

Universidade Federal de
Santa Catarina

Programa de Pós-
Graduação em Ecologia

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Campus Universitário
Florianópolis- SC

Dissertação apresentada ao Programa de Pós-
graduação em Ecologia, Departamento de
Ecologia e Zoologia, do Centro de Ciências
Biológicas da Universidade Federal de Santa
Catarina, como requisito para obtenção do
Título de Mestre em Ecologia.

Orientador: Prof. Dr. Paulo A. Horta

Coorientadora: Profa. Dra. Nadine Schubert

Florianópolis, 2018

IMPLICAÇÕES FISIOLÓGICAS E ECOLÓGICAS DE INTERAÇÕES INTERESPECÍFICAS NOS BENTOS MARINHO:
SUBSÍDIO PARA O ENTENDIMENTO DE CENÁRIOS ATUAIS E FUTUROS
Walter Ambrose Rich IV

IMPLICAÇÕES FISIOLÓGICAS E ECOLÓGICAS DE
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MARINHO: SUBSÍDIO PARA O ENTENDIMENTO DE
CENÁRIOS ATUAIS E FUTUROS

Walter Ambrose Rich IV

Estudo fisiológico das
algas marinhas e um
ouriço-do-mar

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Dissertação submetido(a) ao
Programa de ecologia da
Universidade Federal de Santa
Catarina para a obtenção do
Grau de mestre em ecologia
Orientador: Prof. Dr. Paulo
Antunes Horta Jr.
Coorientadora: Prof. Dra.
Nadine Schubert

Florianópolis
2018

Ficha de identificação da obra elaborada pelo autor
através do Programa de Geração Automática da Biblioteca
Universitária da UFSC.

Rich IV, Walter Ambrose

Implicações fisiológicas e ecológicas de interações
interespecíficas nos bentos marinhos : subsídio para
o entendimento de cenários atuais e futuros /
Walter Ambrose Rich IV ; orientador, Paulo Horta,
coorientador, Nadine Schubert, 2018.

101 p.

Dissertação (mestrado) - Universidade Federal de
Santa Catarina, Centro de Ciências Biológicas,
Programa de Pós-Graduação em Ecologia, Florianópolis,
2018.

Inclui referências.

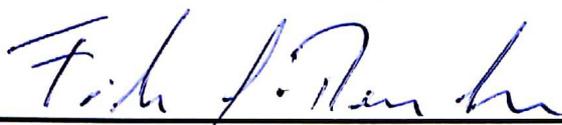
1. Ecologia. 2. Fisiologia. 3. Mudanças
Climáticas. 4. Herbivoria. 5. Algas Marinhas. I.
Horta, Paulo. II. Schubert, Nadine. III.
Universidade Federal de Santa Catarina. Programa de
Pós-Graduação em Ecologia. IV. Título.

“Implicações fisiológicas e ecológicas de interações interespecíficas nos bentos marinho-subsídio para o entendimento de cenários atuais e futuros”

Por

Walter Ambrose Rich IV

Dissertação julgada e aprovada em sua forma final pelos membros titulares da Banca Examinadora (11/2018/PPGECO) do Programa de Pós-Graduação em Ecologia - UFSC.



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Florianópolis, 02 de março de 2018.

Este trabalho é dedicado aos meus colegas de classe e aos meus queridos pais.

AGRADECIMENTOS

Agradeço acima de tudo aos meus pais que sempre estiveram ao meu lado, me dando todo suporte para que eu pudesse seguir esse sonho. Obrigada pelo apoio incondicional e confiança que vocês sempre depositaram em mim.

Às minhas colegas do LAFIC, especialmente Lídi Gouvea, Vanessa Carvalho, Giulia Costa, Eduardo Bastos, Thais Massocato, Caroline Schmitz, Manuela Batista, Antonella Almeida e Manuel Bercovich que me ajudaram com as coletas e com as análises de laboratório, sempre proporcionando momentos de descontração, alegria e muitas risadas.

Aos professores do LAFIC, especialmente ao Prof. Dr. José Bonomi Barufi e Prof. Dr. Leonardo Rörig, que sempre se colocaram a disposição para me ajudar e ensinar um pouco mais sobre as algas.

Aos meus colegas do Programa de Pós-Graduação em Ecologia, gostaria de agradecer em especial a Clarissa Teixeira, Lorena Pinheiro, Alessandra Carneiro, Ricardo Freitas e Lucas Machado; a amizade de vocês fez dessa experiência no Brasil um período único e inesquecível!

Por fim agradeço meus orientadores, Prof. Dr. Paulo Horta e Profa. Dra. Nadine Schubert pela oportunidade, paciência, ensinamentos e conselhos que me ajudaram e vieram a contribuir para minha formação pessoal e profissional. Esse projeto não seria possível sem a ajuda de vocês!

“Many times I’ve wondered how much there is to know”-Led Zeppelin, 1973

RESUMO

As interações bióticas são cada vez mais conhecidas para moldar a estrutura das comunidades e o funcionamento de ecossistemas. Recentemente, as interações estiveram no foco de um bom número de esforços acadêmicos considerando as implicações das mudanças globais, especialmente o aquecimento do oceano (OW) e a acidificação dos oceanos (OA) nos ecossistemas marinhos. Nos ambientes costeiros, as macroalgas estão entre os taxons mais importantes, pois são frequentemente os produtores primários mais abundantes e formam a base das redes alimentares. No entanto, devido à sua natureza sésstil, elas são especialmente vulneráveis aos efeitos das mudanças climáticas. A fim de compreender melhor como as interações das espécies serão afetadas pelos estressores globais, é necessária uma linha de base, caracterizando como as interações entre espécies operam nas condições atuais.

O primeiro capítulo desta tese tenta caracterizar os efeitos das interações interespecíficas na fisiologia das macroalgas e os efeitos subsequentes na palatabilidade para um herbívoro, o ouriço-do-mar *Echinometra lucunter*. Os espécimes de *Jania rubens*, *Sargassum cymosum* e *Ulva lactuca* foram coletados de manchas monoespecíficas ou de "associações", onde os indivíduos estavam em contato físico com uma das outras respectivas espécies, tanto no verão como no inverno. A fotossíntese líquida, a atividade da redutase de nitrogênio e o conteúdo de pigmento, fenol e carbonato de algas foram avaliados entre associações diferentes nas duas estações. Os resultados indicam que, além da variação sazonal na maioria dos parâmetros medidos, as interações entre algas podem mudar em magnitude e sinal (positivo, negativo ou neutro) em diferentes estações. O teste de herbivoria sem escolha revelou que *Jania* e *Ulva* foram consumidos em taxas mais elevadas quando estavam associados um ao outro, enquanto que *Sargassum* não foi afetado. Esses resultados sugerem que interações interespecíficas de macroalgas podem influenciar a fisiologia dos protagonistas das interações, o que pode afetar sua palatabilidade, interferindo de maneira importante na estrutura e papel ecológico das comunidades fitobênticas.

No Segundo capítulo avaliamos os efeitos dos estressores globais relacionados as mudanças climáticas sobre as espécies e suas interações. Para isso em mesocosmo avaliamos a biologia de algas calcárias e entre esta e herbívoros em condições de OW e OA. A alga preferencialmente consumida do primeiro experimento foi selecionada para este teste

(*Jania rubens*) com o ouriço-do-mar *E. lucunter*. Para isso em tanque foram estabelecidas em quadruplicate, por 21 dias, condições controle (atual), condição OW (aquecida), OA (acidificada) e OW + OA (aquecida e acidificada). A fisiologia de *Jania* não foi afetada pelo aumento da temperatura (+ 4 ° C) e adição de CO₂ (1,000 ppm), mas as alterações na composição bioquímica do tecido algal foram encontradas. As taxas metabólicas do ouriço-do-mar *E. lucunter* foram maiores no tratamento de temperatura ambiente e alto pCO₂, e ensaios de alimentação mostraram que isso influenciou o consumo, com taxas de alimentação aumentadas neste tratamento. Os resultados aqui mostram que, embora a composição bioquímica de algas tenha sido afetada pelo futuro pCO₂, pelo menos no curto prazo, os efeitos diretos para o metabolismo do ouriço do mar foram mais importantes para impactar essa interação com algas e herbívoros.

Palavras-chave: Mudanças climáticas 1. Fisiologia 2. Interações entre espécies 3.

ABSTRACT

Biotic interactions are increasingly known to shape ecosystem community structure. Recently, there has been a renewed focus on species interactions in light of global change, especially ocean warming (OW) and ocean acidification (OA) in marine ecosystems. In coastal environments, macroalgae are among the most important taxa as they are often the most abundant primary producers and form the base of food webs. However, due to their sedentary nature, they are also vulnerable to the effects of climate change. In order to better understand how species interactions will be affected by climate change stressors, a solid understanding of how interspecies interactions operate under present-day conditions is needed.

The first chapter of this thesis attempts to characterize seasonal variation in macroalgal physiology and biochemistry, and how interspecific interactions might affect algal fitness and palatability to a sea urchin herbivore (*Echinometra lucunter*). Specimens of *Jania rubens*, *Sargassum cymosum*, and *Ulva lactuca* were collected from monospecific patches or from “associations”, where individuals were in physical contact with another species, in both summer and winter. Net photosynthesis, nitrogen reductase activity, and pigment, phenolic and carbonate content of algae were evaluated among different associations across the two seasons. The results indicate that in addition to seasonal variation in most parameters measured, interactions between algae could change in both magnitude and sign (positive, negative or neutral) in different seasons. The no-choice herbivory assay (conducted in winter) revealed that both *Jania* and *Ulva* were consumed at higher rates when they were associated with each other, whereas *Sargassum* was not affected. These results suggest that macroalgae may influence the physiology and biochemical composition of neighboring species and subsequently affect their palatability, which may influence local community structure.

To further evaluate effects of species interactions under climate change stressors, an experiment was performed to assess algal-herbivore interactions under OW and OA conditions. The most preferentially consumed algae from the first experiment (*Jania rubens*) and the sea urchin *E. lucunter* were evaluated in a 21-day mesocosm study with treatments of control, OW, OA, and OW+OA. Algal physiology was unaffected by increased temperature (+4°C) and pCO₂ (1,000 ppm), but changes in the biochemical composition of the algal tissue were found. Metabolic rates of the sea urchin *E. lucunter* were higher in the ambient

temperature, high pCO₂ treatment, and feeding assays showed that this influenced consumption, with increased feeding rates in this treatment. The results here show that although algal biochemical composition was affected by future pCO₂, at least in the short term, direct effects to sea urchin metabolism were more important for impacting this algae-herbivore interaction.

