the time was the repeated factor. The effect size is represented as ‘‘E.S.” and the observed power is represented as ‘‘O.P. The multiple comparisons were performed by post-hoc Newman-Keuls test ($p<0.05$). Statistical tests were performed with the use of Statistica 12 software (Statsoft), whereas graphs and calculations from P-E curve parameters were made with Excel 2013 (Microsoft).

RESULTS

Photosynthetic efficiency (α_{ETR}) did not present effect in each of the isolated factors (life stage, treatment, time). However, two-way ANOVA showed interaction between the measures of time *vs* life stage [F(1,24)=16.93; p=0.0004; E.S.=0.41; O.P.=0.98] and among time *vs* life stage *vs* treatment [F(2,24)=4.06; p=0.03; E.S.=0.25; O.P.=0.67].

Similarly, maximum electron transport rate (ETR_{max}) had significant interactions between time *vs* life stage [F(1,24)=16.90; p=0.0004; E.S.=0.41; O.P.=0.98], life stage *vs* treatment [F(2,24)=9.27; p=0.001; E.S.=0.44; O.P.=0.96] and among time *vs* life stage *vs* treatment [F(2,24)=10.87; p=0.0004; E.S.=0.48; O.P.=0.98].

Non-photochemical quenching (*NPQ*) showed a significant effect on life stage [F(1,24)=47.89; p=0.000; E.S.=0.67; O.P.=0.99] and significant interactions between time *vs* life stage [F(1,24)=13.04; p=0.001; E.S.=0.35; O.P.=0.93] and time *vs* treatment [F(2,24)=4.24; p=0.03; E.S.=0.26; O.P.=0.69].

Effective quantum yield of PSII ($\Delta F/F_m'$) presented a significant effect on life stage [F(1,24)=31.22; p=0.000; E.S.=0.57; O.P.=0.99] and significant interactions in all situations: time *vs* life stage [F(1,24)=26.50; p=0.000; E.S.=0.52; O.P.=0.98]; time *vs* treatment [F(2,24)=4.84; p=0.017; E.S.=0.29; O.P.=0.75]; and time *vs* life stage *vs* treatment [F(2,24)=3.92; p=0.034; E.S.=0.25; O.P.=0.65].

Maximal quantum yield of PSII (F_v/F_m) had a significant effect of life stage [F(1,23)=17.47; p=0.0004; E.S.=0.43; O.P.=0.98] and treatment [F(2,23)=5.16; p=0.014; E.S.=0.31; O.P.=0.77], and significant interactions in all situations: life stage *vs* treatment [F(2,23)=7.54; p=0.003; E.S.=0.40; O.P.=0.91]; time *vs* life stage [F(1,23)=102.92; p=0.000; E.S.=0.82; O.P.=1.00]; time *vs* treatment [F(2,23)=7.95; p=0.002; E.S.=0.41; O.P.=0.93]; time *vs* life stage *vs* treatment [F(2,23)=15.95; p=0.000; E.S.=0.58; O.P.=0.99].

Recovery capacity of F_v/F_m presented a significant effect on treatment [F(2,22)=6.71; p=0.005; E.S.=0.38; O.P.=0.87], and significant interactions of life stage *vs* treatment [F(2,22)=6.40; p=0.006; E.S.=0.37; O.P.=0.86]; time *vs* life stage [F(1,22)=20.67; p=0.0002; E.S.=0.48; O.P.=0.99] and time *vs* life stage *vs* treatment [F(2,22)=13.47; p=0.0002; E.S.=0.55; O.P.=0.99].

Two-way ANOVA did not reveal significant effect or interaction for saturation parameter (E_k) and photoinhibition (β_{ETR}).

Photosynthetic response to UV exposure of each stage

Both pre-and post-UV exposure PE curves of *K. ambigua* gametophyte and sporophyte stages revealed typically shaded-adapted red algae response, as indicated by occurrence of photoinhibition (β_{ETR}) and low values for the saturation parameter (E_k), ETR_{max} and photosynthetic efficiency (α_{ETR}) (Fig. 1; Table 1).

Dark/Light induction-recovery curves revealed the same general pattern of variation in gametophytic and 'Chantransia' sporophytic stages in both pre and post UV exposure (Fig. 2). Quantum yield kinetics was characterized by a pronounced decrease and a slight increase and stabilization during the light exposure period (Fig. 2).

The gametophytic stage of *K. ambigua* showed higher values for α_{ETR} (p=0.014), ETR_{max} (p=0.001) and F_v/F_m (p=0.0005) after exposure to UVA. (Fig. 1B; Fig. 2B; Table 1). Higher values was observed for F_v/F_m (p=0.002) and recovery capacity of F_v/F_m (p=0.003) after UVAB exposure (Fig. 2C; Table 1).

The sporophytic stage of *K. ambigua* ('Chantransia' stage) showed lower values for ETR_{max} (p=0.044), $\Delta F/F_m'$ (p=0.0004), *NPQ* (p=0.016) and F_v/F_m (p=0.0001) and higher values of recovery capacity of F_v/F_m (p=0.0002) after exposure to UVA (Fig. 1E; Fig. 2E; Table 1). In contrast, no significant differences were observed in the photosynthetic performance after UVAB exposure, except for recovery capacity of F_v/F_m (p=0.014) (Fig. 1F; Fig. 2F; Table 1).

Photosynthetic responses to UV exposure between the stages

P-E curves comparing P, PA and PAB treatments showed negative slopes (photoinhibition β_{ETR}) and lower values for the saturation parameter (E_k), ETR_{max} and photosynthetic efficiency (α_{ETR}) in both stages, a typical shaded-adapted response (Fig. 3A-B).

Dark/Light induction-recovery curves revealed similar patterns in P, PA and PAB treatments. Quantum yield kinetics was characterized by a pronounced decrease and a slight increase and stabilization during the light exposure period, followed by a recovery period after light is turned off (Fig. 3C-D).

Gametophytic stage showed increased photosynthetic performance in PAR and UVA in comparison to sporophytic stage. Higher values were observed for $\Delta F/F_m'$ (p=0.014), *NPQ* (p=0.0005) and F_v/F_m (p=0.002) in PAR; and for $\Delta F/F_m'$ (p=0.0002), *NPQ* (p=0.0005), F_v/F_m (p=0.0001) in UVA, except recovery capacity percentage of F_v/F_m (p=0.013) (Fig. 3; Table 1). No significant differences were observed in the photosynthetic activity between gametophyte and sporophyte after UVAB exposure (Table 1).

Mycosporine-like amino acids

Shinorine was the only type of MAA identified in the gametophyte (1.37 ± 0.56 $\mu g.g^{-1}$DW), with maximum absorbance at 334 nm and no significant differences among PAR, UVA and UVAB. In contrast, no type of MAA was detected for the 'Chantransia' stage.

DISCUSSION

Photosynthetic performance of the freshwater red alga *K. ambigua* pre and post-exposure to UV radiation under culture conditions was different in the gametophyte and in the ‘Chantransia’ sporophytic stage. Gametophytic stage presented an increased of photosynthetic performance (α_{ETR}, ETR_{max}, F_v/F_m) post-UVA exposure. Higher values of F_v/F_m and recovery capacity of F_v/F_m were observed in UVAB radiation. A distinct pattern was observed for the ‘Chantransia’ stage, i.e, a decrease in the photosynthetic activity

(ETR_{max}, $\Delta F/F_m'$, *NPQ*, F_v/F_m and recovery capacity of F_v/F_m) after UVA-exposure, and no response post UVAB-exposure.

Several studies reported harmful UV radiation effects, especially UVB, with decreased photosynthetic performances in marine red algae (Figueroa *et al.* 1997; Roleda *et al.* 2004; Simioni *et al.* 2014). As a general pattern, evident negative effects (decreased $F_v/F_{m,}$ $\Delta F/F_m'$ and ETR) have been observed after exposure to UVA and UVAB radiation, most notably reported by Figueroa *et al.* (1997) and Simioni *et al.* (2014). Although most studies reported a negative impact on photosynthesis by UVB radiation, some investigations found beneficial effects of UVB by photoprotection of photosynthesis in algae (Xu & Gao 2010).

The exclusion of UVB produced a reduction of photosynthetic activity in tropical algae (Hanelt & Roleda 2009).Vass *et al.* (2013) reported beneficial effects of UVB on the D1 and the D2 proteins of PSII in cyanobacteria. According to Xu and Gao (2010), UVB irradiance might be used as a signal to induce photoprotective and/or photorepair processes. In addition, positive effects of moderate levels of UVA radiation on photosynthesis and growth have been documented that can also stimulate beneficial photorepair of UVB damage, so that no additional UVB effect on growth is observed (Roleda *et al.* 2012). Häder *et al.* (2007) concluded that UVA is not always deleterious but it has some positive effects, as it can be used as a source of energy for photosynthesis, or in DNA-related repair mechanisms.

The comparison between gametophyte and sporophyte ('Chantransia') stage of *K. ambigua* after UV-exposure revealed that haploid individuals had a better performance in UVA (ETR_{max}, $\Delta F/F_m'$, *NPQ*, F_v/F_m) and PAR ($\Delta F/F_m'$, *NPQ*, F_v/F_m) compared to diploid individuals. Studies on the UV effects on different life history phases of macroalgae are

relatively scarce (Roleda *et al.* 2004; Araújo *et al.* 2014; Navarro *et al*, 2016). The diplohaplontic alternation of generations (sporic life history) is widespread in the Florideophyceae, including members of the freshwater red algal order Batrachospermales (Graham *et al.* 2009). The predominance of diploidy has been attributed a role in promoting intrinsic genetic advantages, presumably due to the possession of a double genome, i.e., protection against expression of deleterious recessive mutations and somatic mutations, faster acquisition of new genes and better control of cell differentiation (Richerd *et al.* 1993)

Araújo *et al.* (2014) reported that growth rates, net photosynthesis, ETR_{max}, E_k, and pigment contents in tetrasporophytes were higher than in gametophytes in *Gracilaria caudata* under controlled conditions and UV-B exposure. On the other hand, Navarro *et al.* (2010) reported that *Iridaea cordata* gametophytes were less sensitive than tetrasporophytes after exposure to UV-B, while the the gametophyte of *B. turfosum* showed wide tolerance to irradiation, including UVR (Aigner *et al.* 2017). In this study, we found higher values of maximal and effective quantum yield in the gametophytic stage under UVA, suggesting a less sensitive response in the haploid stage in comparison to the diploid stage.

The synthesis and accumulation of photoprotective compounds, such as mycosporine-like amino acids (MAAs), is one of the strategies used by macroalgae under exposure to high levels of UV (Sinha & Häder 2002; Häder *et al.* 2015; Navarro *et al.* 2016). *Mazzaella laminarioides* presented higher synthesis of MAAs in gametophytes than tetrasporophytes at two days of culture mainly under PAB treatment, and showed higher growth rate than tetrasporophytes at twelve days under PAB, which can suggest that gametophytes would be more adapted to higher UVB irradiances, as long as it is in this

period (Navarro *et al.* 2016). The occurrence of MAA shinorine, although in low concentration, in all treatments of gametophyte of *K. ambigua* and its absence in sporophyte is a plausible evidence of better adaptation of gametophyte in relation to sporophyte. This is the first report of MAA in a member of the freshwater red algal order Batrachospermales, as it was not detected in *B. turfosum* by Aigner *et al.* (2017).

Freshwater red algae are typically shade-adapted plants (Necchi 2005; Bautista & Necchi 2007) and they are expected to have mechanisms to cope with high irradiances and UV radiation (e.g. thermal dissipation, dynamic photoinhibition). The occurrence of photoinhibition in all treatments for both stages indicates that *K. ambigua* is also typically a shade-adapted red alga, in spite of short exposure to high irradiances during some brief periods during the day or season.

High *NPQ* values after UVB exposure have been related to photoprotection in different seaweeds (Figueroa *et al.* 2014). Bautista and Necchi (2007) and Necchi (2005), indicated that thermal dissipation of energy attenuates the negative effect of increased levels of PAR to the photosynthetic apparatus in species of freshwater red algae. This mechanism was observed in the gametophyte that had higher values of non-photochemical quenching (*NPQ*) in UVA and PAR in comparison to sporophyte. This result suggests that gametophyte is apparently better adapted to UV radiation, mainly UVA. High values of NPQ indicate active photoprotective mechanisms, which are highly related to the xanthophyll cycle (Demmig-Adams & Adams 2006). Thermal dissipation measured as-non photochemical PSII fluorescence quenching (NPQ) is triggered by the transthylakoidal proton gradient (ΔpH) and zeaxanthin (ZEA) synthesis through the xanthophyll cycle (Gilmore *et al.* 1994). Although xanthophyll cycle has not been found in red algae, even they presented violaxanthin and anteraxanthin (Schubert *et al.* 2006), interconverson of

these carotenoids has been shown (Ursi *et al.* 2003). Schubert and García-Mendoza (2008) indicated that the degree of decrease and recovery of F_v/F_m and their respective kinetics were related to the carotenoid profile of the species. In species with zeaxanthin or antheraxanthin as the major carotenoid, F_v/F_m reduction and recovery was principally associated with slowly activated and relaxed processes (Schubert & García-Mendoza 2008). To elucidate the role of carotenoids in the NPQ in *K. ambigua*, it is necessary to conduct studies on carotenoid interconversion.

Dark/light induction curves provide valuable information on the photosynthetic performance of dark-acclimated plants (Necchi 2005), allowing to estimate the recovery of photosynthesis as maximal quantum yield of fluorescence (F_v/F_m). Figueroa and Gómez (2001) pointed out that the recovery kinetics gives insights into the photo-adaptive strategies of macroalgae and their light-stress tolerance capacity. Some authors (Jiménez *et al.* 1998; Xu & Gao 2010; Aigner *et al.* 2017) reported higher recovery of F_v/F_m and $\Delta F/F_m'$ in several red algae after exposure to solar UVA or UVAB. Following this same pattern, we found that the gametophyte and 'Chantransia' stage of *K. ambigua* presented high recovery rates (higher than 85%) of F_v/F_m under UVA and UVAB radiation.

In synthesis, this is the first investigation that analyzed UV effects on photosynthetic performance, as well as described the presence of a MAA (shinorine) in a freshwater red algal species. Higher values of *NPQ*, maximal (F_v/F_m) and effective ($\Delta F/F_m'$) quantum yield and the presence of MAAs in gametophyte of *K. ambigua* under UVA partly confirm our hypothesis that effects of UV radiation would be different in both stages in terms of photosynthetic performance and the production of MAAs. In addition, our results suggest that the gametophyte is less sensitive to UV radiation, particularly UVA, in comparison to sporophyte under culture conditions. This finding is relevant for a better

understanding of the adaptation and physiological acclimation of freshwater red algae to varying light climate in terms of global changes.