Keywords: Climate change 1. Physiology 2. Species interactions 3.

LISTA DE FIGURAS

Capítulo 1

- Figure 1** –Examples of algal species collected from different interactions in the field at Barra da Lagoa beach, southern Brazil. A) Monospecific *Jania*, B) Monospecific *Sargassum*, C) Monospecific *Ulva*, D) *Jania* and *Sargassum* in contact with each other, E) *Jania* and *Ulva* in contact with each other, F) *Sargassum* and *Ulva* in contact with each other. 39
- Figure 2** – Monthly mean maximum air temperature and mean daily irradiance in Florianopolis, Brazil from September 2016 to August 2017. 39
- Figure 3** –Seasonal variation in photosynthetic performance (summer- solid bars, winter- striped bars). (a) Maximum photosynthetic efficiency (Fv/Fm) and (b) net photosynthesis of algae in monospecific patches and in association with other species. Data represent mean±SE (n=6) and different letters indicate significant differences within each species (p<0.05, Tukey HSD). 45
- Figure 4** –Total chlorophyll content of algae collected in summer (solid bars) and winter (striped bars). Data represent mean±SE (n=6) and different letters indicate significant differences within each species (p<0.05, Tukey HSD). 47
- Figure 5** –Nitrogen reductase activity of algae collected in summer (solid bars) and winter (striped bars). Data represent mean±SE (n=5) and different letters indicate significant differences within each species (p<0.05, Tukey HSD). 51
- Figure 6** –Carbonate content of *Jania* collected in summer (solid bars) and winter (striped bars). Data represent mean±SE (n=6) and different letters indicate significant differences (p<0.05, Tukey HSD). 52
- Figure 7** –Total phenolic content of algae collected in summer (solid bars) and winter (striped bars). Data represent mean±SE (n=6) and different letters indicate significant differences within each species (p<0.05, Tukey HSD). 53
- Figure 8** –Consumption rates of algae by a sea urchin herbivore (winter only). Data represent mean±SE (n=4) and different letters indicate significant differences within each species (p<0.05, Tukey HSD) 54

Capítulo 2

Figure 1 –Schematic representation of the mesocosm system used for the study. A large tank acted as a water bath (1) in which two treatment tanks (2) were immersed. Temperature of the water bath was maintained by 300W heaters and pumping the water through chillers. Water within treatment tanks was constantly circulated via water pumps (3) into separate mixing tanks (4), where either CO₂-enriched air or ambient air was bubbled to attain the desired pCO₂ concentrations. A total of eight water bath tanks was used for the experiment, with four set to 24°C and four set to 28°C. 77

Figure 2 –Response of *Jania rubens* to different temperature and pCO₂ treatments. (a) Photosynthesis and respiration, (b) carbonate content, and (c) phenolic content. Data present mean±SE (n=4) and different letters indicate significant statistical differences (ANOVA, p<0.05, Newman Keuls). 81

Figure 3 –Sea urchin responses to different temperature and pCO₂ treatments. (a) Sea urchin respiration and (b) weekly feeding rates during the experiment. Sea urchin respiration and feeding are standardized by test diameter of individuals. Data present mean±SE (n=4) and different letters indicate significant statistical differences (ANOVA, p<0.05, Newman Keuls)..... 81

LISTA DE TABELAS

Capítulo 1

Table 1 -ANOVA statistics of physiological parameters of algae from different species interactions (monospecific or in contact with one of the other two species) and seasons (Austral summer= March; Austral winter=September).....	46
Table 2 -ANOVA statistics of biochemical parameters of algae from different species interactions (monospecific or in contact with one of the other two species) and seasons (Austral summer= March; Austral winter=September).....	48
Table 3 -ANOVA statistics of sea urchin feeding rates on algae from different species interactions (monospecific or in contact with one of the other two species). The no-choice feeding assay was performed during Austral winter (September) only.....	50
Table 4 -Seasonal variation in pigment ratios of the studied algal species, in presence or absence of other species. Data represent mean±SE (n=6) and different superscript letters indicate significant differences (Post-hoc TukeyHSD).....	55

Capítulo 2

Table 1 -Carbonate system parameters. Values calculated using CO2Calc with total alkalinity and pH _{tot} as input parameters.....	77
Table 2 -Summary of two- and three-way ANOVAs examining the influence of temperature and pCO ₂ (and time, in case of the feeding rates) on measured parameters.....	82

LISTA DE ABREVIATURAS E SIGLAS

CO₂ – Carbon dioxide

Fv/Fm – Maximum quantum yield

NRA – Nitrogen Reductase activity

OA – Ocean acidification

OW – Ocean warming

ppm – Parts per million

μmol – micromols of photons

SUMÁRIO

INTRODUÇÃO GERAL.....	27
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CAPÍTULO 1

1. INTRODUCTION.....	35
2. MATERIALS AND METHODS	38
3. RESULTS	43
4. DISCUSSION	55
5. REFERENCES.....	64

CAPÍTULO 2

1. INTRODUCTION.....	73
2. MATERIALS AND METHODS	75
3. RESULTS	80
4. DISCUSSION	83
5. CONCLUSION.....	85
6. REFERENCES.....	86

CONCLUSÃO GERAL.....	95
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INTRODUÇÃO GERAL

Interações bióticas são conhecidas por moldarem a estrutura de comunidades em ecossistemas marinhos. Diversos estudos têm demonstrado que fatores abióticos como temperatura, precipitação, disponibilidade de nutrientes, e irradiação solar podem descrever a distribuição e a abundância das espécies (Hurd et al. 2014). Porém, sabe-se também que interações entre espécies são determinantes nos padrões de escala locais de comunidades (Raffaelli & Hawkins 2012).

As interações interespecíficas são definidas como positivas (i.e., facilitação, mutualismo) em que o desempenho de um organismo é aumentado pela interação com outra espécie; negativas (i.e., competição, predação, parasitismo), em que o desempenho de uma das espécies é diminuída; ou neutro (i.e., comensalismo), em que não há efeitos aparentes no desempenho físico. Entretanto, os tipos de interação podem mudar no espaço (ou seja, sobre os gradientes ambientais ou de estresse) e no tempo (sazonalmente ou em diferentes estágios da vida de um mesmo organismo), de modo que uma interação positiva em uma situação pode tornar-se neutra ou negativa em outra (Leonard 2000, Brown et al., 2001, Kordas et al., 2011). Estudos clássicos em habitats intermareais demonstraram a importância das interações bióticas na regulação da riqueza de espécies, abundância e padrões espaciais e temporais na distribuição de espécies (Paine 1966, Lubchenco 1978, Lubchenco & Menge, 1978). Porém, ainda existem lacunas críticas na compreensão de como os efeitos sutis das interações das espécies podem moldar a estrutura da comunidade.

Um dos grupos mais notáveis em comunidades intermareais são as macroalgas. Esses produtores primários formam a base de redes alimentares marinhas em áreas costeiras e são componentes importantes das comunidades bentônicas marinhas (Hurd et al., 2014). As assembleias de macroalgas são muitas vezes diversas em espécies, e com espaço limitado e recursos disponíveis, as algas frequentemente são encontradas em associação (ou seja, em contato físico direto) com outras espécies de algas. Assim, o potencial para as interações das espécies é alto e, de fato, as interações entre algas têm sido bem documentadas. Por exemplo, o gênero oportunista *Ulva* pode superar outras algas na obtenção de recursos em piscinas de maré (Beer et al., 2014), ou produzir compostos alelopáticos que inibem o crescimento de outras espécies próximas (Friedlander et al., 1996). Há também exemplos de interações positivas, nas quais as algas formadoras de copa podem facilitar o crescimento, o recrutamento e a sobrevivência das algas em

baixo do dossel, fornecendo refúgio contra a dessecação e alto estresse de irradiância solar (Bertness & Leonard 1997, Amsler 2008, Barner et al., 2016).

Embora as interações entre espécies de algas possam influenciar a estrutura das comunidades intermareais, é válido ressaltar a importância das interações entre as algas e os herbívoros. A herbivoria é um dos fatores mais importantes que controlam a distribuição e a abundância de macroalgas em ambientes marinhos (Hay & Fenical, 1988). Devido às altas taxas de herbivoria, muitas algas desenvolveram defesas químicas e físicas para minimizar o consumo por herbívoros (Hay & Fenical, 1988). As taxas de herbivoria também podem ser afetadas por outros fatores, tais como temperatura (O'Connor 2009) ou condição relativa do herbívoro e/ou das algas (Duarte et al., 2016); portanto, as interações entre algas e herbívoros são complexas e dinâmicas. A importância das interações entre algas e herbívoros é claramente demonstrada em sistemas perturbados, em que um aumento nas populações de herbívoros causou um declínio nas florestas de algas (Estes & Palmisano 1974) e a sobrepesca em recifes de coral resultou em redução da herbivoria, causando uma mudança de fase de um sistema dominado por corais e algas (Hughes et al., 2007).

Recentemente, diversos estudos têm tido como foco as mudanças globais e suas consequências nas interações entre espécies (Montoya et al., 2010, Kordas et al., 2011, Bozinovic & Pörtner 2015, Riebesell et al., 2015). As atividades antropogênicas, principalmente a queima de combustíveis fósseis, estão causando um rápido aumento nas concentrações globais de dióxido de carbono (CO₂) (IPCC 2014). O ambiente marinho é particularmente impactado, já que os oceanos absorvem cerca de 90% da energia presa por emissões antropogênicas de gases de efeito estufa, causando o aquecimento dos oceanos (OW) (IPCC 2014). Além disso, a química do carbonato dos oceanos está mudando para um novo equilíbrio, causando uma queda no pH de 0,3 unidades desde a revolução industrial e resultando na acidificação dos oceanos (OA) (Doney et al., 2009). Essas mudanças de condições afetarão as interações das espécies (Harley et al., 2012, Harvey et al. 2013, Kroeker et al., 2013). Em particular, as interações salgas-herbívoros marinhos provavelmente serão afetadas por OW e OA, considerando que as algas marinhas muitas vezes contêm defesas químicas complexas de alto custo metabólico (Harley et al., 2012, Poore et al., 2013) e as taxas de herbivoria podem ser parcialmente afetadas pela temperatura (O'Connor 2009).

Embora muitos estudos abordem as interações entre espécies intermareais, ainda há uma escassez de informações básica para as interações entre algas nas condições atuais, especialmente no que se refere a como essas interações podem afetar a herbivoria. Tal informação é especialmente importante para estudos de mudanças globais, nos quais as respostas são comparadas com o conhecimento de base, que atualmente não é totalmente compreendida. Além disso, muitos estudos sobre mudanças climáticas avaliam os efeitos diretos de OW e OA em uma espécie e, em seguida, testam os efeitos indiretos das interações das espécies em condições de controle. Embora informativos, tais estudos não capturam os efeitos diretos de OW e OA em ambas as espécies em questão, o que de fato pode alterar o resultado.