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Table 1. Parameters derived from the photosynthesis-irradiance and dark/light induction-recovery curves comparing (1) pre and post UV-exposure for each life stage of *K. ambigua* and (2) after UV-exposure, by comparison between the gametophyte and sporophyte in the P, PA and PAB treatments. Statistical analysis based on two-way ANOVA (Newman-Keuls Post Hoc)

			Kumanoa ambigua				
UVR	Exposure		Gametophyte	Sporophyte		Gametophyte	Sporophyte
P	Pre	ETR_{max}	1.32 ± 0.10	1.09 ± 0.27	α_{ETR}	0.015 ± 0.003	0.015 ± 0.005
	Post	(µmol electrons m^{-2} s^{-1})	1.49 ± 0.22	1.16 ± 0.26		0.017 ± 0.002	0.014 ± 0.004
PA	Pre		0.66 ± 0.22*	1.58 ± 0.42*		0.009 ± 0.004*	0.017 ± 0.003
	Post		1.57 ± 0.22*#	0.96 ± 0.26*#		0.016 ± 0.002*	0.012 ± 0.003
PAB	Pre		0.70 ± 0.26	1.22 ± 0.37		0.010 ± 0.004	0.017 ± 0.005
	Post		1.12 ± 0.29	1.51 ± 0.27		0.010 ± 0.002	0.016 ± 0.002
P	Pre	E_k	85.85 ± 15.19	74.93 ± 14.69	β_{ETR}	30.08 ± 6.67	34.47 ± 6.97
	Post	(µmol photons m^{-2} $s^{-1)}$	88.45 ± 4.99	82.90 ± 8.05		28.36 ± 1.60	30.42 ± 3.10
PA	Pre		84.59 ± 23.74	91.11 ± 15.41		32.75 ± 6.76	28.01 ± 4.07
	Post		94.71 ± 7.10	80.67 ± 9.86		26.54 ± 2.12	31.38 ± 3.73
PAB	Pre		77.79 ± 41.65	73.62 ± 12.64*		38.95 ± 16.91	34.77 ± 5.81
	Post		77.14 ± 14.11	94.83 ± 6.79*		33.43 ± 6.86	26.49 ± 1.88
P	Pre	*NPQ*	1.27 ± 0.11	0.95 ± 0.19	$\Delta F/F_m'$	0.57 ± 0.03	0.53 ± 0.03
	Post		1.38 ± 0.04#	0.87 ± 0.25#		0.59 ± 0.01#	0.48 ± 0.07#
PA	Pre		1.12 ± 0.10	0.98 ± 0.13*		0.53 ± 0.04	0.53 ± 0.03*
	Post		1.31 ± 0.09#	0.71 ± 0.28*#		0.59 ± 0.01#	0.39 ± 0.09*#
PAB	Pre		1.06 ± 0.14	0.99 ± 0.08		0.51 ± 0.04	0.52 ± 0.06
	Post		1.37 ± 0.12	1.10 ± 0.22		0.60 ± 0.03	0.52 ± 0.06
P	Pre	F_v/F_m	0.53 ± 0.03	0.51 ± 0.06	% recovery	86% ± 4*	88% ± 2*

	Post	0.56 ± 0.01#	0.46 ± 0.05#	91% ± 2*	95% ± 2*
PA	Pre	0.47 ± 0.03*	0.53 ± 0.02*	93% ± 3	88% ± 2*
	Post	0.58 ± 0.01*#	0.34 ± 0.03*#	92% ± 2#	97% ± 2*#
PAB	Pre	0.49 ± 0.04*	0.53 ± 0.05	89% ± 3*	85% ± 2*
	Post	0.56 ± 0.02*	0.52 ± 0.05	93% ± 1*	89% ± 2*

* Significant differences between pre (PAR-only) and post exposure in each life stage ($p < 0.05$).
# Significant differences between gametophyte and sporophyte post UV-exposure ($p < 0.05$).

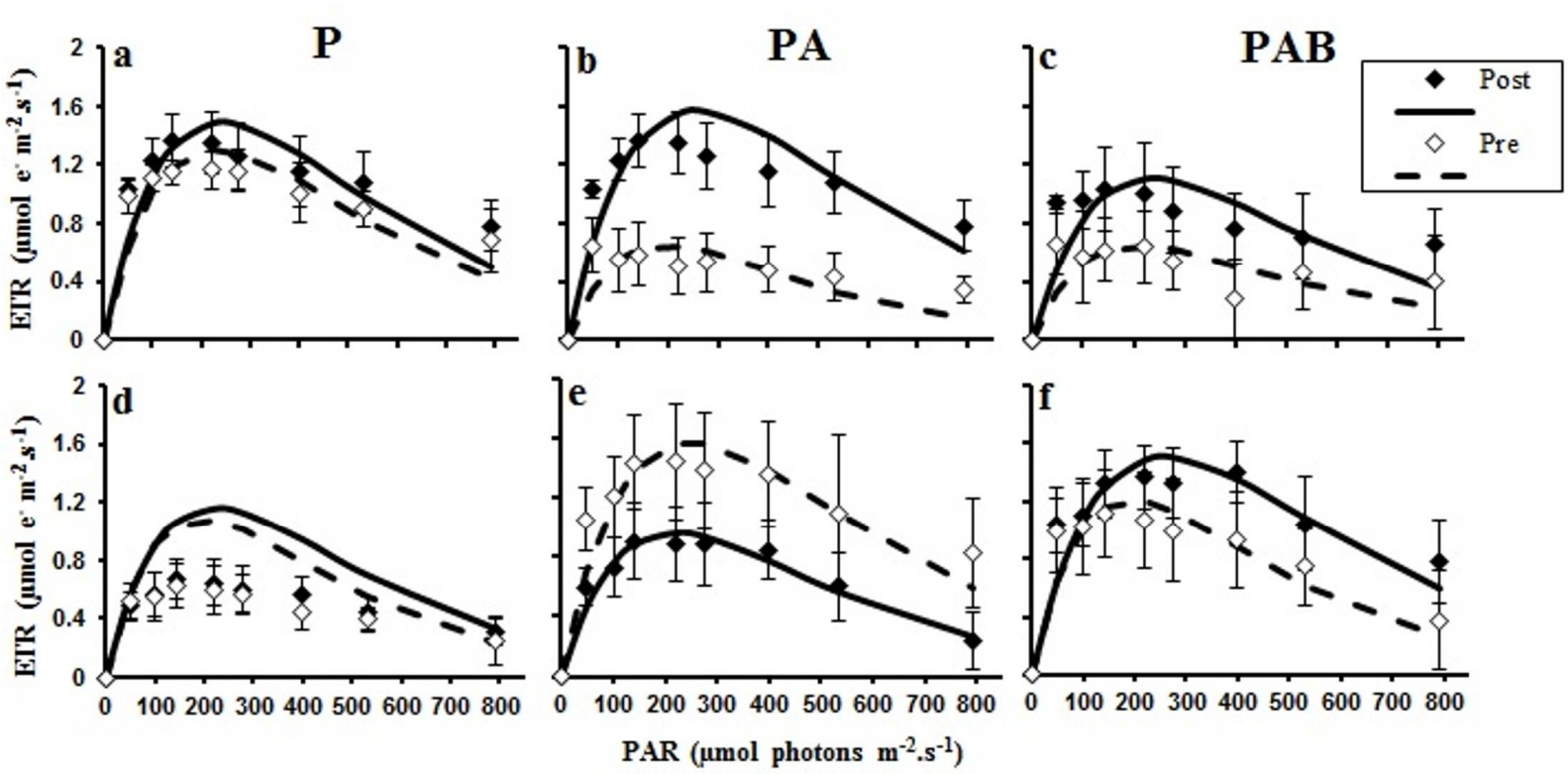

Fig.1. PE curves of *K. ambigua* gametophyte (a, b, c) and sporophyte (d, e, f) stages under UVR treatments. The dotted and solid lines indicate the fitted model for *K. ambigua* pre (PAR-only) and post (after) treatment exposures, respectively

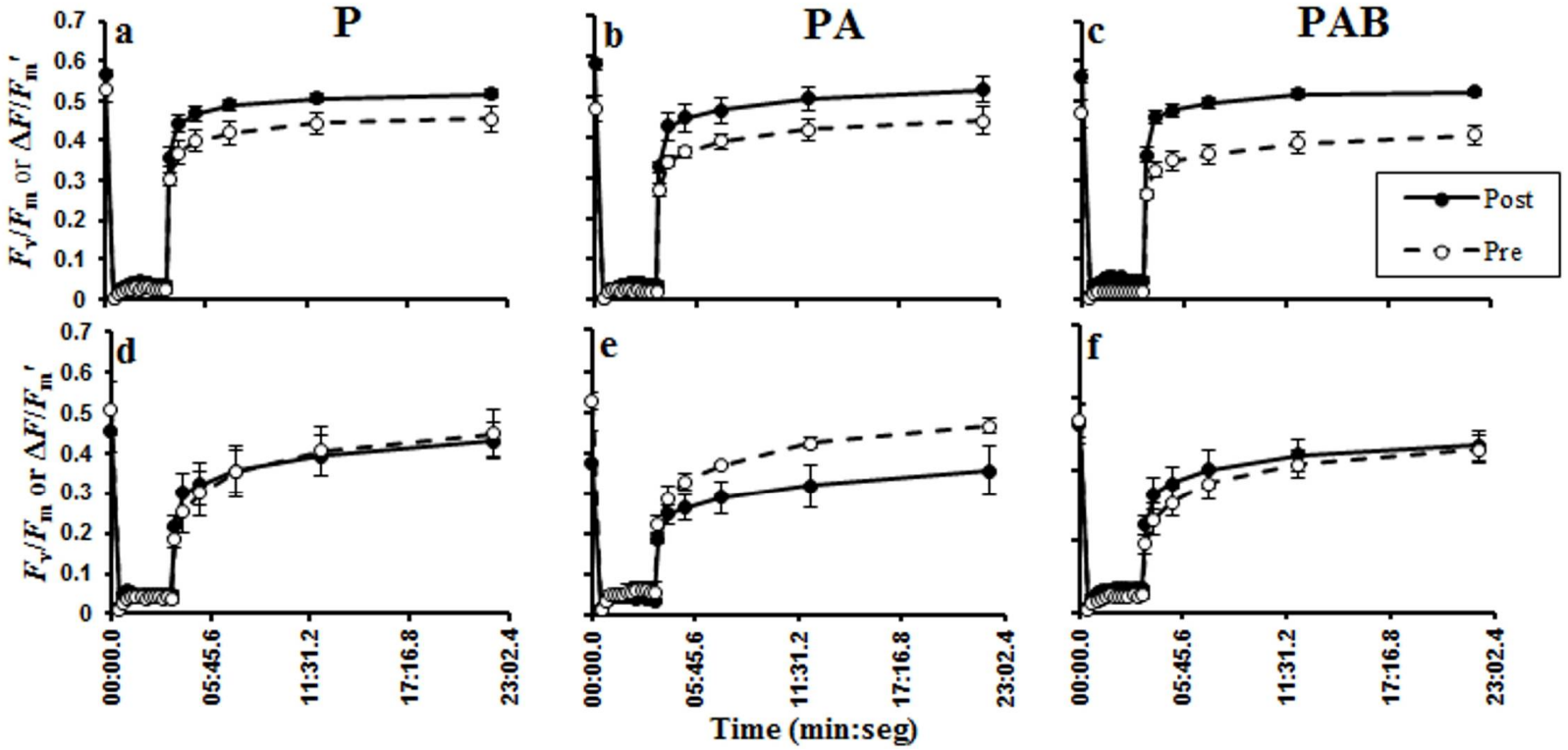

Fig.2. Dark/Light induction-recovery curves of *K. ambigua* gametophyte (a, b, c) and sporophyte (d, e, f) stages under UVR treatments. The dotted and solid lines indicate the fitted model for *K. ambigua* pre (PAR-only) and post (after) treatment exposures, respectively

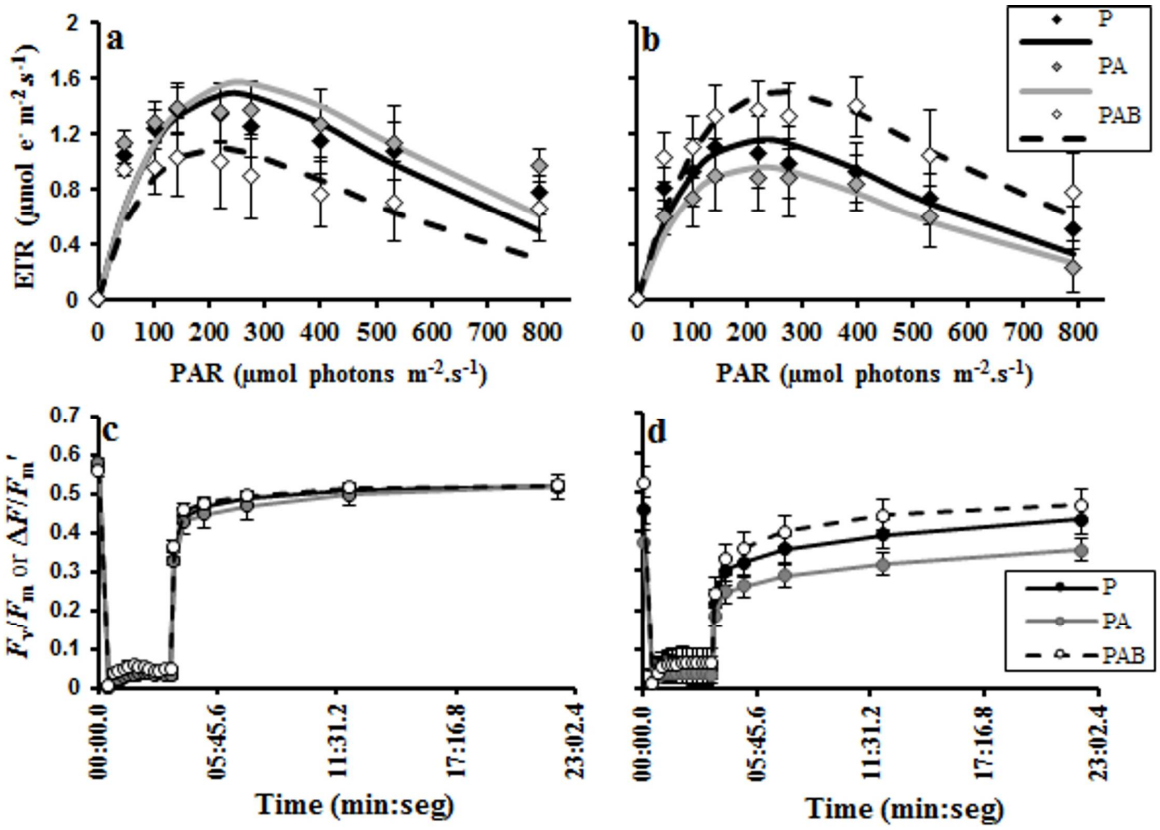

Fig.3. PE (a-b) and Dark/Light induction-recovery (c-d) curves of gametophyte (‘a’ and ‘c’) and 'Chantransia' stage (‘b’ and ‘d’) under UVR treatments

Supporting information

Table S1 Absolute irradiance and daily doses (KJ m^{-2}) of UVB, UVA and PAR applied to *K. ambigua* in different treatments (P, PA and PAB) at laboratory. In addition, data of environmental and UVB / UVA ratio is included

	Absolute Irradiance[a] ($W.m^{-2}$)			**Daily dose ($KJ.m^{-2}$)**			
	P	**PA**	**PAB**	**P**	**PA**	**PAB**	**Environment[b]**
PAR	12.15	12.58	12.65	524.95	543.66	546.61	1762.09
UVA	0.09	1.09	1.20	4.03	47.07	51.99	984.53
UVB	0.10	0.11	0.30	4.12	4.83	13.07	213.75
Total	12.34	13.78	14.15	533.10	595.56	611.67	2960.37
UVB/UVA			0.25				0.22

[a]Measured with a spectroradiometer UV-Visible USB2000 + RAD (Ocean Optics, Dunedin, USA).