Nessa dissertação, eu levanto essas diferentes questões reunindo dados de base sobre as interações entre diferentes espécies de algas e entre algas e herbívoros. Em seguida, demonstrei tais questões em um experimento sobre os efeitos das mudanças climáticas em uma interação alga-herbívoro nas condições OW e OA. Assim, o objetivo geral deste estudo é compreender como as associações interespecíficas afetam a fisiologia dos indivíduos e avaliar como algumas dessas interações podem mudar sob os estressores das alterações climáticas. No primeiro capítulo, eu avaliei interações entre espécies de macroalgas intermareais comuns em condições de campo para testar as seguintes hipóteses: 1) interações interespecíficas alteram a fisiologia e a composição bioquímica de macroalgas individuais, 2) as interações entre algas variam sazonalmente, e 3) interações interespecíficas causa mudanças nas taxas de alimentação das macroalgas para um herbívoro do ouriço-do-mar. No segundo capítulo, eu utilizo a alga *Jania rubens* (a espécie de alga de maior preferência por herbívoros no primeiro capítulo) e ouriços-do-mar para realizar um experimento de mesocosmo em condições de OW e OA. Aqui, teste as hipóteses de que 1) As condições de OW e OA afetam a fisiologia e a composição bioquímica de uma alga calcária e ouriço-do-mar, e 2) As condições de OW e OA alteram as taxas de alimentação de um herbívoro de ouriço-do-mar nesta alga calcária. Os resultados apresentados ajudarão a esclarecer como as interações dessas espécies podem mudar em futuros cenários de mudanças globais.

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CAPÍTULO 1

Seasonal variation in intertidal macroalgal physiology: Exploring the importance for species interactions

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Abstract:

Intertidal macroalgal communities are shaped by various abiotic and biotic factors. For instance, seasonal changes in abiotic conditions cause predictable changes in algal physiology. In addition, biotic interactions can have positive, negative, or neutral effects on species, and are important forces which determine community structure. However, little is known about more subtle effects of species interactions on the physiology of individuals, especially in different seasons. Here, we conducted a seasonal evaluation of the physiological and biochemical effects of interspecific interactions between three common species of macroalgae in the field, and possible indirect effects on their palatability to an herbivore. Specimens of *Jania rubens*, *Sargassum cymosum*, and *Ulva lactuca* were collected from monospecific patches or from “associations”, where individuals were in physical contact with another species, in both summer and winter. Net photosynthesis, nitrogen reductase activity, and pigment, phenolic and carbonate content of algae were evaluated among different associations across the two seasons. The results indicate that in addition to seasonal variation in most parameters measured, interactions between algae could change in both magnitude and sign (positive, negative or neutral) in different seasons. The no-choice herbivory assay (conducted in winter) revealed that both *Jania* and *Ulva* were consumed at higher rates when they were associated with each other, whereas *Sargassum* was not affected. The results here suggest that although predictable variations in macroalgae occur between seasons, species interactions may influence these seasonal changes and can affect their palatability, which may influence local community structure.

Keywords: acidification, algae, climate change, *Echinometra lucunter*, *Jania rubens*, warming

Introduction

Rocky intertidal habitats are dynamic environments whose communities are subject to various biotic and abiotic stressors (Raffaelli & Hawkins 2012). These ecosystems often are characterized by high biomass and diversity of macroalgal species, which create complex habitat for infauna and epiphytes and are highly productive primary producers which form the base of trophic webs (Hurd et al. 2014). The structure and function of intertidal macroalgal communities is variably both spatially and temporally, and depends on abiotic factors including temperature, salinity, wave action, desiccation, irradiance, and nutrient levels (Hurd et al. 2014). These factors influence the physiology and biochemical composition of macroalgae that vary between seasons as individuals acclimate to changing conditions. For example, as photosynthetic organisms, algae must adjust their physiological machinery to account for variable irradiance throughout the year. Such adjustments include regulating production of photosynthetic pigments (chlorophyll and accessory pigments) and photoprotective compounds (i.e., phenolics), which ultimately determine algal photosynthesis (Beer et al. 2014).

In general, intertidal macroalgae produce less phenolics in winter months (Plouguerné et al. 2006, Khairy & El-Sheik 2015, Celis-Plá et al. 2016) as irradiance declines and there is less need for photoprotection, while pigment content increases to improve light-harvesting capacity (Waaland et al. 1974, Ismail & Osman 2016). However, photosynthesis is also affected by changing temperatures and nutrient availability, which often change predictably with the seasons. Thus, other aspects of macroalgal physiology, such as nutrient assimilation and calcification, are dependent on seasonally variable environmental conditions (Wheeler & Weidner 1983, Davidson & Stewart 1984, Gao et al. 1992, Young et al. 2007).

In addition to environmental factors, biotic interactions, such as herbivory, facilitation, and competition play large roles in determining the structure and functioning of algal communities (Lubchenco 1978, Lubchenco and Menge 1978, Cubitt 1984, Bertness & Leonard 1997). Negative interactions (competition) are well-studied, where macroalgae can impede the growth of other species by being superior competitors in terms of resource acquisition or production of allelopathic compounds (Harlin & Rice 1987, Carpenter 1990, Olson 1990, Paine 1990, Friedlander et al. 1996, Amsler 2008, Coombes et al. 2013). Conversely, positive interactions (facilitation) are also important biotic interactions that can foster higher macroalgal fitness by increasing the suitability of habitat (e.g., canopy-forming algae reducing irradiance, desiccation and wave stress for understory species) or “concealing” other macroalgal species from herbivory (Hay 1986, Bertness & Leonard 1997, Amsler 2008, Barner et al. 2016). Biotic interactions can also lead to subsequent knock-on effects with other species, especially with regard to herbivores. For instance, some macroalgae in competition with corals upregulate production of allelopathic compounds but become more susceptible to herbivory, possibly as a consequence of a trade-off between production of allelopathic and anti-herbivory compounds (Rasher & Hay 2014, Longo & Hay 2015). While such macroalgal-coral interactions have been extensively studied, investigations of interactions between two or more macroalgal species are less numerous. Notable exceptions are studies conducted in tidal pools, where some species of macroalgae can alter water quality characteristics that affect the ecophysiology and fitness of other species (Harlin & Rice 1987, Beer et al. 2014).

Importantly, the strength (magnitude of response) and sign (positive, negative or neutral) of species interactions is often context-dependent, and can change depending on life history stage (Kordas & Dudgeon 2011), environmental conditions (Leonard 2000, Chamberlain et al. 2014), or disturbance (Brown et al. 2001). Despite evidence of interspecific interactions affecting algal fitness, there are few studies examining individual intertidal algal-algal interactions in natural field settings. In particular, field studies to date have often evaluated

interactions through the lens of population ecology to assess abundance and growth of individuals, but less often investigate physiological effects of interactions. Furthermore, although general trends of seasonal variation in algal physiology and biochemical composition are well known, there is a paucity of studies evaluating seasonal variation together with biotic interactions. Young & Gobler (2017) showed in field experiments that negative effects of competition with macroalgae only appeared in certain months, suggesting that biotic interactions may be enhanced or moderated depending on season. Indeed, changing environmental conditions, such as temperature and nutrient enrichment, can cause algal blooms as the competitive balance tips in the favor of opportunistic, fast-growing species (Martins et al. 2001, Chavez-Sanchez et al. 2017). Yet, we still know little of how seasonal environmental variation and biotic interactions are related to algal fitness in more stable intertidal communities. In particular, effects of algal-algal interactions

On the sub-tropical southern Brazilian coast, rocky intertidal macroalgal communities are characterized by high abundance and species richness (Horta et al. 2001). However, three genera utilizing different ecophysiological strategies tend to dominate the macroalgal assemblages: *Jania* (Rhodophyta), *Sargassum* (Heterokonta), and *Ulva* (Chlorophyta) (Martins et al. 2012). *Jania* are articulated coralline algae that form dense mats from the subtidal to intertidal regions (Martins et al. 2012), while *Sargassum* are canopy-forming brown algae with a similar range and tend to thrive in more wave-exposed habitats (Mafra & Cunha 2006). *Ulva* are opportunistic and highly productive foliose algae that are considered excellent competitors, and are often the first algae to settle disturbed habitats and tend to be more resistant to anthropogenic stressors (Martins et al. 2012, Scherner et al. 2013). While these three genera often grow in distinct areas and follow zonation patterns seen in other marine intertidal organisms, patches can be found where all three genera are growing together in physical contact with at least one of the other species (Fig. 1).

Despite a long history of studies detailing the ecology of marine intertidal communities, ecophysiological effects of species interactions between dominant macroalgal species, especially in different seasons, are less well understood. Here, we evaluated three common macroalgae of the southern Brazilian coast in monospecific stands or in physical contact with another macroalgal species. Our goals were to assess 1) the seasonal variation in algal physiology and biochemical composition, 2) if the presence of other species affects algal physiology and biochemical composition, and 3) if so, if this causes changes in their fitness and in their palatability to a common herbivore. The results of this evaluation may shed light on the specific mechanisms that, in part, structure macroalgal communities in the rocky intertidal zone.

2. Materials and Methods

2.1 Sample site and collection

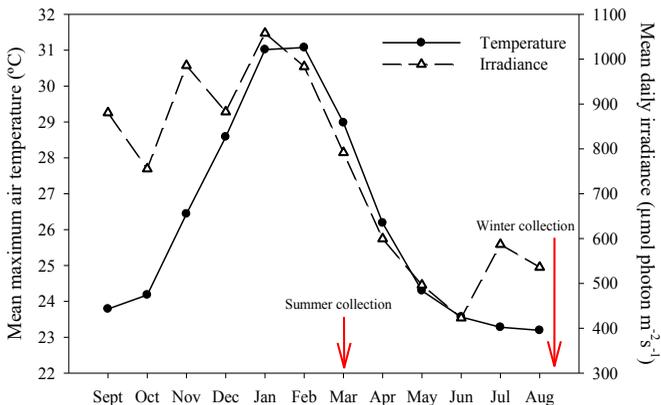
Macroalgae were collected from the upper rocky intertidal region of Barra da Lagoa beach in Florianópolis, Brazil (27.573720°S, 48.419677°W) in March (late austral summer) and September (late austral winter), 2017 (Fig. 1).

Atmospheric temperature and mean daily values for irradiance data from September 2016 to August 2017, obtained from the state of Santa Catarina's Agricultural Research and Outreach division (EPAGRI), showed mean maximum temperatures at the sampling site varied between 23.2°C and 31.1°C, while irradiance varied between ~400-1100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Fig. 2). For the sampling periods, the recorded average maximum temperature was 29°C and 23°C, for summer and winter sampling, respectively. The average daily irradiance for summer-collected algae was 790 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, whereas the average daily irradiance for the winter-collected algae was 540 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Fig. 2).

Figure 1: Examples of algal species collected from different interactions in the field at Barra da Lagoa beach, southern Brazil. A) Monospecific *Jania*, B) Monospecific *Sargassum*, C) Monospecific *Ulva*, D) *Jania* and *Sargassum* in contact with each other, E) *Jania* and *Ulva* in contact with each other, F) *Sargassum* and *Ulva* in contact with each other.



Figure 2: Monthly mean maximum air temperature and mean daily irradiance in Florianopolis, Brazil from September 2016 to August 2017.



Three common species of macroalgae in the area were chosen for the experiments: *Jania rubens*, *Sargassum cymosum*, and *Ulva lactuca* (here after referred to as *Jania*, *Sargassum* and *Ulva*). All species were collected from the same tidal elevation and in the same area to minimize effects of environmental variation on algal physiology. The three species were commonly found in contact with each other and samples were collected from three “associations”; monospecific patches of the species, and the species in physical contact with one of the other two species (for example, *Jania* only, *Jania* in contact with *Sargassum*, and *Jania* in contact with *Ulva*) (Fig. 1). Collections were made at low tide in early morning, and macroalgae were placed in separate bags in coolers and immediately transported to the Phycology Laboratory at the Federal University of Santa Catarina for same-day incubations.

2.2 Photosynthetic performance

Incubations with airtight chambers were performed on macroalgae from each association (Fig. 1) to evaluate photosynthetic performance via oxygen production/consumption (n=6). Algal species were carefully cleaned of epibionts before being incubated individually in custom-made acrylic chambers (960 ml) that were filled with filtered seawater and placed in a water bath to control water temperature (summer: 24°C; winter: 20°C). Initial oxygen concentration of the seawater was measured with a benchtop oximeter (YSI 5000, Ohio, USA) before organisms were placed in the chambers and quickly sealed. The algae were incubated for 20 min at 650 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, after which the final oxygen concentration was measured. Afterwards, the chambers were sealed again and incubated in darkness for 20 min for respiration measurements. At the conclusion of the incubations macroalgae were weighed to determine biomass and subsequently frozen at -80°C for further chemical analyses. In addition to incubations, separate samples (n=5) of algae were dark acclimated for 1 h and the maximum photosynthetic efficiency (Fv/Fm) was assessed using a Diving-PAM (Walz, Germany).

2.3 Pigment analysis

Samples of *Ulva* and *Sargassum* (20-100 mg fresh weight) were pulverized with liquid nitrogen and 3 ml of 100% methanol (*Ulva*) or 90% acetone (*Sargassum*) was added to extract the pigments. Samples were kept in the dark at 4°C for 24 h before being centrifuged for 2 min at 3,500 rpm. The supernatant was measured spectrophotometrically and the chlorophyll concentrations were calculated following Porra et al. (1989) and Richie (2008).

In the case of *Jania*, the hydrophilic (phycobilipigments) and lipophilic pigments (chlorophyll a) were determined by a 2-step extraction, according to Kursar et al. (1983), with slight modifications.

Phycobilipigment extraction was done by grinding the tissue with liquid nitrogen and maintaining the sample in potassium phosphate buffer (0.1 mol L⁻¹, pH 6.8) at 4°C for 2 h in darkness. Subsequently, the extract was centrifuged (3,500 rpm, 2 min), and phycobilipigment content was determined in the supernatant. The pellet was resuspended with methanol overnight in darkness at 4°C to extract lipophilic pigments. The phycobilipigment content was calculated according to Kursar et al. (1983), and chlorophyll a was calculated using the equation of Porra et al. (1989).

2.4 Nitrate reductase enzyme assay

The activity of the nitrate reductase (NR) was measured in situ, based on the method of Corzo and Niell (1991). Fresh algal tissue (150–300 mg) was incubated with assay buffer (0.1 M phosphate buffer, pH 8.0, 0.5 mM EDTA, 0.1% 1-propanol, 30 mM KNO₃, 10 μM glucose) that had been previously bubbled with N₂ gas for 2 min. After inserting the tissue into the vials with the assay buffer, they were bubbled with N₂ gas for an additional 2 min before being placed into a 30°C water bath in the dark for 1 h. After the incubation, 2 ml of the assay buffer was

removed and after the addition of 4% sulphanilamide and 0.1% n-(1-naphthyl) ethylenediamine dihydrochloride the nitrite concentrations were determined spectrophotometrically (Biospectro SP220) at 543 nm (Snell and Snell 1949). Following the assay, the algal tissue was weighed and nitrate reductase activity was calculated as $\mu\text{mol NO}_2 \text{ g FW}^{-1} \text{ h}^{-1}$.

2.5 Carbonate content

Samples of *Jania* (~1.0 g) were dried at 60°C for 24 h and weighed before being submerged in a 10% HCL solution for 30 min. Samples were rinsed with distilled water, dried again at 60°C for 24 h, and re-weighed. Carbonate content was calculated as the difference between final and initial weights.

2.6 Phenolic analysis

Extraction of phenolic compounds followed Schiavon et al. (2012) with some modifications. Samples (~300 mg fresh weight) were ground with liquid nitrogen and 8 ml of 80% methanol solution were added. After a 1 h dark incubation, samples were centrifuged for 10 min at 4,400 rpm. 200 μl of the supernatant was pipetted to a separate glass tube; then, 150 μl of folin and 1,650 μl of a 2% NaCO_3 solution were added. Following a 1 h dark incubation, absorbance of samples was read in a spectrophotometer at 750 nm.

2.7 Herbivory assay

Feeding rates were assessed for the winter experiment in a no-choice test with the intertidal sea urchin *Echinometra lucunter*. Sea urchins ($n=36$; test diameter=30-40 mm) were collected from Pântano do Sul beach in Florianópolis, Brazil (27.787728°S, -48.507530°W),

immediately transported to the Phycology laboratory and placed individually into plastic containers containing 1.5 L of filtered seawater. The containers were maintained in a temperature- and light-controlled walk-in chamber (24°C, photoperiod 12:12). Aeration was provided for all containers and sea urchins were starved for three days before commencing the experiment, at which time the water was replaced. Algae from each of the nine associations were dabbed dry with paper towels and weighed before being placed with the sea urchins. A control was used to monitor background algal growth rates. The feeding assay was concluded after three days, in which the algae were re-weighed. Herbivory rates were calculated as the difference between initial and final weights and corrected for algal growth rates and urchin size. The herbivory assay consisted of four replications for each of the nine algal treatments.

2.8 Statistical analyses

All data were checked for normality prior to analyses; when needed, a log-transformation was performed to conform to assumptions of normality. A two-way ANOVA with species association (three levels) and season (two levels) as factors was used. For the feeding assay, a one-way ANOVA considering species association was performed. When significant differences were found ($p < 0.05$), a TukeyHSD post-hoc analysis was performed.

3. Results

3.1 Photosynthetic performance

Maximum photosynthetic efficiency and oxygen production rates of algae varied significantly by season and when in contact with other algal species (Fig. 3a, Table 1). In general, all three species exhibited higher Fv/Fm values in winter, compared to summer (Fig. 3a, Table 1).

In *Jania*, associations with both *Sargassum* and *Ulva* resulted in lower Fv/Fm values compared to monospecific *Jania* in the summer (Fig. 3a, Table 1). However, during the winter these effects disappeared and Fv/Fm was not significantly different between species associations (Fig. 3a, Table 1). Fv/Fm of *Sargassum* was lowest when in contact with *Jania* and highest when in contact with *Ulva* in the summer (Fig. 3a, Table 1). Again, effects of species associations disappeared during the winter, and there were no differences in Fv/Fm between species associations (Fig. 3a, Table 1). *Ulva* did not exhibit significant effects of species associations on Fv/Fm in either season (Fig. 3a, Table 1).

Net photosynthesis was higher for all three species of algae in the winter months (Fig. 3b, Table 1). *Jania* was negatively affected by the association with *Ulva* in the summer (Fig. 3b, Table 1). Though net photosynthesis was marginally lower for *Jania* in contact with *Sargassum* compared to monospecific *Jania*, they were not significantly different (Fig. 3b, Table 1). In the winter, however, *Jania* with *Sargassum* had the lowest net photosynthesis, while *Jania* alone and with *Ulva* were not significantly different. Compared to summer, winter-collected monospecific *Jania* and *Jania* in contact with *Ulva* had much higher net photosynthesis; however, *Jania* in contact with *Sargassum* did not have significantly different net photosynthesis between the two seasons (Fig. 3b, Table 1). Net photosynthesis of *Sargassum* during the summer was not significantly different between species associations (Fig. 3b, Table 1). *Sargassum* associated with *Ulva* had substantially higher net photosynthesis than *Sargassum* alone or when in contact with *Jania* (Fig. 3, Table 1). Summer-collected *Ulva* did not have significant differences in net photosynthesis between species associations (Fig. 3b, Table 1). Likewise, net photosynthesis of *Ulva* in the winter was not affected by species associations (Fig. 3b, Table 1).

Figure 3: Seasonal variation in photosynthetic performance (summer- solid bars, winter- striped bars). (a) Maximum photosynthetic efficiency (Fv/Fm) and (b) net photosynthesis of algae in monospecific patches and in association with other species. Data represent mean±SE (n=6) and different letters indicate significant differences within each species (p<0.05, Tukey HSD).