[b]Measured on 29 July 2013 at São José do Rio Preto, São Paulo State, southeastern Brazil (20º47’3.21’’S, 49º21’32.04’’W)

CAPÍTULO 2

UV-radiation effects on photosynthesis and photoprotection in two species of Cyanobacteria

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With 2 figures and 2 tables

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Abstract: Cyanobacteria have evolved a number of mitigation strategies to minimize the negative effects of UV and investigations are relevant for a better understanding of the different mechanisms of aerophytic cyanobacteria to varying light climates in terms of global changes. The aim of this study was to analyze the photosynthetic performance (parameters derived of chlorophyll fluorescence), pigment concentrations (chlorophyll *a* and carotenoids) and the presence of UV-absorbing compounds (mycosporine-like aminoacids and scytonemin) in two terrestrial cyanobacteria, *Leptolyngbya* cf. *henningsii* and *Scytonema* sp. in response to UV radiation exposure (UV-A, UV-AB and PAR-only) under laboratory culture conditions. Experiments were performed under three conditions: (1) PAR (400-700nm) treatment, control, P; (2) PAR+UV-A (320-700nm), PA treatment; (3) PAR+UV-A+UV-B (280-700nm), PAB treatment. *Scytonema* sp. and *L*. cf. *henningsii* showed distinct strategies to deal with UV radiation. Response of *L*. cf. *henningsii* was characterized essentially as insensitive to exposure to both UV-A and UV-AB radiations, and the lack of MAAs and scytonemin, but there was an increase of carotenoid contents suggesting that their roles as complementary mechanism to avoid the effect of UV radiation. An increase of photosynthetic activity (higher values of ETR_{max}, E_k, F_v/F_m and $\Delta F/F_m'$) in UV-A, and a decrease of carotenoids and chlorophyll *a*, suggest that *Scytonema* sp. is capable to use UV-A as a source of energy for photosynthesis, and/or in DNA-related repair mechanisms. In addition, a decrease of photosynthetic performance (lower values of α_{ETR}, F_v/F_m and $\Delta F/F_m'$) and significantly higher values of MAAs under UV-AB, indicate that *Scytonema* sp. was more sensitive to UVAB radiation.

Keywords: chlorophyll fluorescence, cyanobacteria, photosynthesis, screening substances, UV radiation

Introduction

The depletion of the ozone layer caused by release of anthropogenic atmospheric pollutants has increased solar UV radiation on Earth's surface, including aquatic systems (Häder et al. 2007; Mckenzie et al. 2011). Since the amount of UV radiation is influenced by global climate changes, this makes the decrease of UV radiation on the Earth's surface more doubt and emphasizes the need to study of effects of UV radiation on biological systems (Rastogi et al. 2014). In the tropics, studies projected higher levels at the end of the 21st century than in 1980 (Mckenzie et al. 2011). Bais et al. (2011) estimated 2-3% of UV-Ery (erythermal solar irradiance) increments, while Hegglin & Shepherd (2009) predicted that UV indices may increase by about 4% in the tropics in the next decades.

It is widely accepted that UV radiation can adversely affect aquatic organisms (Häder et al. 2007). UV radiation causes chronic and physiological stress in cyanobacteria (Richa & Sinha 2011). Growth, pigment concentration, photosynthetic performance and nitrogen fixation are affected by UV-B radiation in several cyanobacteria and microalgae (Xue et al. 2005; Singh et al. 2008, 2010; Babele et al. 2012). Singh et al. (2010) evaluated the effects of UV radiation, mainly UV-B, in species of cyanobacteria, and concluded that DNA and photosynthesis are the main targets of damage from UV-B. Likewise, photosynthesis, growth rate and chlorophyll synthesis are negatively affected by UV-A. Although UV-A can provoke the same effects as UV-B, they are with less consequences (Castenholz & Garcia-Pichel 2012). On the other hand, Richa & Sinha (2011) reported that effect of UV-B radiation in cyanobacteria can be attenuated by UV-A or PAR.

Cyanobacteria occur in varied habitats in terrestrial, freshwater and marine systems (Richa & Sinha 2011; Rastogi et al. 2014), and play a significant role in biogeochemical

cycle of nitrogen, carbon and oxygen, contributing to global biomass production (Häder et al. 2007; Sinha et al. 2003). These organisms are one of the oldest life forms and the first photosynthetic oxygen prokaryotes, evolved when the ozone shield was absent (Singh et al. 2010). For this reason, it is believed that they had to face high concentrations of UV radiation and, as a consequence, they have developed a variety of protecting mechanisms to minimize damaging effects of UV (Babele et al. 2012; Singh et al. 2010). A number of studies (Rastogi et al. 2014; Babele et al. 2012; Castenholz & Garcia-Pichel 2012; Richa & Sinha 2011; Singh et al. 2010; Garcia-Pichel 1998) described the main defense mechanisms: (1) Avoidance, including motility and mat forming ability; (2) Enzymatic/non enzymatic antioxidative systems. Once UV penetrates inside the cell, it interacts with oxygen and other organic compounds to produce toxic reactive oxygen species (ROS) resulting in oxidative stress, which can disarrange cellular membranes and internal proteins. To overcome this stress, antioxidant systems involving enzymatic and non-enzymatic (e.g. carotenoids) antioxidants have been developed by cyanobacteria. For example, carotenoids protect cells against photooxidative damage by absorbing triplet state energy from chlorophyll and quenching singlet state oxygen; (3) Synthesis of UV-absorbing compounds. Mycosporine-like amino acids (MAAs) and scytonemin are well known UV-absorbing/screening compounds that provide photoprotection against UV-B and/or UV-A radiation. MAAs's absorption spectra range from 310-362 nm and they protect the cells absorbing UV and dissipating the high energy as heat or acting as antioxidants. Scytonemin is a yellow-brown sunscreen pigment, exclusively produced by cyanobacteria that shows absorption maximum at 386 nm, although there is also substantial absorbances at 252, 278 and 300 nm; (4) DNA repair. Photoreactivation, excision, repair and recombinational repair are some DNA repair mechanisms reported in cyanobacteria, driven by photolyases.

Many studies on responses to UV radiation in cyanobacteria focus on the synthesis of UV-absorbing/screening substances (Ehling-Schulz & Scherer 1999; Gao & Garcia-Pichel 2011; Babele et al. 2012; Rastogi & Incharoensakdi 2014). Some investigations have also reported the effects of UV radiation on photosynthetic performance of cyanobacteria using *in vivo* chlorophyll *a* fluorescence (George et al. 2001; Han et al. 2003; Gao et al. 2007; Lesser 2008).

The aim of this study was to analyze the photosynthetic performance (parameters estimated by chlorophyll fluorescence), pigment concentrations (chlorophyll *a* and carotenoids) and the presence of UV-absorbing compounds in two cyanobacteria, *Leptolyngbya* cf. *henningsii* (non-heterocystous) and *Scytonema* sp. (heterocystous) in response to UV radiation exposure (PAR+UV-A, PAR+UV-AB and PAR-only) under culture conditions. These two species are typical soil crust-forming cyanobacteria and grow aerophytically or sub-aerophytically on rocks and soil sites (Dojani et al. 2014, Guiry and Guiry 2017). Considering the environmental factors of their habitats, such as water availability and exposure to solar radiation, they presumably have developed a variety of protecting mechanisms to cope with such unfavorable conditions.

Based on the assumption that: 1) *L.* cf. *henningsii* and *Scytonema* sp. are presumably adapted to their habitat conditions (particularly of exposure to solar radiation); 2) cyanobacteria have evolved a number of mitigation strategies to minimize the negative effects of UV (Babele et al. 2012; Singh et al. 2010); 3) distinct species differ with respect to their tolerance to UV-B radiation and even closely related strains show differential sensitivity (Richa & Sinha 2011); we hypothesized that the responses to exposure of UV-A and UV-AB radiation will be similar in both species for photosynthetic performance but they will differ in terms of pigment concentrations and UV-absorbing compounds.

Material and Methods

Biological material

Leptolyngbya cf. *henningsii* (Lemmermann) Anagnostidis was collected in Brazil, São Paulo State, Furnas do Bom Jesus (20º13'S; 47º27'W) on April, 2013. *Scytonema* sp. was collected in Brazil, Rio Grande do Sul State, Santana do Livramento (30º35'S, 55º67'W) on March 27, 2012. They were isolated in culture under irradiance of photosynthetic active radiation (PAR, λ=400-700 nm) of 50 µmol photons m^{-2} s^{-1}, 20.0±1.0ºC and photoperiod of 12 hours and kept in BG11 Medium (Allen, 1968) in Revco RI 12-555 incubators.

Experimental design

Three treatments (with five replicates) were performed (1) PAR (400-700nm) treatment, control, P; (2) PAR+UV-A (320-700nm), PA treatment; (3) PAR+UV-A+UV-B (280-700nm), PAB treatment. All treatments were provided by two cool white fluorescent lamps Osram L 15W "cool daylight" (Osram, Hildesheim, Germany), covered with neutral black mesh and by one Ultra-Vitalux 300W lamp, 230V E27/ES (Osram, Hildesheim, Germany). Radiation treatments were obtained with placing cut off filters in front of the samples (Rastogi et al. 2010, 2014). PAR treatment (1) used filter Lee n. 226, blocking UV-A (320-400nm) and UV-B radiation (280-320nm). PA treatment (2) used filter Lee n.130, blocking UV-B radiation. PAB treatment (3) did not received any filter, being submitted to the entire spectra provided by the lamps.

Average irradiance level in each treatment was 179±18 µmol photons m^{-2} s^{-1} in order to adjust the irradiance described in the literature for freshwater macroalgae (Necchi

2004). Absolute irradiance ($W.m^{-2}$) was measured by the spectroradiometer UV-Visible USB2000 + RAD (Ocean Optics, Dunedin, USA). The UV-B/UV-A ratio was 0.25 in laboratory, whereas in the field it was 0.22 (TableS1).

Incubations were performed at a temperature of 22.0±2.0ºC in refrigerated incubator Marconi, MA mod 830/A, with orbital agitation of 100±5 rpm. To ensure uniform irradiation of all samples, replicates positions were changed each two days during the experiment. Thalli were incubated in UV-transparent 300 mL metacrylate plastic vessels. Incubations were done in BG11 Medium changed every three days in order to avoid nutrients and CO_2 limitation, and water evaporation. The initial fresh weights for *L*.cf. *henningsii* and *Scytonema* sp. were 45.0±5.0 mg and 55.0±5.0 mg fresh weight, respectively, for each replicate. Before experiments, all samples were acclimated for 24 hours under the control conditions. The incubation time in each treatment was ten days with a photoperiod of 12h.

Photosynthetic performance as in vivo chlorophyll *a* fluorescence

The photosynthetic activity was evaluated: 1) before (pre, PAR only) and after (post) UV-exposure (after 10 days of incubation) for each specie to UV radiation; 2) after UV-exposure, by comparison among P, PA and PAB treatments for each species. *In vivo* chlorophyll *a* fluorescence was measured using a Diving-PAM underwater fluorometer (Walz, Effeltrich, Germany). Algal thalli were placed directly on the tip of the fluorometer optic fiber using the supplied magnet sample holder. The biomass was high enough to cover the area of the fluorometer optic fibertip.

Photosynthesis-irradiance (P-E) curves, as rapid light curves (White & Critchley 1999), consisted of the fluorescence responses to eight increasing irradiances from 0 to 792

μmol m^{-2} s^{-1}, using the "light curve" option of the Diving-PAM. The exposure time at each irradiance was 15 s, each separated by a saturating flash (0.8 s, ~6,000 μmol m^{-2} s^{-1}). The calculations and terminology followed Schreiber et al. (1994) and van Kooten & Snel (1990), respectively. The following parameters were determined from each sample: 1) Effective quantum yield of PSII, $\Delta F/F_m' = (F_m' - F_t)/F_m'$, being F_m' maximal fluorescence of light acclimated thalli and F_t the transient fluorescence of light-acclimated thalli; 2) Non-photochemical quenching (*NPQ*) was determined as $NPQ = (F_m - F_m')/F_m'$. 3) Electron transport rate (ETR) was calculated as $ETR = \Delta F/F_m' \times E_{PAR}$(μmol photons m^{-2} s^{-1})$\times A \times F_{II}$ being E_{PAR} the incident irradiance of PAR in μmol m^{-2} s^{-1}. A was the absorptance, or fraction of light absorbed by PSII in an optical cross section. It was estimated for each alga tested (0.68 to *L.* cf. *henningsii* and 0.87 to *Scytonema* sp.), based on measurements made with and without the alga within a circle of 0.5 cm in diameter, similar to the area of the fluorometer optic fibertip (Necchi 2004; Vieira & Necchi 2006; Bautista and Necchi 2007). F_{II} is the fraction of chlorophyll *a* associated to photosystem II. F_{II} in red macroalgae and cyanobacteria have a value of 0.15 (Johnsen & Sakshaug 2007). Based on the ETR versus irradiance curves measured for each treatment, maximum electron transport rate (ETR_{max}), photosynthetic efficiency (α_{ETR}), saturation parameter (E_k) and the slope of photoinhibition (β_{ETR}) were obtained by fitting these curves to the tangential fitting reported by Platt et al. (1980).

Dark/light induction-recovery (Kautsky) curves were performed on thalli dark-acclimated. For dark acclimation, apices of algal thalli were placed for 10 min directly on the tip of the fiberoptic using the supplied dark leaf clip determining the basal fluorescence (F_o) and maximal fluorescence (F_m) after a saturating light pulse (0.8 s, ~6,000 μmol m^{-2} s^{-1}). First, a saturation pulse was applied for determination of maximal quantum yield of

PSII, F_v/F_m, being $F_v=F_m-F_o$ (Schreiber et al. 1994). Then, a constant actinic irradiance (221 μmol photons m^{-2} s^{-1}, close to the incubation irradiance) was applied using the halogen light source of the Diving PAM, separated by eight saturating light pulses at 15 s intervals, and initiated 30 s after the first saturation pulse. After recording of dark/light induction, six saturation pulses were applied at successive intervals (10, 30, 60 seconds and 2, 5 and 10 minutes) to assess the dark recovery of F_v/F_m, by comparing the initial and final values.

Determination of pigments and MAA-like substances

After incubations, samples of 6.0±0.5 mg DW were used for the extraction of mycosporine-like amino acids (MAAs) and photosynthetic pigments. The methodology for determination of MAAs followed Garcia-Pichel & Castenholz (1993). Extraction was made in 1 ml of 20% aqueous methanol (v/v) for 2 h at 45°C. This procedure did not extract photosynthetic pigments because the cells were not distrupted (ground or sonicated). Specific contents were determined spectrophotometrically in a Libra S60 spectrophotometer (Biochrom) from the aqueous methanol extracts by measuring the absorbance at the wavelength of MAA maximum absorbance, and corrections were made according to the expression $A_\lambda^*=A_\lambda- A_{260}(1.85 - 0.005\lambda)$, where A_λ^* is the corrected value of the absorbance at the maximum, A_λ is the measured value of absorbance at the maximum, and λ is the wavelength (in nanometers) of maximal absorbance. Specific contents were measured with a 1cm-pathlength cuvette in 1mL 20% aqueous methanol (v/v) and were expressed as A x milligrams (dry weight)$^{-1}$.

Subsequent extraction of extracellular scytonemin and photosynthetic pigments was conducted in 100% acetone overnight in darkness at 4ºC. The material was grinded in a Precellys 24 tissue homonogenizer (Bertin Technologies) and centrifuged in a Mikro 220R

centrifuge (Hettich) at 4000 rpm, 4°C for 20 minutes. The determination of scytonemin, chlorophyll *a* and carotenoids contents were done spectrophotometrically by using the trichromatic equations according to Garcia-Pichel & Castenholz (1991): $A^*_{384}(Scyt)=1.04A_{384}–0.79A_{663}–0.27A_{490}$; $A^*_{663}(Chl\textit{a})=1.02A_{663}–0.027A_{384}+0.01A_{490}$; and $A^*_{490}(Car)=1.02A_{490}–0.08A_{384}–0.026A_{663}$. Data were expressed in A x milligrams (dry weight)$^{-1}$ for comparison.

Data analysis

Parametric statistical tests were applied considering that the variables followed a normal distribution according to graphical assessment of normality based on the regression curves. Student's *t* test (Zar 1999) was used to evaluate significant differences ($P< 0.05$) in photosynthetic parameters comparing pre and post treatment for each species. The comparison of the three treatments, P, PA and PAB, after the 10 day period, was done by one-way ANOVA, followed by Student-Newman-Keuls Post-hoc. Statistical tests were performed using GraphPad Prism 5.01 software, whereas graphs and calculations from P-E curve parameters were made using Excel 2013.

Results

Photosynthetic response to UV exposure

The photosynthetic performance of *L.* cf. *henningsii* was characterized essentially as insensitive to changes in response to UV-exposure (Figs. 1A and 1C; Tables 1 and 2). Pre (PAR only) and post UV-exposure did not reveal significant differences after PA, except for ETR_{max} (t=2.90; $p<0.05$) (Table 1). Significantly higher values were observed for β_{ETR}

(t=3.32; p<0.05) and *NPQ* (t=3.46; p<0.05) and significantly lower values for E_k (t=3.72; p<0.05) (Table 1) after PAB exposure. After UV-exposure, no significant differences were found among PAR, PA and PAB, except for β_{ETR} (F=18.56; p<0.05) (Table 2, Fig. 1A). Dark/Light induction-recovery curves revealed the same general pattern of variation for pre and post UV-exposure and among treatments (Fig. 1C). Quantum yield kinetics was characterized by a small decrease, followed by a slight increase and stabilization during the light exposure period and after the recovery period (Fig. 1C). No significant differences in recovery capacity of F_v/F_m values were found between pre and post UV-exposure and among treatments (Tables 1 and 2). Moreover, recovery capacity of F_v/F_m values was higher than 80% in *L.* cf. *henningsii* (Tables 1 and 2).

Increases of photosynthetic performance and heat dissipation (*NPQ)* in *Scytonema* sp. were observed after PA exposure (Fig. 1B and 1D, Tables 1 and 2), whereas under PAB there was a decrease of photosynthetic activity (Fig. 1B and 1D; Tables 1 and 2). Significantly higher values were observed post PA exposure for ETR_{max} (t=4.16; p<0.05), E_k (t=3.30; p<0.05), $\Delta F/F_m'$ (t=4.79; p<0.05), F_v/F_m (t=3.51; p<0.05) and *NPQ* (t=3.58; p<0.05) (Fig. 1B; Table 1). Pre and post PAB exposure revealed significantly lower values for α_{ETR} (t=3.45; p<0.05), $\Delta F/F_m'$ (t=7.14; p<0.05) andF_v/F_m (t=5.29; p<0.05) (Fig. 1B; Table 1). Similar results were observed after UV-exposure among treatments (Fig. 1B; Table 2). Significantly higher and lower values were found in PA and PAB, respectively, for ETR_{max} (F=25.91; p<0.05) and $\Delta F/F_m'$ (F= 51.69; p<0.001), whereas α_{ETR} lower values were found in PAB (F=7.76; p<0.05) and higher values of F_v/F_m in PA (F= 54.07; p<0.001) (Table 2). Dark/Light induction-recovery curves revealed different patterns of variation pre and post PA and PAB exposure (Fig. 1D). Post PA exposure presented higher values of quantum yield during the dark recovery of F_v/F_m (Fig. 1D), while post PAB showed an

opposite result (Fig. 1D). The same pattern was observed among P, PA and PAB exposure (Fig. 1D). Quantum yield kinetics was characterized by a pronounced decrease, followed by a slight increase and stabilization during the light exposure period and after the recovery period (Fig. 1D). No significant differences in recovery capacity of F_v/F_m values were found between pre and post UV-exposure and among treatments (Tables 1 and 2). Values of recovery capacity of F_v/F_m were ≤ 62% in *Scytonema* sp. (Tables 1 and 2).

Pigments and MAAs

Significantly higher values of carotenoids ($F=10.72$; $p<0.05$) and chlorophyll *a* ($F=12.05$; $p<0.05$) were observed in *L.* cf. *henningsii* under PA and PAB in comparison to PAR (Fig. 2A). An opposite response was observed in *Scytonema* sp. which showed significantly lower values of carotenoids ($F=22.17$; $p<0.01$) and chlorophyll *a* ($F=7.75$; $p<0.05$) in treatments containing PA and PAB (Fig. 2B) in comparison to PAR conditions

Contents of MAAs were significantly higher in PAB for *Scytonema* sp. in comparison to samples which received P or PA ($F=44.71$; $p<0.001$) (Fig. 2B). In contrast, MAAs were lacking in *L.* cf. *henningsii* (Fig. 2A). Scytonemin was not found in any of the two species.

Discussion

The responses to exposure to UV-A and UV-AB radiation on photosynthetic performance, pigment concentration and MAAs were distinct under culture conditions in the two cyanobacteria tested: the non-heterocystous *L.* cf. *henningsii* and the heterocystous *Scytonema* sp. The photosynthetic activity *L.* cf. *henningsii* was characterized essentially to

be insensitive to exposure to both UV-A and UV-AB radiations, although it had increased chlorophyll *a* and carotenoid concentrations. A different response was observed in *Scytonema* sp. evidenced by an increase of photosynthetic performance (higher values of ETR_{max}, E_k, F_v/F_m and $\Delta F/F_m'$) after exposure to UV-A, and a decrease (lower values of α_{ETR},F_v/F_m and $\Delta F/F_m'$) under UV-AB. Carotenoids and chlorophyll *a* concentrations were lower in UV-A and UV-AB, whereas MAAs content was higher in UV-AB.

Several studies have reported harmful UV radiation effects, especially UV-B, as indicated by decreased photosynthetic performances in cyanobacteria. Gao et al. (2007) observed significantly lower values of F_v/F_m in *Anabaena* sp. under solar PAR or PAR + UVR with F_v/F_m of cells exposed to UVR recovering their initial values. Different degrees of photoinhibition with a greater decrease in the $\Delta F/F_m'$ under PAR+UV than in PAR were observed in *Anabaena* sp. (Han et al. 2003), whereas Lesser (2008) reported that ETR_{max} and α_{ETR} were significantly reduced by UVR. UV-B exposure reduced the F_v/F_min the desert cyanobacterium *Scytonema javanicum* (Wang et al. 2012), similarly to *Scytonema* sp. in this study, which had significantly lower values in α_{ETR}, F_v/F_m and $\Delta F/F_m'$ under UV-AB radiation. Our results are consistent with the previous studies reporting mostly negative effects under UV-AB for *Scytonema* sp. but not for *L.* cf. *henningsii.*

DNA and photosynthesis have been recognized the main targets of UV-B damage in literature (Singh et al. 2010). The photoinhibition of photosynthesis by intense light or UV radiation has been shown to occur in two steps: damage of the oxygen-evolving complex (oxidative stress) with release of magnesium ions, and secondly, degradation of the D1 protein which, together with the D2 protein, are the major constituent of photosystem II (Latifi et al. 2009). However, Richa & Sinha (2011) reported that UV-B damage can be reduced by UV-A and PAR. In our study, we observed that *Scytonema* sp. presented an

increase of its photosynthetic performance (ETR_{max}, F_v/F_m and $\Delta F/F_m'$) after UV-A exposure. However, a considerable number of studies have reported the negative effects of UV-A radiation in cyanobacteria (Richa & Sinha 2011; Castenholz & Garcia-Pichel 2012; Moon et al. 2012). Both UV-B and UV-A causes chronic and physiological stress in cyanobacteria either by direct or indirect effects (Richa & Sinha, 2011). To our knowledge, there are no published investigations dealing with the positive effects of UV-A specific to cyanobacteria, but Häder et al. (2007) stated that UV-A is not always deleterious but can have some positive effects in aquatic organisms, as it can be used as a source of energy for photosynthesis, or in DNA-related repair mechanisms.

The relative insensitivity observed in *L.* cf. *henningsii* after exposure to both UV-A and UV-AB radiations can be explained by investigations reporting insensitivity of cyanobacteria and eukaryotic algae to UV radiation are scarce. The terrestrial cyanobacterium *Nostoc flagelliforme* showed insensitivity by both UV-A and UV-B (Gao and Ye 2007) in terms of photosynthetic CO_2 uptake by the desiccated colonies and the photosynthetic activity by rehydrated colonies. The physiological performance of freshwater green alga *Zygnema* was showed to be insensitive to experimental UV exposure (Holzinger et al. 2009).

Since cyanobacteria are generally exposed to high UV radiation, they have evolved strategies to reduce direct and indirect damaging effects of UV (Rastogi et al. 2014; Babele et al. 2012; Castenholz & Garcia-Pichel 2012; Richa & Sinha 2011; Singh et al. 2010; Garcia-Pichel 1998). One of the most common is the synthesis of UV-absorbing compounds (MAAs and scytonemin), which protect cells by screening out deleterious UVR (Gao et al. 2007). The role that MAAs play as sunscreen compounds to protect against damage by harmful levels of UV radiation is well-known (Garcia-Pichel & Castenholz

1993; Rastogi et al. 2014). MAAs are widely distributed among cyanobacteria and the relative defense against UV-B damage provided by MAAs depends on the species and the location of the pigments in the cells (Ehling-Schulz & Scherer, 1999; Rastogi et al. 2014). The induction of MAAs was reported to be more effective under UV-B by Sinha et al (2003), which agrees with our finding of a significant increase of MAAs in *Scytonema* sp. under UV-AB. However, our results suggest only a partial role of these pigments in *Scytonema* sp. as a shield under UV radiation, since there was a decrease in photosynthetic performance under UV-AB. According to Oren and Gunde-Cimerman (2007) some MAAs may protect the cell not only against UV radiation by absorbing the high-energy photons and dissipating the energy as heat but also by scavenging reactive oxygen species (ROS) such as singlet oxygen, superoxide anions, hydroperoxyl radicals, and hydroxyl radicals. Garcia-Pichel & Castenholz (1993) and Asencio & Hoffman (2013) investigated many *Scytonema* species and found that all species presented scytonemin and/or MAAs, but never the lack of these two pigments. This is in agreement with our data for *Scytonema* sp. that presented MAAs but not scytonemin. On the other hand, MAAs and scytonemin are lacking in some cyanobacteria, such as *Leptolyngbya* from hot springs (Castenholz & Garcia-Pichel 2012), which is also consistent with our observations in the aerophytic *L.* cf. *henningsii*.

We observed a decrease of carotenoids and chlorophyll *a* in *Scytonema* sp. under UV-A and UV-AB, whereas in *L.* cf. *henningsii* there was an increase in their concentrations under the same treatments. The distinct responses between these two cyanobacteria can be explained by additional mechanisms to reduce damaging effects of UV, consisting in non-enzymatic and enzymatic antioxidants systems, such as carotenoids (Babele et al. 2012). Studies have indicated that UV-B radiation affects chlorophyll and

carotenoids contents in cyanobacteria (Han et al. 2003; Gao et al. 2007; Lesser 2008; Yu & Liu 2013; Rastogi et al. 2014). The cyanobacterium *Anabaena* sp. increased its carotenoid concentration, but decreased chlorophyll *a* content after UV-AB radiation, suggesting that carotenoids were not effective to prevent the breakdown of chlorophyll *a* by the levels of UV (Gao et al. 2007). Total carotenoids and chlorophyll decreased with increasing irradiance in *Aphanocapsa* (Nonnengieber et al. 1996). Our results suggest a protective role in *L.* cf. *henningsii*, but not in *Scytonema* sp. In prokaryotes most carotenoid absorption is in the violet/blue/green region of the visible spectrum, and, thus, the effect of these pigments as a UV screen is minimal (Castenholz & Garcia-Pichel 2012). An increase of chlorophyll *a* content in *N. flagelliforme* under short period of exposure to UV-B was attributed to adaptation strategy, i.e. the cells irradiated by UV-B could produce more chlorophyll *a* in order to offset the reduction after a long period of radiation, and this might make that cells live longer under UV-B (Yu and Liu 2013). Chlorophyll *a* decreased in cultures of *Anabaena* sp. exposed to UV (Lesser 2008). Han et al. (2003) proposed that a strategy for adaptation to prevent over-excitation in the reaction center may be by a decrease of chlorophyll *a* content.