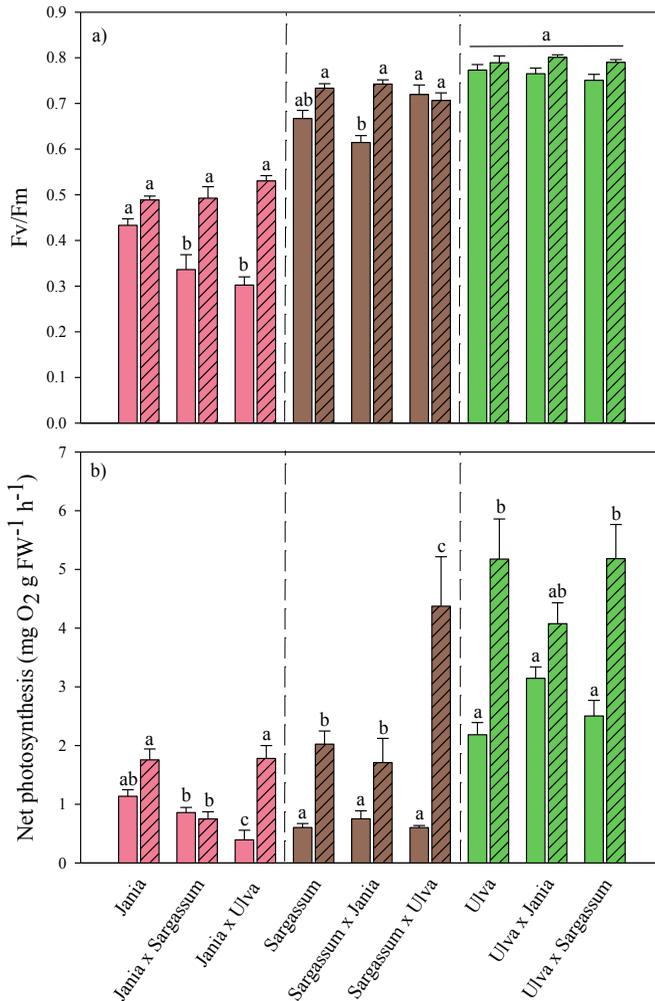


Table 1. ANOVA statistics of physiological parameters of algae from different species interactions (monospecific or in contact with one of the other two species) and seasons (Summer= March; Winter=September).

Response	MS	F-value	P-value
Fv/Fm			
<i>Jania</i>			
Species association	0.00689	2.716	0.0864
Season	0.18129	63.613	< 0.0001
Species association × Season	0.18129	7.410	0.0031
<i>Sargassum</i>			
Species association	0.00305	2.013	0.1555
Season	0.02740	18.081	< 0.0001
Species association × Season	0.01252	8.258	0.0019
<i>Ulva</i>			
Species association	0.00047	0.594	0.5600
Season	0.00070	8.765	0.0068
Species association × Season	0.00039	0.489	0.6193
Photosynthesis			
<i>Jania</i>			
Species association	2.481	7.571	0.0022
Season	3.603	21.902	< 0.0001
Species association × Season	3.352	10.189	< 0.0001
<i>Sargassum</i>			
Species association	0.414	1.754	0.1910
Season	13.476	57.095	< 0.0001
Species association × Season	0.969	4.106	0.0269
<i>Ulva</i>			
Species association	0.04	0.030	0.9703
Season	41.70	34.303	< 0.0001
Species association × Season	3.67	3.021	0.0643
Nitrogen Reductase activity			
<i>Jania</i>			
Species association	12.268	120.69	< 0.0001
Season	2.789	27.40	< 0.0001
Species association × Season	8.301	81.53	< 0.0001
<i>Sargassum</i>			
Species association	0.21361	2.123	0.1420
Season	0.20922	2.079	0.1620
Species association × Season	0.00585	0.058	0.9440
<i>Ulva</i>			
Species association	1.892	17.906	< 0.0001
Season	0.269	2.637	0.1190
Species association × Season	3.867	37.868	< 0.0001

3.2 Pigment content

Pigment content of algae varied between seasons and by species associations (Fig. 4, Tables 2, 4). Total chlorophyll of *Jania* was not affected by season or by species association, but the interaction of these two factors was significant (Fig. 4, Table 2). In general, *Jania* with *Sargassum* had higher chlorophyll content than monospecific *Jania* in the summer, but this trend was reversed in the winter (Fig. 4, Table 2). Total chlorophyll of *Sargassum* was lower in summer compared to winter, but was unaffected by species association (Fig. 4, Table 2). *Ulva* tended to have higher chlorophyll content when in contact with *Jania*, particularly in the summer, and was unaffected by season (Fig. 4, Table 2).

Figure 4: Total chlorophyll content of algae collected in summer (solid bars) and winter (striped bars). Data represent mean \pm SE (n=6) and different letters indicate significant differences within each species ($p<0.05$, Tukey HSD).

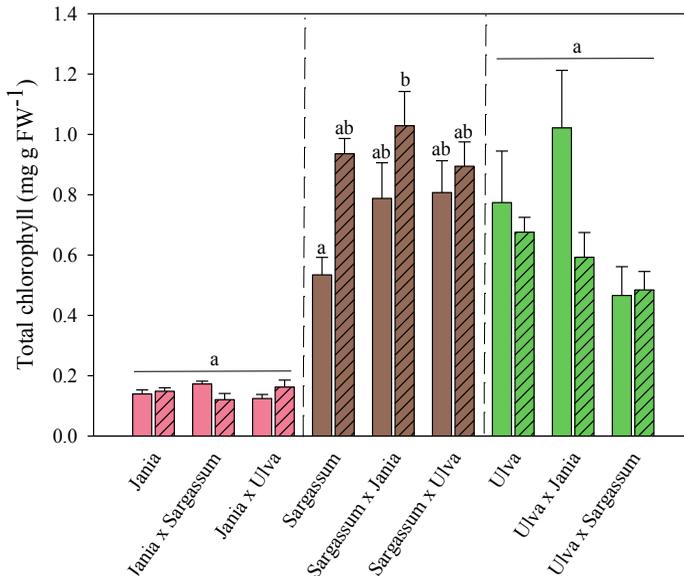


Table 2: ANOVA statistics of biochemical parameters of algae from different species interactions (monospecific or in contact with one of the other two species) and seasons (Summer= March; Winter=September).

Response	MS	F-value	P-value
Carbonate content			
<i>Jania</i>			
Species association	0.07786	7.155	0.0023
Season	0.30135	27.693	<0.0001
Species association × Season	0.02837	2.607	0.0866
Phenolic content			
<i>Jania</i>			
Species association	0.3613	5.367	0.0106
Season	0.8919	13.248	0.0011
Species association × Season	0.2374	3.526	0.0431
<i>Sargassum</i>			
Species association	4.394	47.504	<0.0001
Season	6.765	73.236	<0.0001
Species association × Season	0.808	8.744	0.0011
<i>Ulva</i>			
Species association	6.40	3.922	0.0311
Season	40.44	24.798	<0.0001
Species association × Season	5.09	3.121	0.0592
Total chlorophyll			
<i>Jania</i> (Chl <i>a</i>)			
Species association	0.00004	0.025	0.9751
Season	0.00003	0.021	0.8859
Species association × Season	0.00633	3.624	0.0394
<i>Sargassum</i> (Chl <i>a+c</i>)			
Species association	0.0933	1.545	0.2298
Season	0.5332	8.827	0.0058
Species association × Season	0.0742	1.128	0.3072
<i>Ulva</i> (Chl <i>a+b</i>)			
Species association	0.3587	3.440	0.0452
Season	0.2587	2.418	0.1258
Species association × Season	0.1618	1.551	0.2285
Pigment ratios			
<i>Jania</i> (Phycobilipigments: Chl <i>a</i>)			
Species association	33.39	2.399	0.1099
Season	145.30	10.440	0.0032
Species association × Season	5.61	0.403	0.6720

Table 2 continued

Response	MS	F-value	P-Value
<i>Jania</i> (PE:PC)			
Species association	67.47	8.676	0.0012
Season	74.88	9.629	0.0045
Species association × Season	39.81	5.119	0.0130
<i>Sargassum</i> (Chl <i>a</i> : Chl <i>c</i>)			
Species association	2.168	1.028	0.3701
Season	18.188	8.622	0.0063
Species association × Season	2.721	1.290	0.2901
<i>Ulva</i> (Chl <i>a</i> : Chl <i>b</i>)			
Species association	0.790	1.364	0.2711
Season	4.380	7.567	0.0010
Species association × Season	0.719	1.242	0.3033

Pigment ratios also varied by season and species association. For *Jania*, the ratio of total phycobilipigments to total chlorophyll (Chlorophyll *a*) was unaffected by species association, but was higher in winter (Table 2, Table 3). The ratio of phycoerythrins to phycocyanins (PE: PC) in *Jania* was higher when in contact with *Sargassum*, but only in the winter (Table 2, Table 3). Ratios of chlorophyll *a* to chlorophyll *c* in *Sargassum* were not affected by species associations in either season; however, overall ratios were higher in summer (Table 2, Table 3). Ratios of chlorophyll *a* to chlorophyll *b* in *Ulva* were unaffected by species association for both seasons, but were higher in winter (Table 2, Table 3).

Table 3: Seasonal variation in pigment ratios of the studied algal species, in presence or absence of other species. Data represent mean \pm SE (n=6) and different superscript letters indicate significant differences (Post-hoc TukeyHSD).

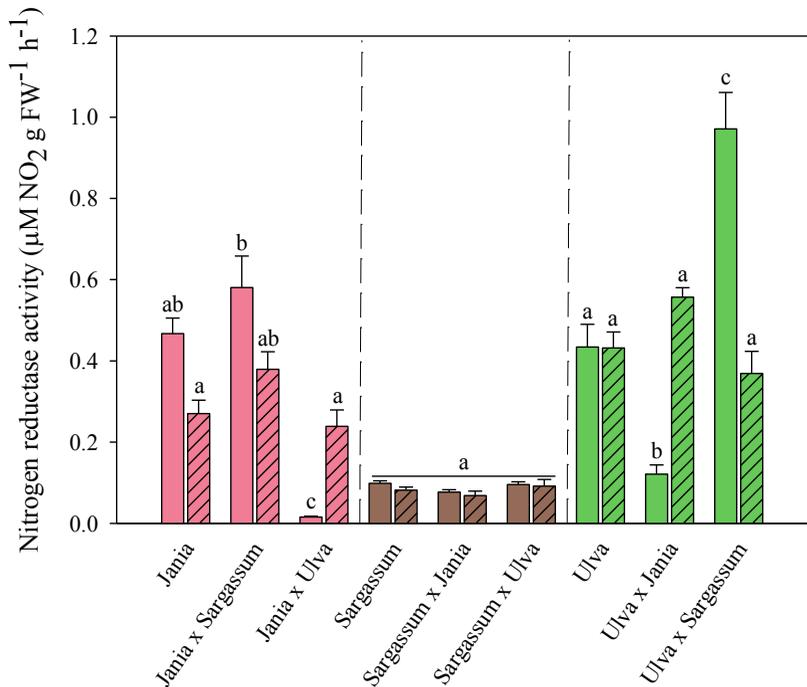
Pigment ratios	Summer	Winter
<i>Jania</i> (Phycobilipigments: Chl <i>a</i>)		
Monospecific	11.00 \pm 0.89a	15.79 \pm 1.82b
+ <i>Sargassum</i>	8.69 \pm 0.52a	18.23 \pm 4.41b
+ <i>Ulva</i>	11.22 \pm 2.37a	15.00 \pm 3.67a
<i>Jania</i> (PE:PC)		
Monospecific	8.99 \pm 0.70a	9.36 \pm 0.88a
+ <i>Sargassum</i>	10.32 \pm 1.15a	15.89 \pm 2.27b
+ <i>Ulva</i>	10.28 \pm 1.34a	12.41 \pm 1.95a
<i>Sargassum</i> (Chl <i>a</i> : Chl <i>c</i>)		
Monospecific	7.64 \pm 0.59ab	5.27 \pm 0.41b
+ <i>Jania</i>	8.01 \pm 0.52a	6.59 \pm 0.69ab
+ <i>Ulva</i>	7.16 \pm 0.32ab	6.69 \pm 0.62ab
<i>Ulva</i> (Chl <i>a</i> : Chl <i>b</i>)		
Monospecific	1.42 \pm 0.15a	2.16 \pm 0.28a
+ <i>Jania</i>	1.41 \pm 0.21a	2.58 \pm 0.33a
+ <i>Sargassum</i>	2.20 \pm 0.44a	2.39 \pm 0.22a

3.3 Nitrogen reductase enzyme activity

Nitrogen reductase activity (NRA) differed between seasons and by species association (Fig. 5, Table 1). Overall, NRA of *Jania* for all species associations was lower in the winter (Fig. 5, Table 1). Summer-collected *Jania* had markedly lower NRA when in contact with *Ulva* (Fig. 5, Table 1). In the winter, NRA of *Jania* was not affected by species association (Fig. 5, Table 1).