Several studies have identified diverse effects of UVR on the pigmentation and photosynthetic parameters/performances of cyanobacteria (Rastogi et al. 2014). Although *Scytonema* sp. and *L.* cf. *henningsii* were found as typical aerophytic cyanobacteria, and thus both subjected to high irradiances, they presented distinct responses to exposure of UV radiation as regard to photosynthetic performance, pigment concentrations and UV-absorbing compounds. As a consequence, our initial hypothesis was partly confirmed. Ehling-Schulz & Scherer (1999) proposed that a combination of different strategies may be used by photosynthetic organisms to acclimate to UV irradiation. The first protective

defense includes mat or crust formation (Richa & Sinha, 2011) and both *Scytonema sp.* and *L.* cf. *henningsii* present this characteristic in their habitats. The absence of MAAs and scytonemins, the insensitivity of photosynthetic performance and the increase of carotenoid contents in *L.* cf. *henningsii* under UV-A and UV-AB suggests that carotenoids acted as and effective shield for UV radiation. An increase of photosynthetic activity in UV-A, although decrease of carotenoids and chlorophyll, suggest that *Scytonema* sp. is capable to use UV-A as a source of energy for photosynthesis, and/or in DNA-related repair mechanisms. The observed decrease of photosynthetic performance under UV-AB, in spite of significantly higher values of MAAs have been observed under UV-AB, indicate that *Scytonema* sp. was more sensitive to UV radiation. In addition, this UV-absorbing compound was not sufficiently effective to avoid the negative action by harmful levels of UV.

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Table 1. Parameters derived from the photosynthesis-irradiance and dark/light induction-recovery curves comparing pre (PAR-only) and post exposure conditions in P, PA and PAB for the *Leptolyngbya* cf. *henningsii* and *Scytonema* sp. Data are expressed as means ± standard-deviation (n=5): ETR_{max} as µmol electrons m^{-2} s^{-1} and E_k as µmol photons m^{-2} s^{-1}. Distinct letters indicate significant differences ($p<0.05$) by Student's t test.

Radiation	Exposure	Parameter	*Leptolyngbya* cf. *henningsii*	*Scytonemav* sp.	Parameter	*Leptolyngbya* cf. *henningsii*	*Scytonema*sp.
P	Pre	ETR_{max}	1.76±0.19[a]	1.43±0.19[a]	α_{ETR}	0.018±0.001[a]	0.018±0.001[a]
	Post		1.60±0.36[a]	1.79±0.08[b]		0.019±0.001[a]	0.022±0.005[a]
PA	Pre		1.58±0.14[a]	1.46±0.24[a]		0.016±0.003[a]	0.018±0.003[a]
	Post		1.41±0.17[b]	2.34±0.24[b]		0.018±0.001[a]	0.021±0.002[a]
PAB	Pre		1.44±0.17[a]	1.60±0.02[a]		0.018±0.002[a]	0.018±0.002[a]
	Post		1.45±0.22[a]	1.18±0.30[a]		0.019±0.002[a]	0.014±0.002[b]
P	Pre	E_k	100.92±12.28[a]	82.32±15.46[a]	β_{ETR}	26.22±3.67[a]	31.32±6.19[a]
	Post		76.50±12.05[a]	79.01±14.57[a]		21.97±3.27[a]	32.61±6.43[a]
PA	Pre		104.99±15.02[a]	81.03±15.26[a]		25.89±4.84[a]	31.87±6.71[a]
	Post		79.87±7.76[a]	110.69±16.65[b]		31.57±3.22[a]	25.44±6.34[a]
PAB	Pre		82.11±7.35[a]	87.57±13.20[a]		30.67±2.76[a]	29.16±4.91[a]
	Post		72.49±7.81[b]	87.46±22.23[a]		34.85±3.90[b]	28.46±7.52[a]
P	Pre	*NPQ*	0.38±0.02[a]	0.44±0.06[a]	$\Delta F/F_m'$	0.45±0.01[a]	0.40±0.02[a]
	Post		0.32±0.03[b]	0.58±0.06[b]		0.44±0.02[a]	0.39±0.02[a]
PA	Pre		0.29±0.04[a]	0.45±0.07[a]		0.45±0.02[a]	0.39±0.05[a]
	Post		0.34±0.05[a]	0.52±0.06[b]		0.42±0.04[a]	0.49±0.01[b]
PAB	Pre		0.33±0.09[a]	0.46±0.04[a]		0.43±0.04[a]	0.43±0.01[a]
	Post		0.39±0.07[b]	0.49±0.01[a]		0.46±0.02[a]	0.32±0.03[b]
P	Pre	F_v/F_m	0.39±0.04[a]	0.38±0.05[a]	% recovery	82%±7[a]	57%±2[a]
	Post		0.42±0.03[a]	0.28±0.03[a]	F_v/F_m	83%±4[a]	62%±13[a]
PA	Pre		0.43±0.03[a]	0.34±0.06[a]		80%±2[a]	57%±11[a]
	Post		0.42±0.03[a]	0.44±0.02[b]		84%±5[a]	60%±7[a]
PAB	Pre		0.41±0.05[a]	0.37±0.03[a]		86%±4[a]	60%±4[a]
	Post		0.42±0.04[a]	0.26±0.04[b]		83%±3[a]	62%±8[a]

Table 2. Parameters derived from the photosynthesis-irradiance and dark/light induction-recovery curves comparing P, PA and PAB in *Leptolyngbya* cf. *henningsii* and *Scytonema* sp. after 10 days experiment. Data are expressed as means ± standard-deviation (n=5): ETR_{max} as µmol electrons m^{-2} s^{-1} and E_k as µmol photons m^{-2} s^{-1}. Distinct letters indicate significant differences ($p<0.05$) by ANOVA (Newman-Keuls Post-hoc)

Parameter	Radiation	*Leptolyngbya* cf. *henningsii*	*Scytonema* sp.
ETR_{max}	P	1.60±0.36[a]	1.79±0.08[a]
	PA	1.41±0.17[a]	2.34±0.24[b]
	PAB	1.45±0.22[a]	1.18±0.30[c]
E_k	P	76.50±12.05[a]	79.01±14.57[a]
	PA	79.87±7.76[a]	110.69±16.65[a]
	PAB	72.49±7.81[a]	87.46±22.23[a]
NPQ	P	0.32±0.03[a]	0.58±0.06[a]
	PA	0.34±0.05[a]	0.52±0.06[a]
	PAB	0.39±0.07[a]	0.49±0.01[a]
F_v/F_m	P	0.42±0.03[a]	0.28±0.03[a]
	PA	0.42±0.03[a]	0.44±0.02[b]
	PAB	0.42±0.04[a]	0.26±0.04[a]
α_{ETR}	P	0.019±0.001[a]	0.022±0.005[a]
	PA	0.018±0.001[a]	0.021±0.002[a]
	PAB	0.019±0.002[a]	0.014±0.002[b]
β_{ETR}	P	21.97±3.27[a]	32.61±6.43[a]
	PA	31.57±3.22[b]	25.44±6.34[a]
	PAB	34.85±3.90[b]	28.46±7.52[a]
$\Delta F/F_m'$	P	0.44±0.02[a]	0.39±0.02[a]
	PA	0.42±0.04[a]	0.49±0.01[b]
	PAB	0.46±0.02[a]	0.32±0.03[c]
% recovery F_v/F_m	P	83%±4[a]	62%±13[a]
	PA	84%±5[a]	60%±7[a]
	PAB	83%±3[a]	62%±8[a]

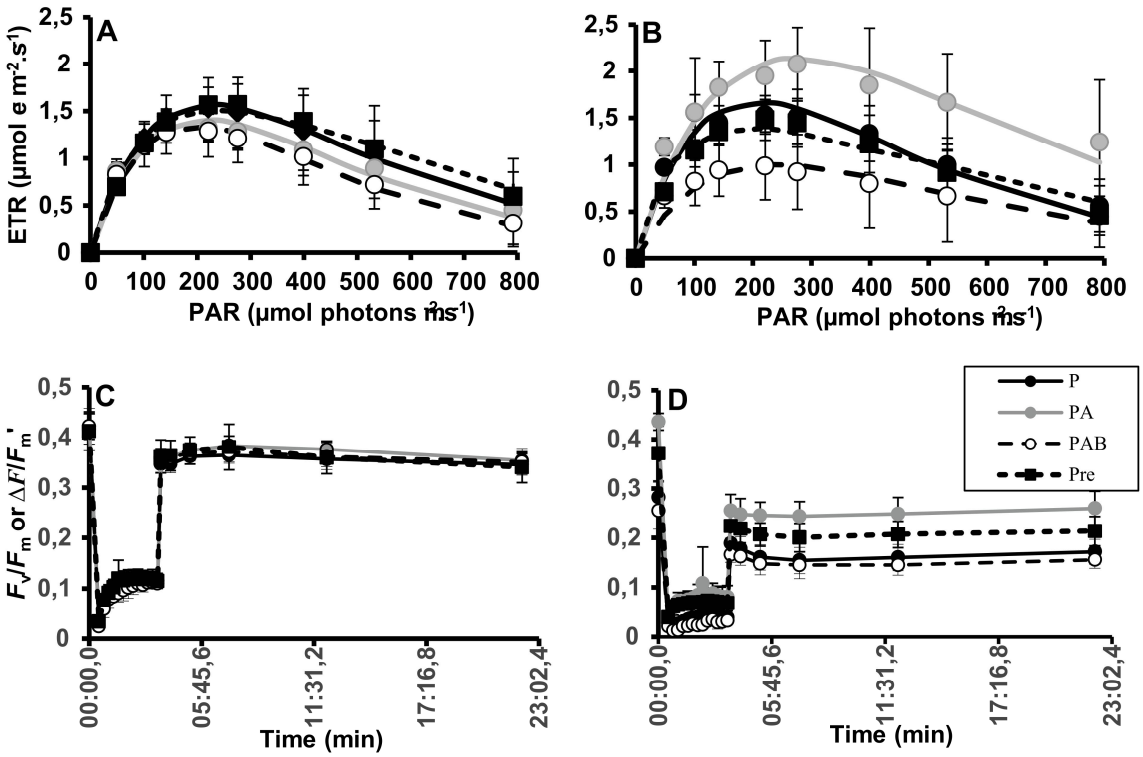

Fig.1 PE (A-B) and Dark/Light induction-recovery (C-D) curves of *Leptolyngbya* cf. *henningsii* (A and C) and *Scytonema* sp. (B and D) under Pre (PAR-only) and (P, PA and PAB). Data are expressed as means ± standard-deviation. (n=5 for P, PA and PAB; n=15 for Pre)

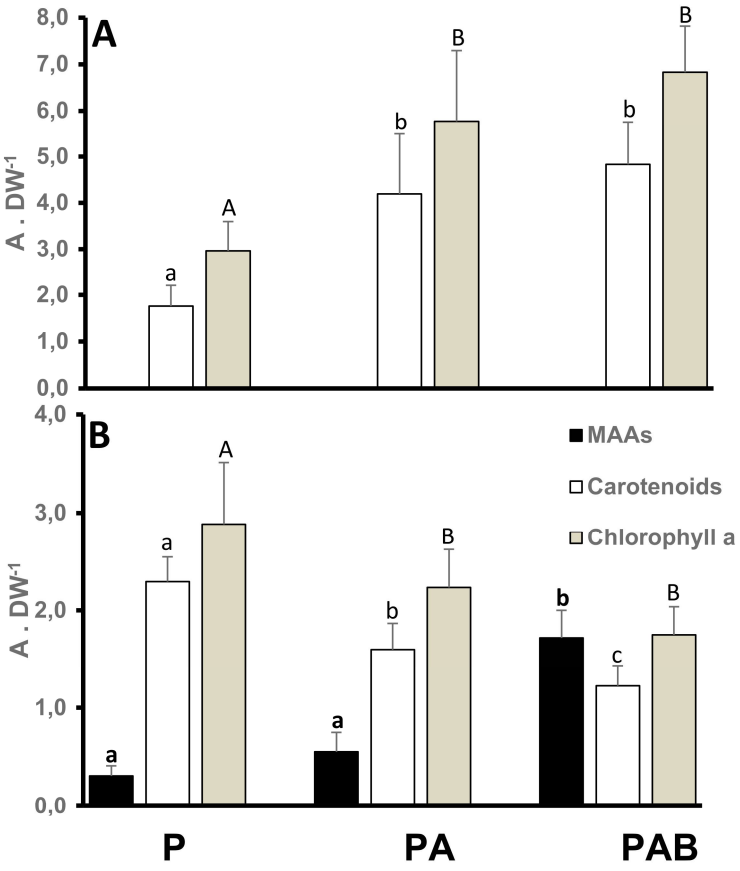

Fig. 2 Mycosporine-like amino acids (MAAs), total carotenoids and chrorophyll *a* contents of *Leptolyngbya* cf. *henningsii* (A) and *Scytonema* sp. (B) comparing P, PA and PAB treatments. Data are expressed as means ± standard-deviation (n=5). Distinct letters indicate significant differences ($p<0.05$) by ANOVA (Newman-Keuls Post-hoc).

Supporting information

Table S1 Absolute irradiance and daily doses (KJ m^{-2}) of UVB, UVA and PAR applied to *Leptolyngbya* cf. *henningsii* and *Scytonema* sp. in different treatments (P, PA and PAB) at laboratory. In addition, data of environmental and UVB / UVA ratio is included

	Absolute Irradiance[a] ($W.m^{-2}$)			**Daily dose ($KJ.m^{-2}$)**			
	P	**PA**	**PAB**	**P**	**PA**	**PAB**	**Environment[b]**
PAR	12.15	12.58	12.65	524.95	543.66	546.61	1762.09
UVA	0.09	1.09	1.20	4.03	47.07	51.99	984.53
UVB	0.10	0.11	0.30	4.12	4.83	13.07	213.75
Total	12.34	13.78	14.15	533.10	595.56	611.67	2960.37
UVB/UVA			0.25				0.22

[a] Measured with a spectroradiometer UV-Visible USB2000 + RAD (Ocean Optics, Dunedin, USA).

[b]Measured on 29 July 2013 at São José do Rio Preto, São Paulo State, southeastern Brazil (20º47’3.21’’S, 49º21’32.04’’W)

CAPÍTULO 3

UV-radiation effects on photosynthesis in three species of tropical lotic macroalgae

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Abstract

Algae in shallow rivers are affected by many environmental factors, including UV radiation. The aim of this study was to analyze the photosynthetic performance (parameters derived of chlorophyll fluorescence), chlorophyll *a* concentrations and the presence of UV-absorbing compounds (mycosporine-like aminoacids - MAAs) in three tropical lotic macroalgae, *Cladophora glomerata* (Chlorophyta), *Spirogyra* sp. (Streptophyta) and *Sirodotia delicatula* (Rhodophyta) in response to UV radiation exposure (PAR+UV-A, PAR+UV-AB and PAR-only) under laboratory conditions. Experiments were performed under three treatments: (1) PAR (400-700nm), P; (2) PAR+UV-A (320-700nm), PA; (3) PAR+UV-A+UV-B (280-700nm), PAB. *Cladophora glomerata* and *Spirogyra* sp., typical sun-adapted lotic macroalgae, had distinct responses to UV radiation exposure. A more pronounced decrease of F_v/F_m and increase of *NPQ* under UV-A than UV-AB was observed, whereas chlorophyll *a* content was lower under UV-AB in *C. glomerata*. *Spirogyra* sp. had a decrease of photosynthesis ($\Delta F/F_m'$ and F_v/F_m) under PAR, indicating that UV-AB radiation may be an important source of energy in the photosynthetic apparatus. Surprisingly, *S. delicatula*, a shade-adapted alga, exhibited less sensitivity to UV exposure. These results suggests that the presence of MAAs (shinorine and palythine) in *S. delicatula* is a significant shield of protection against UV radiation.

Keywords: chlorophyll fluorescence, photosynthesis, screening substances, UV radiation, Chlorophyta, Rhodophyta.

Introduction

The stratospheric ozone layer is no longer decreasing during the coming decades, in response to the reduction in the atmospheric concentrations of ozone depleting substances (Bais et al. 2011). However, it is known that climate changes, caused mainly by increase in greenhouse gas concentrations, have strong interactions with ozone depletion, causing important consequences for UV exposure in aquatic ecosystems (Häder et al. 2015). In addition, changes in other factors, such as clouds and air pollution, can alter UV radiation on the Earth's surface (Mckenzie et al. 2011). As a consequence, this scenario emphasizes the need for studies on effects of UV radiation on biological systems (Rastogi et al. 2014).

Photosynthesis is the one of main metabolic functions of algae, and it is responsible to transfer energy for their maintenance and growth (Sabater et al. 2016), and among various physiological processes, it is potentially the main target of UV irradiation, not only in higher plants, but also in algae (Schreiber et al. 1994). Damage in D1 protein of photosystem II or in the Rubisco enzyme, decrease of photosynthetic pigments and reduced expression of genes involved in photosynthesis are the main mechanisms responsible for decrease in photosynthetic activity after UV treatment (Holzinger and Lütz 2006). However, UV radiation does not always have a deleterious effect on aquatic photosynthetic organisms. Positive effects of UV-B radiation were observed in the green alga *Zygnemopsis decussata* (Figueroa et al. 2009), while positive effects of moderate levels of UV-A radiation on photosynthesis and growth were reported in the red alga *Gracilaria vermiculophylla* (Roleda et al. 2012).

Algae in rivers are affected by many environmental factors, including light (Sabater et al. 2016). UV susceptibility was determined to be most pronounced in deep-water

species and reduced in shallow-water species (Peschek et al 2010). To deal with extreme variations in PAR (photosynthetically active radiation) and UV radiation, macroalgae in shallow environments have set different physiological acclimation mechanisms, that are important in determining variations in photosynthetic responses (Figueroa et al. 2003). Likewise, Germ (2005) observed that the capability for acclimation of photosynthesis to changing radiation environments is an important prerequisite for macroalgae, especially in areas with high natural radiations.

Periphyton and benthic algae communities are the most successful primary producers exploiting habitats and they are the main source of energy for higher trophic levels in many lotic ecosystems (Stevenson 1996). Periphyton includes several microscopic organisms (algae, bacteria, and fungi) growing as biofilms or thicker matrices with flow, nutrient diffusion, and exposure to other organisms very different to those experienced by macroscopic algae (Stevenson 1996). Among benthic algae, macroalgae represent important component of the communities in rivers and streams. The macroalgae most commonly found in rivers are cyanobacteria (blue-green algae), green algae, red algae, diatoms, and rarely brown algae (Necchi 2016). Yellow-green algae and yellow-golden algae and some flagellated algae are minor components of the benthic algal flora of rivers. Green algae tend to predominate (35–37 % of species) in floras of well-studied regions of the world, with contributions of other algal groups varying considerably: cyanobacteria (24–35 %), red algae (14–20 %), diatoms, and yellow-green algae (14–21 %). Among the green algae, filamentous forms are very common in lotic habitats, with some genera reported in several regions of the world (e.g. *Cladophora* and *Spirogyra*), whereas freshwater red algae occur almost exclusively in streams and rivers with members of the order Batrachospermales being widespread.

Spirogyra Link (1820) is an unbranched filamentous green alga that forms free-floating mats in shallow freshwaters (Graham et al. 1995). Some investigations stated that *Spirogyra* can not maintain optimal rates of photosynthesis at high temperatures and low light, but can tolerate cool water and high irradiances (Graham et al. 1995), while Necchi (2004) reported *Spirogyra* sp. as a typical sun-adapted lotic macroalgae. Germ (2005) have reported the effects of UV radiation (specifically UV-B) on *Spirogyra* sp., and concluded that this alga have physiological plasticity and resistance under enhanced UV-B radiation.

Cladophora glomerata (L.) Kützing 1843 is one of the most widespread freshwater macroalgal species in the world (Dodds and Gudder 1992) and can be found almost throughout the year in shaded or open sites (Ensminger et al. 2000). Many aspects of the ecophysiology of *C. glomerata* have been summarized by Ensminger et al. (2000), Necchi et al. (2004) and Bautista and Necchi (2008), as a macroalga either shade or sun-adapted and could be classified as a stress-tolerant species regarding to temperature and light. The only specific study about photosynthetic performance under UV radiation was conducted by Choo et al (2005), who have studied different stress conditions in two green macroalgae and have reported that photosynthesis in *C. glomerata* was inhibited by UV-B radiation.

Freshwater red algae have been indicated as shade-adapted in previous studies (Necchi and Zucchi 2001; Necchi 2004, 2005), although some species can tolerate high irradiances. Photosynthetic characteristics of *Sirodotia delicatula* (treated as *Batrachospermum delicatulum*) exhibited a wide range of responses to irradiance (Necchi and Alves 2005), suggesting they have mechanisms that enable them to avoid photodamage of the photosynthetic apparatus (Necchi 2005). Several studies reported harmful UV radiation effects, especially UVB, with decreased photosynthetic performances in marine red algae (Figueroa et al. 1997; Roleda et al 2004; Simioni et al. 2014), but there is only

one study dealing with the responses to UV radiation for freshwater red algae (Aigner et al. 2017). They reported that exposure to either UV-A or UV-AB led to a strong transient drop in effective quantum yield in *Batrachospermum turfosum* Bory, but the alga was capable of recovering it after being removed from the UVR treatment.

The aim of this study was to analyze the photosynthetic performance (parameters estimated by chlorophyll fluorescence), chlorophyll *a* concentrations and production of MAAs (mycosporine-like amino acids) in three lotic macroalgae, *C. glomerata* (Chlorophyta), *Spirogyra* sp. (Streptophyta) and *S. delicatula* (Rhodophyta) in response to UV radiation exposure (PAR+UV-A, PAR+UV-AB and PAR-only).

Based on the assumptions that: 1) *C. glomerata*, *Spirogyra* sp. and *S. delicatula* are freshwater macroalgae regularly exposed to high solar radiation, including high doses of UV-B and UV-A, due to their wide distribution in shallow habitats; 2) compared to marine macroalgae, information on the UV-protective mechanisms in freshwater macroalgae is scarce (Roleda et al. 2010); 3) red and green are typically shade and sun-adapted algae, respectively (Necchi 2004, 2005), we hypothesized that: i) *Spirogyra* sp. and *C. glomerata* would present similar photosynthetic performance with slight responses to exposure of UV-A and UV-AB radiation; and ii) *S. delicatula* would exhibit a higher sensitivity to UV exposure.

Material and Methods

Biological material

Cladophora glomerata (Linnaeus) Kützing (Chlorophyta) was collected on 29 August 2014 in Preto River (20º52’S, 49º19’W), Engenheiro Schmidt District, municipality

of São José do Rio Preto, São Paulo State, Brazil,, São Paulo State, São José do Rio Preto, Engenheiro Schmidt, Preto River (20º52'S, 49º19'W), in a shaded river segment (radiation of 104 ± 11 µmol photons m^{-2} s^{-1}) and under temperature of 19.2 ºC. *Spirogyra* sp. (Streptophyta) was collected on 19 April 2016 and *Sirodotia delicatula* Skuja (Rhodophyta) was collected on 11 September 2013. Both species were collected in Talhadinho Stream (20º43'S, 49º13'W), São José do Rio Preto, São Paulo State, Brazil., São Paulo State, São José do Rio Preto, Talhadinho Stream (20º43'S, 49º13'W). Both species were collected in different times in a partly shaded stream segment with the following radiation and temperature: 910 ± 163 µmol photons m^{-2} s^{-1} and 25.3 ºC (*Spirogyra* sp.) and 1049 ± 86 µmol photons m^{-2} s^{-1} and 22.6 ºC (*S. delicatula*).

Experimental design

After collection, algal thalli were transported to the laboratory and were cleaned of debris and visible epiphytes under a stereoscope. Then, and they were transferred to 1 liter vessels containing water from the collecting site, previously filtered and autoclaved. They were acclimated for 24 hours under PAR conditions (179 ± 18 µmol photons m^{-2} s^{-1}, 22.0 ± 2.0ºC, 12:12h photoperiod). Radiation was supplied by cool-white fluorescent lamps Osram L 15W "cool daylight" (Osram, Hildesheim, Germany). Algal thalli under these conditions were used as the control (designated Pre).

Three treatments (with five replicates) were performed for each species: (1) PAR (400-700nm), designated P treatment; (2) PAR+UV-A (320-700nm), designated PA treatment; (3) PAR+UV-A+UV-B (280-700nm), designated PAB treatment. All treatments were provided by two cool white fluorescent lamps Osram L 15W "cool daylight" (Osram, Hildesheim, Germany), covered with neutral black mesh and by one Ultra-Vitalux 300W

lamp, 230V E27/ES (Osram, Hildesheim, Germany). Radiation treatments were obtained with placing cut off filters in front of the samples (Figueroa et al. 1997, 2003; Roleda et al. 2010). P treatment (1) used filter Lee n. 226, blocking UV-A (320-400nm) and UV-B radiation (280-320nm). PA treatment (2) used filter Lee n.130, blocking UV-B radiation, whereas for PAB treatment (3) no filter was applied, being submitted to the entire spectra provided by the lamps. Algal thalli were maintained for 10 days under these conditions with a photoperiod of 12h.

Average irradiance level in each treatment was 179 ± 18 µmol photons m^{-2} s^{-1} in order to adjust the irradiance described in the literature for freshwater macroalgae (Necchi 2004). Absolute irradiance (W m^{-2}) was measured by the spectroradiometer UV-Visible USB2000 + RAD (Ocean Optics, Dunedin, USA). The UV-B/UV-A ratio was 0.25 in laboratory, whereas in the field it was 0.22 (Table S1).

Incubations were performed at a temperature of 22.0 ± 2.0ºC in refrigerated incubators Marconi, MA mod 830/A, with orbital agitation of 100 ± 5 rpm. To ensure uniform irradiation of all samples, replicates positions were changed each two days during the experiment. Thalli were incubated in UV-transparent 300 mL metacrylate plastic vessels. Incubations were done in water from the collecting site, previously filtered and autoclaved. The water was changed every two days in order to avoid nutrients and CO_2 limitation, as well as water evaporation. The initial fresh weights for each replicate of *C. glomerata*, *Spirogyra* sp. and *S. delicatula* were 35.0±5.0 mg, 235.0±5.0 mg and 145.0±5.0 mg fresh weight (FW), respectively.

Photosynthetic performance as in vivo chlorophyll *a* fluorescence

In vivo chlorophyll *a* fluorescence was measured using a Diving-PAM underwater fluorometer (Walz, Effeltrich, Germany). Algal thalli were placed directly on the tip of the fluorometer optic fiber using the supplied magnet sample holder. The biomass was high enough to cover the area of the fluorometer optic fibertip.

Photosynthesis-irradiance (P-E) curves, as rapid light curves (White and Critchley 1999), consisted of the fluorescence responses to eight increasing irradiances from 0 to 792 μmol m^{-2} s^{-1}, using the "light curve" option of the Diving-PAM. The exposure time at each irradiance was 15 s, each separated by a saturating flash (0.8 s, ~6,000 μmol m^{-2} s^{-1}). The calculations and terminology followed Schreiber et al. (1994) and van Kooten and Snel (1990), respectively. The following parameters were determined from each sample: 1) Effective quantum yield of PSII, $\Delta F/F_m' = (F_m' - F_t)/F_m'$, being F_m' maximal fluorescence of light acclimated thalli and F_t the transient fluorescence of light-acclimated thalli; 2) Non-photochemical quenching (*NPQ*) was determined as $NPQ = (F_m - F_m')/F_m'$. 3) Electron transport rate (ETR) was calculated as $ETR = \Delta F/F_m' \times E_{PAR}$(μmol photons m^{-2} s^{-1})$\times A \times F_{II}$ being E_{PAR} the incident irradiance of PAR in μmol m^{-2} s^{-1}. A was the absorptance, or fraction of light absorbed by PSII in an optical cross section. It was estimated for each alga tested (0.89 to *C. glomerata*, 0.69 to *Spirogyra* sp. and 0.78 to *S. delicatula*), based on measurements taken with and without the alga within a circle of 0.5 cm in diameter, similar to the area of the fluorometer optic fibertip (Necchi 2004; Bautista and Necchi 2007). F_{II} is the fraction of chlorophyll *a* associated to photosystem II. F_{II} in red and green macroalgae is assumed to have a value of 0.15 and 0.5, respectively (Johnsen and Sakshaug 2007). Based on the ETR versus irradiance curves measured for each treatment, maximum electron transport rate (ETR_{max}), photosynthetic efficiency (α_{ETR}), saturation parameter (E_k)

and the slope of photoinhibition (β_{ETR}) were obtained by fitting these curves to the tangential fitting reported by Platt et al. (1980).

Dark/light induction-recovery (Kautsky) curves were performed on thalli dark-acclimated to determinate of maximal quantum yield and to assess the recovery capacity . For dark acclimation, apices of algal thalli were placed for 10 min directly on the tip of the fiberoptic using the supplied dark leaf clip determining the basal fluorescence (F_o) and maximal fluorescence (F_m) after a saturating light pulse (0.8 s, ~6,000 μmol m^{-2} s^{-1}). First, a saturation pulse was applied for determination of maximal quantum yield of PSII, F_v/F_m, being $F_v=F_m-F_o$ (Schreiber et al. 1994). Then, a constant actinic irradiance (221 μmol photons m^{-2} s^{-1}, close to the incubation irradiance) was applied using the halogen light source of the Diving PAM, separated by eight saturating light pulses at 15 s intervals, and initiated 30 s after the first saturation pulse. After recording of dark/light induction, six saturation pulses were applied at successive intervals (10, 30, 60 seconds and 2, 5 and 10 minutes) to assess the dark recovery of F_v/F_m, by comparing the initial and final values.

Chlorophyll *a* content

Chlorophyll *a* (Chl *a*) concentrations were evaluated at the end of experiment, after measurements of the photosynthetic parameters. They were calculated according to the protocol by Ritchie (2006). Plants were kept frozen until the analysis. The material was grinded in the darkness in a Precellys 24 tissue homonogenizer (Bertin Technologies). Subsequent extraction of chlorophyll *a* was conducted in 1mL of 90% alkaline acetone in darkness at 4°C. Samples were centrifuged in a Mikro 220R centrifuge (Hettich) at 4000 rpm, 4°C for 20 minutes and chlorophyll *a* was quantified according to the spectrophotometric technique using the equation according to Ritchie (2006, 2008).