NRA of *Sargassum* did not differ between seasons or in species associations (Fig. 5, Table 1). Compared to *Ulva* alone, *Ulva* with *Jania* had lower NRA, while *Ulva* with *Sargassum* had substantially higher NRA in the summer (Fig. 5, Table 1). However, these effects of species associations disappeared in the winter (Fig. 5, Table 1).

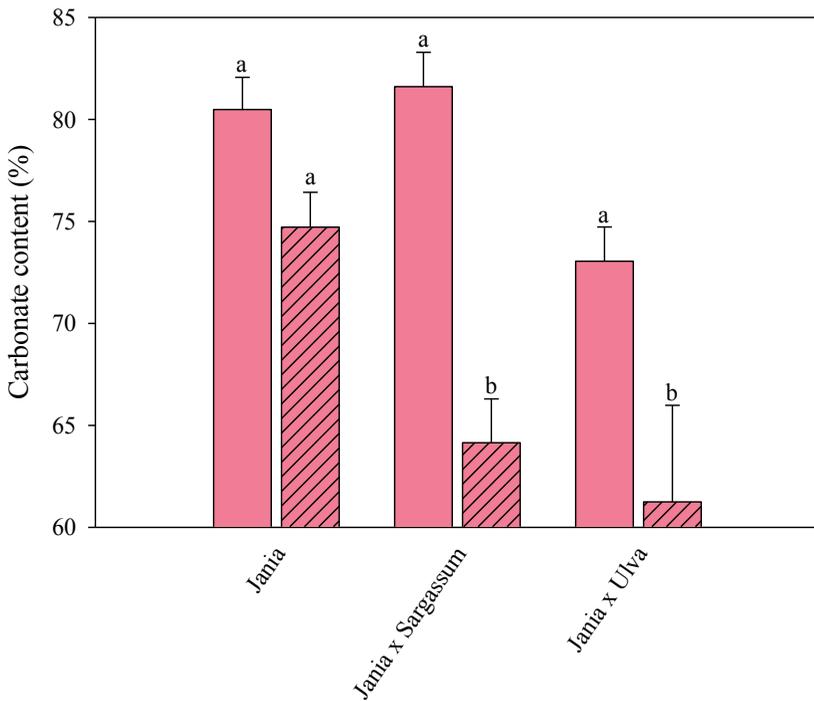
Figure 5: Nitrogen reductase activity of algae collected in summer (solid bars) and winter (striped bars). Data represent mean \pm SE (n=5) and different letters indicate significant differences within each species ($p < 0.05$, Tukey HSD).



3.4 Carbonate content

Overall, carbonate content of *Jania* tended to be lower in winter compared to summer (Fig. 6, Table 1). Carbonate content of *Jania* was unaffected by the presence of other species in the summer (Fig. 6, Table 1). Winter-collected *Jania* showed lower carbonate content when in contact with *Sargassum* and *Ulva* compared to monospecific *Jania* (Fig. 6, Table 1).

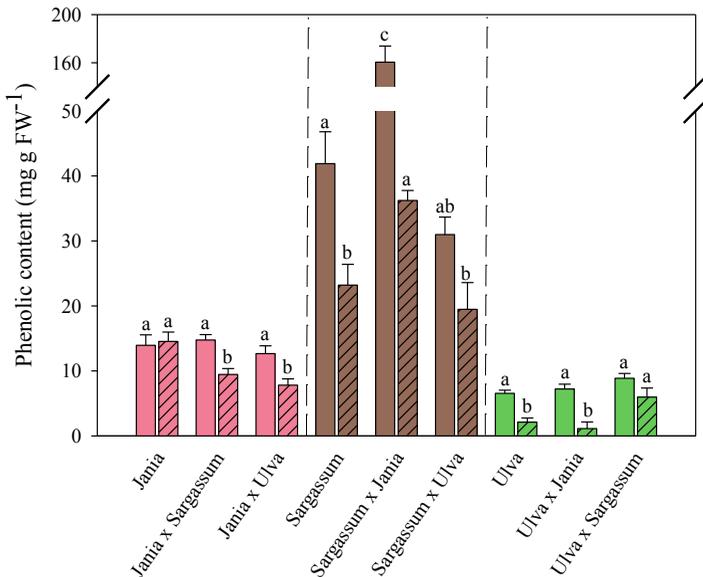
Figure 6: Carbonate content of *Jania* collected in summer (solid bars) and winter (striped bars). Data represent mean \pm SE (n=6) and different letters indicate significant differences ($p < 0.05$, Tukey HSD).



3.5 Phenolics

Phenolic content of *Jania*, *Sargassum* and *Ulva* was lower in winter compared to summer (Fig. 7, Table 2). In *Jania*, phenolic content was unaffected by species association in summer; however, in winter phenolic content was lower when it was in contact with *Sargassum* and *Ulva* (Fig. 7, Table 2). Phenolic content of *Sargassum* was nearly four times higher when in contact with *Jania* in the summer (Fig. 7, Table 2). Similarly, *Sargassum* with *Jania* had higher phenolic content in the winter, but to a lesser extent than in summer (Fig. 7, Table 1). *Ulva* was unaffected by species association in the summer, but tended to have higher phenolic content when in contact with *Sargassum* in the winter (Fig. 7, Table 2).

Figure 7: Total phenolic content of algae collected in summer (solid bars) and winter (striped bars). Data represent mean \pm SE (n=6) and different letters indicate significant differences within each species ($p < 0.05$, Tukey HSD).



3.6 Herbivory assay

Feeding rates of sea urchins on algae were variable but were significantly affected by species association. Sea urchins consumed more *Jania* which had been in contact with *Ulva* than monospecific *Jania* or *Jania* with *Sargassum* (Fig. 8, Table 3). Consumption rates of *Sargassum* were not significantly different between species associations (Fig. 8, Table 3). Sea urchins consumed significantly more *Ulva* that had been in contact with *Jania* than monospecific *Ulva* or *Ulva* with *Sargassum* (Fig. 8, Table 3).

Figure 8: Consumption rates of algae by a sea urchin herbivore (winter only). Data represent mean \pm SE (n=4) and different letters indicate significant differences within each species (p<0.05, Tukey HSD).

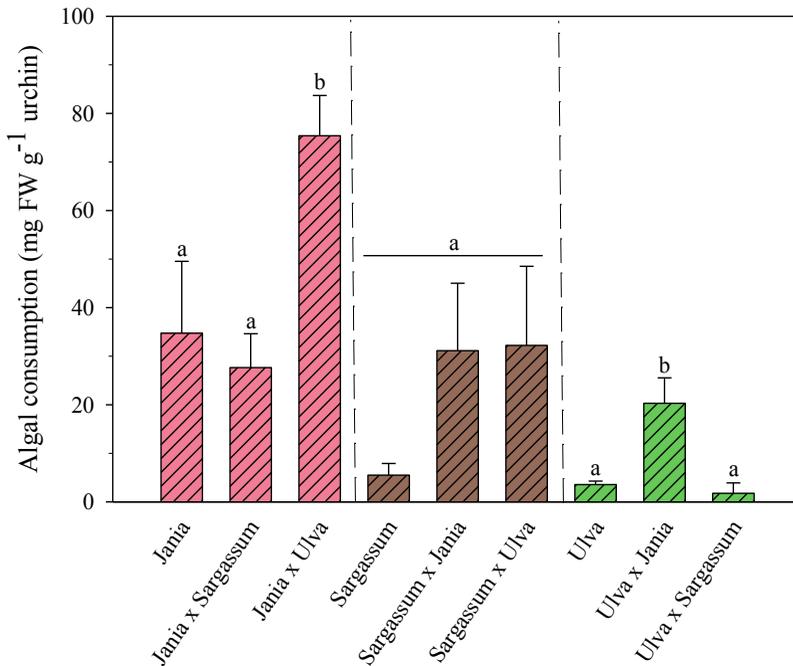


Table 4: ANOVA statistics of sea urchin feeding rates on algae from different species interactions (monospecific or in contact with one of the other two species). The no-choice feeding assay was performed during Austral winter (September) only.

Response	MS	F-value	P-value
Urchin feeding rates			
<i>Jania</i>			
Species association	0.00218	5.931	0.0263
<i>Sargassum</i>			
Species association	0.00091	1.11	0.371
<i>Ulva</i>			
Species association	0.00040	8.741	0.0097

4. Discussion

Upper intertidal macroalgal communities are structured by various abiotic and biotic factors. Here, our results suggest that seasonal changes in ecophysiology may be affected by interspecific associations between dominant macroalgae, which seem to at least partially influence palatability to an herbivore. Based on the degree to which algae were affected, it appears *Jania* is more susceptible to negative interactions by *Ulva* and *Sargassum*, while both *Sargassum* and *Ulva* usually experienced neutral or positive effects from associations with other algal species. This may be a reflection of the differing ecophysiological strategies of the algae: *Jania*, as a calcareous alga, must invest more energy in growth and maintenance compared to *Ulva* (an opportunistic and fast-growing species) and *Sargassum* (a more robust and competitive climax species).