Mycosporine-like amino acids - MAAs

Considering that Rhodophyta has the highest percentage of species that synthesize MAAs (Barufi et al. 2011) among the various groups of macroalgae, while only a few macroscopic green algae investigated so far contain MAA-like UV-absorbing compounds, (Holzinger et al. 2006; Pescheck et al. 2010),. quantification of this parameter was carried out only for the red alga *S. delicatula*

After the experimental periods, samples of 20.0 ± 5.0 mg DW (dry weight procedure according to Necchi et al., 2010) were used for the extraction and identification of photoprotective substances, mycosporine-like amino acids (MAAs). They were extracted in 1 mL of 20% aqueous methanol (v/v) for 2 h at 45 °C. Aliquots of 600 µL were transferred to new tubes and the liquid contents of the tubes removed by a vacuum microcentrifuge. 600 µL of 100% chromatographic methanol was added to this solid residue to remove salts and the extracted proteins, mixed by vortex, and centrifuged at 13000 g, 4 ° C for 10 min. A total of 100 µL of the supernatant was filtered and transferred to glass tubes in the HPLC Waters system (Barcelona, Spain).

MAAs were detected by using an isocratic run containing 2.5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in distilled water as the mobile phase. The flow rate was 0.5 mL min^{-1} and each run took 20 min; 30 µL of the each sample was injected into a Sphereclone C8 column (Phenomenex, Germany) with a pre-column attached (5-mm packing; 250×4 mm I.D.). MAAs were detected with a Waters Photodiode Array Detector 996 (Barcelona, Spain). The absorption spectra were recorded between 290 and 400 nm at each second. Finally, a chromatogram selected for absorbance at 330 nm was obtained, and the observed peaks were identified according to the spectrum and retention time, compared

with a secondary standard of *Porphyra leucosticta*. MAAs were quantified according to Korbee-Peinado et al. (2004). Data were collected and analyzed with the Millennium 3.2 software.

Data analysis

Data set was analyzed using one-way ANOVA and post hoc multiple comparisons were made using Newman-Keuls multicomparative analysis to identify differences in photosynthetic parameters and pigments (chlorophyll *a* and MAAs). The factor evaluated was the difference among Pre (control), P, PA and PAB treatments. Statistical tests were performed using GraphPad Prism 5.01 software, whereas graphs and calculations from P-E curve parameters were made using Excel 2013.

Results

P-E curves revealed different responses to UV radiation among the three freshwater macroalgae (Fig. 1). *Cladophora glomerata* had higher ETR_{max} (22.37-19.69µmol e^{-} $m^{-2}.s^{-1}$), α_{ETR} (0.178-0.014) and E_k (125.51-141.38) (Fig.1A, Table 1) than *Spirogyra* sp. (Fig. 1B, Table 1), whereas *S. delicatula* presented lower values of ETR_{max} and α_{ETR} (Fig. 1C, Table 1).

C. glomerata presented significantly higher values in Pre (control) for α_{ETR} ($F=7.84$, $p < 0.01$), and no significant difference were found for ETR_{max} (Fig.1A; Table 1). As a consequence, higher values of E_k were observed among treatments, but significant differences were observed only in P treatment ($F=4.3$, $p < 0.05$) (Fig.1A; Table 1). Higher values of β_{ETR} were observed in control, but significant difference was observed in P

(F=3.89, $p < 0.05$) (Fig.1A; Table 1). *NPQ* was significantly higher in PA and PAB than in control and P (F=54.06, $p < 0.0001$), while significantly lower values of $\Delta F/F_m'$ (F=13.56, $p < 0.001$) and of F_v/F_m (F=4.70, $p < 0.01$) were observed in PA (Fig.2). Higher recovery capacity of F_v/F_m was found in PA (F=21.97, $p < 0.0001$) (Fig.2).

Spirogyra sp. presented significantly higher values in control for ETR_{max} (F=19.10, $p < 0.0001$) and α_{ETR} (F=7.84, $p < 0.01$), (Fig.1B; Table 1) and significant differences for E_k were found only in PA (F=4.86, $p < 0.01$) (Table 1). No significant differences were found in *NPQ*, except in P that presented lower values of NPQ (F=7.41, $p < 0.05$) (Fig.2). Although *Spirogyra* sp. generally showed higher values in control, an increase of photosynthetic performance ($\Delta F/F_m'$ and F_v/F_m) was observed in PA and PAB (F=32.48, $p < 0.0001$ for $\Delta F/F_m'$ and F=18.95, $p < 0.0001$ for F_v/F_m) (Fig. 2). In contrast, significant lower values of recovery capacity of F_v/F_m were found in PA and PAB (F=4.37, $p < 0.05$) (Fig. 2).

Sirodotia delicatula had significantly higher values in control for ETR_{max} (F=44.96, $p < 0.0001$), α_{ETR} (F=58.45, $p < 0.0001$) and β_{ETR} (F=21.85, $p < 0.0001$) (Fig.1C; Table 1), while lower values in control were found for E_k (F=54.32, $p < 0.0001$) (Table 1). *NPQ* was significantly higher in P, PA and PAB treatments (F=13.48, $p < 0.0001$), while significantly lower values were observed for F_v/F_m for all treatments (F=24.66, $p < 0.0001$) (Fig.2). No trend of increase or decrease among treatments was observed for $\Delta F/F_m'$ and recovery capacity of F_v/F_m (Fig.2).

Chlorophyll *a* concentrations were significantly lower (F=9.56; $p<0.01$) in PAB for *C. glomerata*, whereas in *Spirogyra* sp. and *S. delicatula* no significant differences were observed among treatments (Table 1).

Shinorine and palythine were the two type of MAAs identified in *S. delicatula*, with shinorine corresponding about 80% of the total MAAs found in this alga (Fig. 3). In addition, a significant decrease of MAAs concentrations were found among UV treatments ($F=14.17$; $p<0.01$ for shinorine; $F=11.18$; $p<0.01$ for palythine; $F=22.28$; $p<0.01$ for total MAAs) (Fig.3).

Discussion

The three tropical lotic macroalgae studied were characterized as either sun-adapted (*C. glomerata*) and shade-adapted (*S. delicatula*) or with intermediate characteristics according to their responses of photosynthetic parameters to artificial UV exposure. *Sirodotia delicatula* showed lower values of ETR_{max} and E_k and higher values of $\beta_{ETR,}$ typical as shaded-adapted algae. Adaptations to low irradiance were consistently observed for Rhodophyta (Necchi and Zucchi 2001; Necchi 2004). In contrast, most species of Chlorophyta has been reported as sun-adapted algae (Graham et al. 1995; Ensminger et al. 2001; Necchi 2004). While *C. glomerata* revealed typical responses of a sun-adapted plant, with higher ETR_{max} and E_k, and lower values of β_{ETR} ,*Spyrogyra* sp. could be considered an intermediate group in terms of light adaptations.

Most of studies on UV effects on macroalgae focused on UV-B radiation (Altamirano 2000; Hanelt et al. 2006), and a question, which is still a matter of debate, is whether all the different bands of UV spectra (UV-A and UV-B) are damaging or possibly beneficial (Altamirano et al. 2000). Although PE curves have revealed different trends to PAR acclimations among *C. glomerata*, *Spirogyra* sp. and *S. delicatula*, the way that

photosynthesis was affected by UV radiation was distinct, no matter if they were sun- or shade-adapted algae.

A decrease of the F_v/F_m level and an increase of *NPQ* values were more pronounced under UV-A than UV-AB radiation in the three algae tested, although significant lower values in recovery capacity of F_v/F_m and in chlorophyll *a* content under UV-AB radiation had been showed only for *C. glomerata*. Ensminger et al. (2001) found that *NPQ* values increased rapidly with the increase of irradiance for both high-light (open site) and shaded plants of *C. glomerata*. In addition, a decrease of F_v/F_m was interpreted as an indicator of the photoinhibition effect on photosynthesis (Figueroa et al. 2009), revealing amplified excitation pressure on photosystem II in *C. glomerata*. The decrease in F_v/F_m under different conditions of UV radiation was associated with a gradual decrease in chlorophyll contents in two species of the marine green alga *Ulva* (Figueroa et al. 2003). Pigment contents give us important elements about the state of the photosynthetic apparatus. However, because the pigments are not directly linked to photosynthetic UV acclimation, their concentration is usually considered to be a weak biological indicator of the effects of UV radiation in this type of experimental designs (Figueroa et al. 2003).

We generally observed higher values of *NPQ*, $\Delta F/F_m'$ and F_v/F_m, under UV-A and UV-AB under PAR-only in *Spirogyra* sp, while no significant differences among treatments were observed in chlorophyll *a* content. In addition, although the recovery capacity of F_v/F_m have been lower under UV-A and UV-AB, they had 100% of recovery, indicating a high capacity to cope with UV radiation. The mostly good photosynthetic performance in *Spirogyra* sp. under UV radiation could be related to their capacity to tolerate high irradiances (Graham et al. 1995; Necchi 2004). Germ (2005) concluded that *Spirogyra* sp. have high physiological plasticity and resistance under which may be

enhanced by UV-B radiation. Although most studies reported a negative impact on photosynthesis by UV radiation, some investigations found beneficial effects of UV-B and UV-A by photoprotection of photosynthesis in algae (Figueroa et al. 2009). Photosynthesis in the green alga *Dasycladus vermicularis* significantly increased for plants exposed to PAR+UV-A radiation (Perez-Rodriguez et al. 1998). According to Figueroa et al. (2009), UV-B could have a role in inducing and maintaining photoprotective mechanisms under high UV exposure. The exclusion of UV-B was shown to produce a reduction of photosynthetic activity in tropical algae (Hanelt and Roleda 2009). The photosynthetic performance of *Ulva rigida* (Chlorophyta) was always negatively affected under PAR alone compared to that in the presence of UV radiation (Altamirano 2000).

High values of *NPQ* may indicate active photoprotective mechanisms, which are highly related to the xanthophyll cycle (Demmig-Adams and Adams 2006). Schubert and García-Mendoza (2008) reported that the degree of decrease and recovery of F_v/F_m and their respective kinetics were related to the carotenoid profile of the species. Choo et al. (2005) stated that photosynthesis in *C. glomerata* was inhibited by UV-B radiation, and a defense mechanism against UVR-induced oxidative stress is enabled through an increase in carotenoid concentration and a functional xanthophyll cycle. It is plausible to expect an important role of carotenoids and *NPQ* under UV radiation in *C. glomerata* and *Spirogyra* sp. but this will require further studies on carotenoid quantification, identification and interconversion.

Freshwater red algae have a typical response to irradiance, most being considered as shade-adapted algae (Necchi 2005; Bautista and Necchi 2007), although they are typically found in shallow parts of streams and rivers and, thus, are exposed to high solar radiation during some periods (Sheath and Hambrook 1990; Necchi 2005; Bautista and Necchi

2007). Thus, they are expected to have mechanisms to cope with such high irradiances, as shown in some previous studies (Necchi 2005; Bautista and Necchi 2007; Aigner et al. 2017). Necchi and Alves (2005) observed that *S. delicatula* (treated as *Batrachospermum delicatulum*) exhibited a wide range of responses to irradiance regarding to photosynthetic characteristics. In this study, values of *NPQ*, $\Delta F/F_m'$, F_v/F_m and recovery capacity of F_v/F_m revealed that the alga is practically insensitive to UV-A and UV-AB radiation, corroborating studies that this red alga species can tolerate not only high irradiances but also UV radiation. Aigner et al. (2017) reported that exposure to either UV-A or UV-AB led to a strong transient drop in effective quantum yield in *B. turfosum*, but the alga was capable of recovering it after being removed from UVR treatment.

The synthesis and accumulation of photoprotective compounds, such as mycosporine-like amino acids (MAAs), is one of the strategies used by macroalgae under exposure to high levels of UV (Sinha and Häder 2002; Häder et al. 2015; Navarro et al. 2016). Rhodophyta is the algal group that has the highest percentage of species that synthesize MAAs (Barufi et al. 2011). Two types of MAAs were identified in *S. delicatula* - shinorine and palythine – in very low quantities in comparison to marine red algae (reference), with the former corresponding about 80% of the total MAAs found in this alga. Although a decrease of MAAs concentrations were found among UV treatments, the presence of MAAs suggests that this substance was produced to avoid photodamage of the photosynthetic apparatus. This is the second report of MAA in a member of the freshwater red algal order Batrachospermales. Gametophytes of *Kumanoa ambigua* presented the occurrence of MAA shinorine under PAR and UV-A and UV-AB radiation (Bautista-Saraiva et al., *unpublished data*), whereas MAAs were not detected in *B. turfosum* by Aigner et al. (2017).

Typical sun-adapted lotic macroalgae, *C. glomerata* and *Spirogyra* sp., presented distinct responses to exposure of UV radiation as regard to photosynthetic performance and chlorophyll *a* concentrations. A more pronounced decrease of F_v/F_m and increase of *NPQ* under UV-A than UV-AB was observed, although chlorophyll *a* content was lower under UV-AB in *C. glomerata*. *Spirogyra* sp. had a decrease of photosynthesis ($\Delta F/F_m'$ and F_v/F_m) indicating that UV-AB radiation is an important source energy in the photosynthetic apparatus. Surprisingly, *S. delicatula*, a typical shaded-adapted alga, exhibited less sensitivity to UV exposure. These results suggest that the presence of MAAs is a significant shield of protection against UV radiation. The plasticity of photosynthesis is a major attribute that enables autotrophic organisms to balance energy conversion and energy consumption by acclimation of the photosynthetic apparatus (Ensminger et al. 2001) and, thus, they are able to cope with light climate variations in terms of global changes.

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Table 1. Parameters derived from the P-E curves, corresponding to Fig.1, and Chlorophyll *a* content (Chl*a)* comparing Pre (initial-control), P, PA and PAB in *Cladophora glomerata, Spirogyra* sp. and *Sirodotia delicatula* after 10 days. Data are expressed as means ± standard-deviation (n=5 for P, PA and PAB; n=15 for control). Distinct letters indicate significant differences ($p < 0.05$) by ANOVA (Newman-Keuls Post-hoc).