All three species of algae showed some general seasonal trends that have been previously reported. Net photosynthesis tended to be higher in the winter compared to the summer (Fig. 3b), agreeing with similar studies for *Jania* (Saroussi & Beer 2007), *Sargassum* (Baer & Stengel 2010), and *Ulva* (Vergara et al. 1997, Kim et al. 2004, Saroussi & Beer 2007). High irradiance and temperatures during the summer (i.e., Fig. 2), especially in tropical and sub-tropical areas, can stress algae and

result in lower net photosynthesis, as also indicated by the lower overall Fv/Fm values. This is also supported by the general higher phenolic content in summer in all three species (Fig. 7), a feature that has been also reported previously in species of the genera studied here (Plouguerné et al. 2006, Khairy et al. 2015). Phenolics can play a role in photoprotection (Abdala-Diaz et al. 2006), which might explain the higher content found during summer when irradiance was higher. Besides these general trends, seasonal variability also was found in the physiological and/or biochemical algal responses in different species associations.

4.1 *Jania* and *Sargassum* species association

Both *Jania* and *Sargassum* affected each other. For *Jania*, the presence of *Sargassum* had a significant effect on its biochemical composition (pigment ratios, phenolic content and carbonate content), but only during the winter. Based on the changes in the parameters measured, we suggest the most likely explanation for these results is that *Sargassum* was shading *Jania* in the winter months, which may have negatively impacted biosynthesis of phenolics and calcification.

Net photosynthesis tended to be lower when associated with *Sargassum*, although this was not significant (Fig. 3b). Photosynthesis in macroalgae is affected by many factors including light intensity, carbon and nitrogen availability, and pollutants or allelopathic compounds (Rai et al. 1981, Friedlander et al. 1996, Beer et al. 2014). Here, the higher phycobilipigment to chlorophyll a ratio (Table 3) indicates larger light-harvesting antennae, which suggests *Jania* was most likely light-limited in this species association. When light is limiting, red algae increase their accessory pigments relative to chlorophyll to improve light harvesting capacity (Waaland et al. 1974). Although in this particular association *Sargassum* did not form a continuous canopy, the *Jania* collected here were in contact with it and could easily be shaded (Fig. 1d). Photosynthetic rates have direct effects on production of phenolic

compounds (Pavia & Toth 2000, Cabello-Pasini et al. 2011, Celis-Plá et al. 2016) and calcification (i.e., carbonate content) (Borowitzka 1981); therefore, the trend for lower net photosynthesis may explain lower phenolic and carbonate content. The lack of such effects in the summer is puzzling, but the increased irradiance (Fig. 2) in this season may have been at saturating levels for *Jania*, allowing it to maintain higher rates of photosynthesis even in shaded conditions (i.e., Beer et al. 2014).

While light limitation is an appealing explanation, we cannot rule out other possible stressors. A second possibility is that *Sargassum* caused mechanical stress on *Jania* via abrasion. For example, *Sargassum* from Hawaii has been shown to reduce nearby turf algal biomass via abrasive action caused by its stiff thalli (Cheroske et al. 2000). More intense storms tend to occur in the winter in southern Brazil (Barletta & Calliari 2001), and could theoretically increase abrasive action by *Sargassum* on surrounding *Jania*, which could stress the algae and result in less allocation to phenolics, pigments and calcification. However, a study focused on the effects of mechanical stress to the physiology of *Jania* is needed.

For *Sargassum*, the only factor that was significantly affected by the presence of *Jania* was phenolic content, which in both seasons, but especially in summer, was markedly higher when in contact with *Jania* (Fig. 7). The exact mechanism by which the species association with *Jania* caused increased phenolic content is unclear. In addition to seasonal variation related to irradiance, phenolic content has been shown to be inversely correlated with nitrogen content in the brown alga *Fucus vesiculosus* (Ilvessalo & Tuomi 1989). However, in this study there was no evidence of nitrogen limitation in *Sargassum* as NRA was unaffected by season or species association (Fig. 4). In addition to their possible role as photoprotective compounds, phenolics also serve as anti-herbivory compounds in brown algae, and herbivory pressure can induce algae to produce more phenolic compounds (Van Alstyne 1988). In fact, Peckol et al. (1996) found more evidence for herbivory-induced defense than nitrogen limitation in explaining the production of phlorotannins, which are among the most common phenolic compounds

in brown algae. Though herbivore abundance was not quantified in the current study, *Jania* has been shown to host higher herbivore abundance and diversity than *Sargassum* or *Ulva* (Fishelson & Haran 1987). It is possible that infauna herbivores hosted by *Jania* were causing higher herbivory pressure on *Sargassum*, inducing higher phenolic content; however, more detailed studies of herbivore density and herbivory pressure in different algal species associations are needed to verify this hypothesis.

4.2 *Jania* and *Ulva* species association

The presence of *Ulva* affected *Jania* in both seasons. During the summer, *Jania* associated with *Ulva* had lower net photosynthesis and lower NRA. Unlike the aforementioned case of lower photosynthesis in *Jania* associated with *Sargassum*, a lack of changes in total chlorophyll and pigment ratios here suggests that *Ulva* was not causing a shading effect on *Jania*. NRA was also lower in this association, which can be due to nitrogen availability (Turnpin 1991, Berges 1997), but is also directly related to rates of photosynthesis (Davidson & Stewart 1984, Gao et al. 1992, Berges 1997); hence, the two-fold decrease in photosynthesis compared to monospecific *Jania* likely caused a decline in NRA. One possibility is that *Ulva* may have been producing allelopathic compounds that negatively affected the performance of *Jania*. *Ulva* has been shown to negatively affect other red algae (*Gracilaria conferta*) via allelopathy in laboratory studies (Friedlander et al. 1996), which here might have hindered *Jania* photosynthesis and subsequently affected NRA. As allelopathic compounds were not measured in the current study, it remains uncertain whether this was the cause of *Jania* physiology.

In the winter, net photosynthesis and NRA were unaffected, but relative to monospecific *Jania*, *Ulva* caused a decrease in phenolic content and carbonate content. Why these two factors alone changed is difficult to explain, but again may be due to allelopathic activity caused by *Ulva*,

resulting in less energy allocated by *Jania* to calcification and production of phenolics.

While *Ulva* negatively affected *Jania* in both seasons, it was probably more strongly affected during the summer months, when photosynthesis was two times less than monospecific *Jania*. Photosynthesis is the ultimate source of energy for algae and therefore reduced rates of net photosynthesis can affect all other metabolic processes (Beer et al. 2014, Hurd et al. 2014). The seasonal difference in responses by *Jania* when in contact with *Ulva* may be due to abiotic stressors experienced during the summer months. Compared to *Ulva* which is more tolerant of stress (Beer et al. 2014), coralline algae such as *Jania* often bleach in the summer months due to high temperatures and irradiance (Lathman 2008, Martone et al. 2010), indicating it is already subjected to high stress. Though we did not quantify bleaching occurrence in this study, and all *Jania* sampled did not show signs of bleaching, there were areas of bleaching at the same study site during the summer (pers. obs.). This may predispose *Jania* to be more susceptible to any biotic stressors it may encounter in the summer, but in winter months it may be able to recover.

Jania caused a decrease in NRA (Fig. 4) for *Ulva* during the summer. The cause for this decrease is difficult to explain, as all other measured parameters were not significantly different than monospecific *Ulva*. Decreases in NRA can be due to less nitrate or excess ammonia available in the water column (Turnpin 1991, Berges 1997). Since *Jania* usually host more infauna than *Ulva* or *Sargassum* (Fishelson & Haran 1987), it is possible that ammonia excretion by infauna hosted by *Jania* resulted in lower NRA (Bracken 2004). However, directed studies on invertebrate density and excretion rates would be needed to confirm the mechanism of lower NRA.

4.3 *Sargassum* and *Ulva* species association

Of all species associations, *Sargassum* and *Ulva* had the fewest effects on each other; these effects are also the most difficult to explain. *Ulva* did not significantly affect *Sargassum* in any measured parameters during the summer. In the winter, *Sargassum* had higher net photosynthesis when in the presence of *Ulva*. Photosynthesis is dependent on a variety of factors including temperature, irradiance, nutrient levels and allelochemicals or pollutants (Rai et al. 1981, Friedlander et al. 1996, Beer et al. 2014). While none of the other measured parameters were affected by this association, there may be another advantage conferred to *Ulva* by *Sargassum* that was not considered in this study, such as micronutrients (Beer et al. 2014).

Sargassum caused a nearly two-fold increase in NRA for *Ulva* compared to monospecific *Ulva* in the summer. As previously stated, NRA is positively related to nitrate and negatively related to ammonia availability, and it is possible that in some way *Sargassum* was locally influencing nitrate or ammonia concentrations. *Sargassum* NRA was unaffected in this association, so if this were true, *Sargassum* was apparently unaffected. However, it is unclear why this effect would be present only in the summer months.

In the winter, *Ulva* had higher phenolic content with *Sargassum* compared to monospecific *Ulva* or when with *Jania*. Considering our previous suggestion with *Sargassum* and *Jania*, herbivory rates may have been higher in this association, but *Sargassum* did not see a similar increase in phenolic content. Higher levels of phenolics can sometimes indicate nitrogen limitation (Ilvessalo & Tuomi 1989, Yates & Peckol 1993), but NRA was unaffected here. For both species in the *Sargassum-Ulva* association, we conclude that other factors may be at play affecting photosynthesis, NRA and phenolic contents, and future studies are required to disentangle the mechanisms causing changes in these parameters.

4.4 Herbivory assays

Rates of herbivory often depend on the nutritional value (i.e., nitrogen content) and defensive compounds (i.e., phenolics, carbonate) of the macroalgae (Hay and Fennical 1988). Here, the three-day herbivory assay revealed some differences in feeding rates by the sea urchin *E. lucunter* for algae from different associations.

Jania associated with *Ulva* was consumed at higher rates than monospecific *Jania* or *Jania* with *Sargassum*. The herbivory assay was performed with the winter-collected algae, in which *Jania* in the presence of *Ulva* had lower phenolics and carbonate content, which may have increased the palatability of *Jania* for the sea urchin. However, why *Ulva* caused decreases in these two parameters remains unclear. We also note that both phenolics and carbonate content were lower for *Jania* with *Sargassum*, yet it was consumed at similar rates to monospecific *Jania*. One possibility is that *Sargassum* was leaching phenolics into the water column (i.e., Abdala-Diaz et al. 2006) and nearby *Jania* were benefiting from the defensive role of these compounds. Algae samples used in the phenolic content analysis had been rinsed before extractions began, so possible phenolics from *Sargassum* on *Jania* may have been removed and would not have been apparent in the analysis. Nevertheless, other biochemical compounds not measured in this study may be responsible for the differences in herbivory rates seen here.