	Treatment	*C. glomerata*	*Spirogyra* sp.	*S. delicatula*
ETR_{max} (µmol electrons m^{-2} s^{-1})	Pre	22.37±3.02[a]	15.56±3.37[a]	2.89±0.52[a]
	P	21.43±5.67[a]	5.43±3.45[b]	1.24±0.13[b]
	PA	19.69±1.87[a]	8.43±1.77[b]	1.22±0.16[b]
	PAB	19.86±0.48[a]	6.09±2.30[b]	1.24±0.09[b]
E_k (µmol photons m^{-2} s^{-1})	Pre	125.51±8.61[a]	95.44±17.09[a]	45.98±8.72 [a]
	P	141.38±13.12[b]	83.23±24.52[a]	83.70±9.39[b]
	PA	134.68±6.64[a,b]	127.61±15.49[b]	86.71±2.66[b]
	PAB	133.59±1.95[a,b]	84.27±22.73[a]	86.52±7.23[b]
α_{ETR}	Pre	0.178±0.014[a]	0.158±0.014[a]	0.060±0.010[a]
	P	0.150±0.027[b]	0.061±0.020[b]	0.015±0.002[b]
	PA	0.149±0.011[b]	0.066±0.012[b]	0.015±0.002[b]
	PAB	0.149±0.004[b]	0.073±0.018[b]	0.014±0.001[b]
β_{ETR}	Pre	20.01±1.34[a]	26.44±5.30[a]	48.20±7.76[a]
	P	17.81±1.71[b]	31.62±8.08[a]	30.19±3.35[b]
	PA	18.96±1.23[a,b]	19.79±2.31[a]	30.43±3.35[b]
	PAB	18.72±0.27[a,b]	31.93±10.78[a]	29.09±2.58[b]
Chl*a*	P	0.03±0.01[a]	0.02±0.01[a]	0.08±0.04[a]
	PA	0.04±0.01[a]	0.02±0.01[a]	0.04±0.01[a]
	PAB	0.01±0.01[b]	0.02±0.01[a]	0.05±0.02[a]

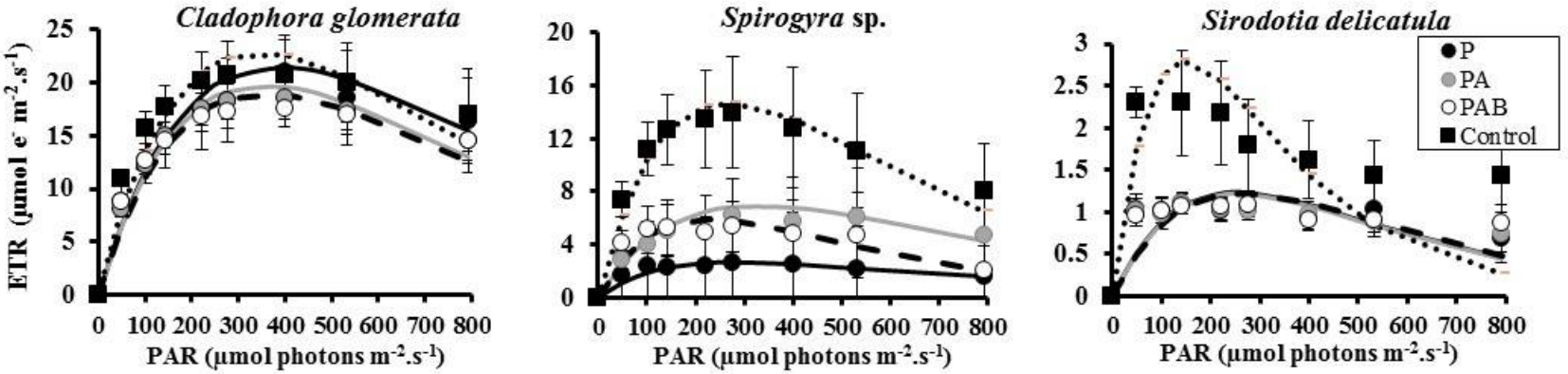

Fig1. P-E curves for *Cladophora glomerata*, *Spirogyra* sp. and *Sirodotia delicatula* under Pre (Control) and UVR treatments (P, PA and PAB). Data are expressed as means ± standard-deviation. (n=5 for P, PA and PAB; n=15 for Control).

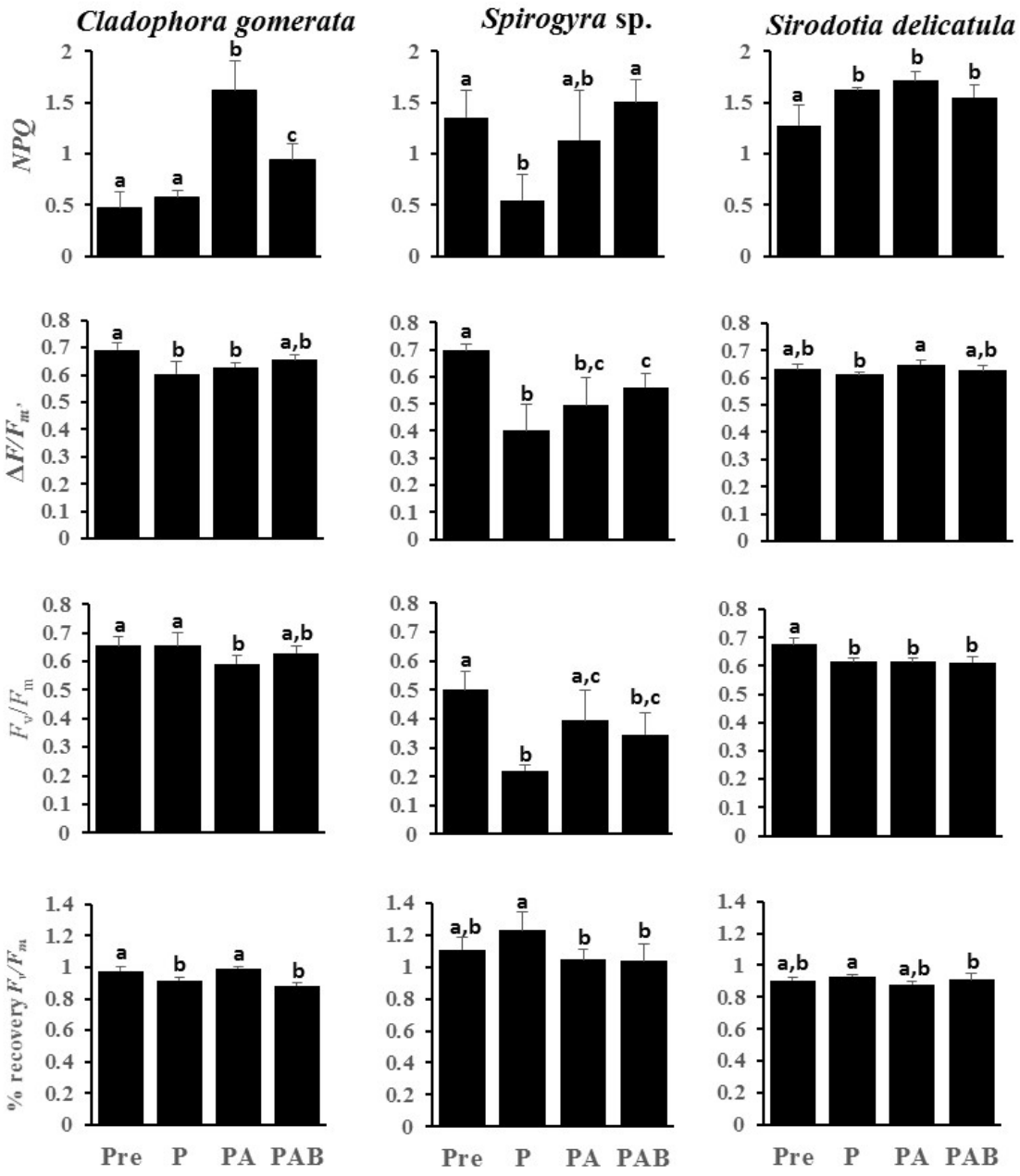

Fig. 2. Variables (NPQ, $\Delta F/F_m'$, F_v/F_m and recovery capacity of F_v/F_m) for *Cladophora glomerata*, *Spirogyra* sp. and *Sirodotia delicatula* under Pre (Control) and UVR treatments (P, PA and PAB). Data are expressed as means ± standard-deviation. (n=5 for P, PA and PAB; n=15 for Control). Distinct letters indicate significant differences ($p < 0.05$) by ANOVA (Newman-Keuls Post-hoc).

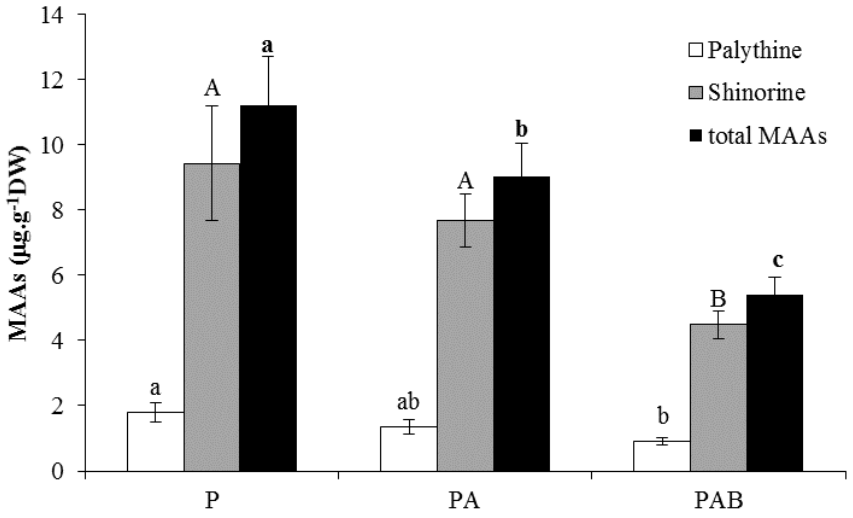

Fig. 3 Mycosporine-like amino acids (MAAs) of *Sirodotia delicatula* in P, PA and PAB treatments. Data are expressed as means ± standard-deviation (n=5). Distinct letters indicate significant differences ($p<0.05$) by ANOVA (Newman-Keuls Post-hoc).

Supporting information

Table S1 Absolute irradiance and daily doses (KJ m^{-2}) of UVB, UVA and PAR applied to *Cladophora glomerata, Spirogyra* sp. and *Sirodotia delicatula* in different treatments (P, PA and PAB) at laboratory. In addition, data of environmental and UVB / UVA ratio is included

	Absolute Irradiance[a] ($W.m^{-2}$)			**Daily dose ($KJ.m^{-2}$)**			
	P	**PA**	**PAB**	**P**	**PA**	**PAB**	**Environment**
PAR	12.15	12.58	12.65	524.95	543.66	546.61	1762.09
UVA	0.09	1.09	1.20	4.03	47.07	51.99	984.53
UVB	0.10	0.11	0.30	4.12	4.83	13.07	213.75
Total	12.34	13.78	14.15	533.10	595.56	611.67	2960.37
UVB/UVA			0.25				0.22

[a] Measured with a spectroradiometer UV-Visible USB2000 + RAD (Ocean Optics, Dunedin, USA).

CONCLUSÃO

Este foi o primeiro trabalho que reportou não somente os efeitos da radiação UV sobre o desempenho fotossintético de macroalgas continentais, bem como descreveu a presença de MAAs em duas espécies de algas vermelhas de água doce. Os estágios do histórico de vida (gametófito e esporófito) de *K. ambigua* em condições de cultura responderam de forma distinta à radiação UV. Observou-se um aumento da performance fotossintética, além da presença de shinorina (MAAs), no gametófito sob UVA. Nossos resultados sugerem que o gametófito é menos sensível à radiação UV, particularmente UVA, em comparação com o esporófito em condições de cultura. Além disso, corroboram estudos que indicam que a radiação UVA, quando moderada, possui efeitos benéficos em alguns organismos aquáticos. Espécimes de campo de *S. delicatula* testados em laboratório apresentaram dois tipos de MAAs (shinorina e palatina) e foram praticamente insensíveis aos tratamentos com radiação UV. Estes resultados sugerem que não somente a presença de MAAs é um importante escudo de proteção contra radiação UV, mas também demonstram que apesar de ser uma rodófita tipicamente adaptada à sombra, pode tolerar altas irradiâncias, incluindo-se a radiação UV. Por outro lado, *C. glomerata* e *Spirogyra* sp., apesar de serem algas verdes adaptadas ao sol, apresentaram respostas distintas no desempenho fotossintético e concentrações de clorofila *a* sob exposição da radiação UV. Em *C. glomerata* foi observada uma diminuição mais acentuada de alguns parâmetros fotossintéticos em UVA em relação a UVAB, apesar de menor teor de clorofila *a* ter sido observado em UVAB. *Spirogyra* sp. apresentou uma diminuição da performance fotossintética principalmente em PAR, sugerindo que a radiação UVAB é importante na indução de fotoreparo e/ou uma fonte energia para o aparelho fotossintético. *Scytonema* sp. e *Leptolyngbya* cf. *henningsii* apesar de serem cianobactérias aerofíticas e, portanto, sujeitas a altas irradiações, também apresentaram respostas distintas à exposição da radiação UV quanto ao desempenho fotossintético, às concentrações de pigmentos e aos compostos absorvedores de UV. A ausência de

MAAs e de scitoneminas e a pouca variação do desempenho fotossintético indicam que *L.* cf. *henningsii* foi insensível à exposição a UV nas condições testadas. Além disso, o aumento do conteúdo de carotenóides sob UVA e UVAB sugerem que os atuaram como escudo eficaz para a radiação UV. Um aumento da atividade fotossintética em UVA sugere que *Scytonema* sp. é capaz de usar UVA como fonte de energia para a fotossíntese, e/ou em mecanismos de reparo relacionados ao DNA. No entanto, a diminuição do desempenho fotossintético observada em UVAB, apesar de valores significativamente mais elevados de MAAs, indicam que *Scytonema* sp. apresentou maior sensibilidade à radiação UVAB. Além disso, sugere que as MAAs presentes não foram suficientemente eficazes para evitar os efeitos negativos da radiação.

Os resultados deste trabalho indicam que as respostas da performance fotossintética, conteúdo de pigmentos e compostos que absorvem UV à radiação UV podem ser espécie-específicas, havendo até mesmo diferenças intra-específicas, como no caso dos estágios de vida de *K. ambigua*. Uma combinação de diferentes estratégias pode ser usada pelos organismos fotossintéticos e isso não está necessariamente associado aos grupos de algas (filos) a que pertencem, mas igualmente influenciado pelas características do hábitat (especialmente da exposição à radiação), estratégias ecológicas e adaptativas, entre outras.

A plasticidade da fotossíntese é um atributo essencial que permite aos organismos autotróficos equilibrarem a conversão de energia e o consumo de energia através da aclimatação do aparelho fotossintético e, portanto, serem capazes de sobreviver às adversidades ambientais, incluindo-se a exposição à radiação UV. Com isso, nossas duas hipóteses iniciais foram refutadas: a primeira, pois nem sempre os indivíduos responderam de forma consistente com tendência à diminuição de sua performance fisiológica à radiação ultravioleta. A segunda, pois *C. glomerata* e *Spirogyra* sp, típicas algas verdes adaptadas ao sol, não mostraram ser mais tolerantes à radiação UV em relação às algas vermelhas, consideradas adaptadas à sombra. Estes resultados são muito relevantes como uma primeira abordagem para melhor compreensão da adaptação e aclimatação fisiológica de macroalgas lóticas tropicais às mudanças globais do clima, notadamente radiação UV.