Consumption of *Sargassum* was not significantly affected by species association. Since NRA, which can be interpreted as a proxy for tissue nitrogen content (Berges & Harrison 1995), was not significantly different between species associations for *Sargassum*, there was likely no difference in nutritional quality for *E. lucunter*. Phenolic content was substantially higher for *Sargassum* with *Jania*, yet this apparently did not affect consumption rates by *E. lucunter*. However, Steinberg (1988) suggests that sea urchins are able to tolerate high phenolic content in algae if more palatable algae are not available. In the future, a choice

test of *Sargassum* from different species associations may be able to resolve the importance of phenolics for sea urchin feeding rates.

Overall, *Ulva* was consumed at much lower rates compared to *Jania* or *Sargassum*. However, more *Ulva* was consumed when in contact with *Jania*. *Jania* caused no significant changes to *Ulva* in the measured parameters during the winter, so it is unclear why *Ulva* from this association was consumed at higher rates. However, we point out that although not statistically significant, NRA tended to be higher for *Ulva* associated with *Jania* in the winter compared to monospecific *Ulva* or *Ulva* with *Sargassum*. Since NRA tends to be correlated with nitrogen tissue content, it is possible that *Ulva* with *Jania* had higher nutritional value for *E. lucunter* and was therefore consumed at higher rates. However, analyses of tissue nitrogen content would be needed to confirm this.

4.5 Implications for intertidal macroalgal community

While macroalgae undergo predictable changes in physiology and biochemical composition between seasons, we demonstrate here that interspecific associations appear to affect the degree to which these changes occur.

Our results indicate that the type and magnitude interactions could change between seasons. Considering *Jania*, there was a neutral effect (no changes in parameters) from *Sargassum* in the summer, but a negative effect (less carbonate content and a trend towards lower net photosynthesis) during the winter. *Ulva* caused a negative effect on *Jania* in both seasons, but was perhaps more strongly negative in the summer (lower net photosynthesis). *Sargassum* was affected negatively by *Jania* in both seasons, with substantially higher phenolic content. *Ulva* had a neutral effect on *Sargassum* in the summer but positively affected *Sargassum* in the winter, (higher net photosynthesis). For *Ulva*, *Jania* seems to have had a neutral effect in both seasons. Meanwhile,

Sargassum had a neutral effect on *Ulva* in summer and a negative effect in the winter (higher phenolic content).

The results of the herbivory assay revealed that *Sargassum* was consumed at similar rates for all species associations, but *Ulva* and *Jania* were consumed at higher rates when they were associated with each other. While we speculate that changes in defensive compounds or possibly nutritional value may be driving differences in consumption, the results presented here offer no definitive answer for why species associations affected herbivory rates. Despite no clear cause for the differences in algal consumption, higher herbivory rates on *Jania* and *Ulva* when associated with each other has clear implications for the functioning of intertidal ecosystems. As herbivory in marine benthic environments is one of the strongest forces shaping community structure (Hay & Fenical 1988), changes in feeding rates can reshape algal assemblages and have subsequent effects on the entire intertidal community (Lubchenco & Gaines 1971, Lubchenco 1978, Lubchenco & Menge 1978, Poore et al. 2012).

While we suggest some mechanisms for the physiological and biochemical changes seen in this study, the cause of differences in some of the parameters is uncertain. Future studies are needed to help elucidate exactly how interspecific associations are affecting macroalgal physiology and biochemical composition, especially in controlled field and laboratory studies where such parameters can be measured before and after initiating algal-algal contact. Regardless, we highlight that such associations do appear to influence algal physiology in the field, and any studies using intertidal macroalgae should consider that interspecific interactions can alter “baseline” measurements in physiology and biochemistry, and may even affect palatability in herbivory studies. Moreover, studies of seasonal variation in algal physiology should take care to collect individuals from monospecific stands to ensure other algal species are not confounding their measurements. Looking at the big picture, long-term evaluations of species associations in the field may help explain how finer-scale patterns of macroalgal community structure arise and are regulated.

Finally, a current area of intense study is how anthropogenic changes are currently affecting coastal communities, and how future changes will impact ecosystem structure and function. We reaffirm that it is important to consider that species interactions may moderate or intensify individual species' responses to stressors (i.e., Montoya & Raffaelli 2010, Kordas et al. 2011).

5. Acknowledgements

The authors are grateful Bruno Castro, Tamela Madaloz, Lidiane Gouvêa, Letícia Peres, Antonella Almeida, and Manuel Bercovich (Federal University of Santa Catarina) for assistance with field collections, incubations and the herbivory assays. We also thank Epagri for supplying the temperature and irradiance data for Florianópolis. Funding for this project was provided by CNPq.

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CAPÍTULO 2

Physiological and biochemical responses of a coralline alga and a sea urchin to climate change: Implications for herbivory

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Abstract

Direct responses to rising temperatures and ocean acidification are increasingly well known for many single species. However, recent reviews have highlighted the need for climate change research to consider a broader range of species, how stressors may interact, and how stressors may affect species interactions. The latter point is important in the context of plant-herbivore interactions, as increasing evidence shows that ocean warming and/or acidification can alter the traits of algae that dictate their susceptibility to herbivores, and subsequently, community and ecosystem properties. To better understand how marine rocky shore environments will be affected by a changing ocean, in the present study we investigated the direct effects of co-occurring warming and ocean acidification on a coralline alga (*Jania rubens*) and a sea urchin herbivore (*Echinometra lucunter*) and assessed the indirect effects of these factors on the algal-herbivore interaction. A 21-day mesocosm experiment was conducted with both algae and sea urchins exposed to ambient (24°C, 390 ppm), warming (28°C, 390 ppm), acidified (24°C, 1000 ppm), or warming plus acidified (28°C, 1000 ppm) conditions. Algal photosynthesis, respiration, and phenolic content were unaffected by increased temperature and pCO₂, but calcium carbonate content was reduced under high pCO₂ treatments in both temperatures. Metabolic rates of the sea urchin were elevated in the ambient temperature, high pCO₂ treatment, and feeding assays showed that consumption rates also increased in this treatment. Despite some changes to algal chemical composition, it appears that at least under short-term exposure to climate change conditions, direct effects on herbivore metabolism were more important in determining herbivory rates than indirect effects caused by changes in algal palatability.

Keywords: acidification, algae, climate change, *Echinometra lucunter*, *Jania rubens*, warming

1. Introduction

Current changes in temperature and chemistry of the world's oceans related to anthropogenic activities, chiefly the burning of fossil fuels, have been strongly associated with rapid alterations of marine ecosystems at a global scale (Hoegh-Guldberg & Bruno 2010, Doney et al. 2012). A major consequence of fossil fuel combustion is the release of CO₂ into the atmosphere, resulting in an increase from an historical 280 parts per million (ppm) to the present-day level of roughly 400 ppm CO₂ in the past 150 years (IPCC 2014). This is predicted to cause the pH of seawater to drop by ~0.3-0.5 units by 2100, accompanied by a rise in seawater temperature of up to 4°C (Caldeira & Wickett 2005, IPCC, 2014).

Studies show that these changes are likely to have profound consequences for the physiology of individual species, where some taxa may benefit and others will suffer negative consequences (e.g., Harvey et al. 2013, Kroecker et al. 2013a). While the direct effects of ocean warming (OW) or acidification (OA) on a wide variety of marine organisms is increasingly well known (e.g., Kroecker et al. 2010, Byrne 2011), fewer studies have examined the interactive effects of these two factors, or their effects on the interaction between species (Harley et al. 2012, Wernberg et al. 2011, Kroecker et al. 2013b). For example, OW has been shown to affect species interactions, resulting in drastic impacts to marine communities, particularly in coastal benthic ecosystems (Vergés et al. 2016, Wernberg et al. 2016). To complicate predictions even further, by affecting species interactions, indirect effects can reverse the direct effects of climate change (Ghedini et al. 2015, Kordas et al. 2017) and greatly complicate predictions of impacts based on single abiotic variables (Connell et al. 2011).

In the case of calcareous macroalgae, it is generally understood that they will be highly affected by the predicted increase in seawater temperature and OA (Doney et al. 2009), experiencing a reduction in biomineralization, whereas non-calcareous algae will become more productive (Doney et al. 2009, Harley et al. 2012, Kroecker et al. 2013b,

Johnson et al. 2014). In particular, the increased CO₂ concentrations will increase the solubility of calcium carbonate, resulting in lower calcification rates or even net dissolution of carbonate in algae (Campbell et al. 2014, Comeau et al. 2014, Johnson et al. 2014, Kram et al. 2016). Given that CaCO₃ content contributes to the structural integrity and herbivore defenses of calcareous algae (Littler et al. 1983, Lewis 1985, Paul & Hay 1986, Hay et al. 1994, Schupp & Paul 1994), these OA-induced shifts in phytochemistry may play a prominent role in the ability to resist mechanical damage and deter grazing. In addition, calcareous algae exhibit potent herbivore feeding deterrents related to the chemical properties of the algal tissue, such as the concentration of secondary metabolites (Hay & Fenical 1988) which have been shown to respond to OW and OA. The response of these metabolites (e.g., phenolic compounds) to climate change factors seems to be species-specific, ranging from an increase in content (Celis-Plá et al. 2015, 2017, Hargrave et al. 2016, Gouvêa et al. 2017) to no response (e.g., Endo et al. 2013, Campbell et al. 2014, Vizzini et al. 2017) or a decrease in content (e.g., Endo et al. 2015, Yildiz & Dere 2015). Given that herbivory is a key structuring agent in algal community composition (Lubchenco & Gaines 1981, Hay & Fenical 1988), especially in rocky intertidal zones (Poore et al. 2012), both qualitative and quantitative distinctions in the production of phytochemical compounds and the effect on herbivore feeding is of primary.

Studies of acidification and warming on plant-herbivore interactions have given much attention to the role of defenses against herbivory. Some studies state these conditions will cause a strengthened interaction, with increasing algal consumption rates by herbivores by either OW (O'Connor 2009, Poore et al. 2013), OA conditions (Campbell et al. 2014, Duarte et al. 2016) or a combination of both factors (Johnson & Carpenter 2012). These studies often focused on subjecting only plants or only herbivores to climate change conditions, and subsequently testing interactions in control conditions (i.e., Johnson & Carpenter 2012, Campbell et al. 2014). However, in recent experiments, when both plants and herbivores were subjected to experimental conditions, it has been revealed that indirect effects caused

by biotic interactions may amplify or moderate effects to individual species (i.e., Alsterberg et al. 2013, Brown et al. 2014, Poore et al. 2016, Cardoso et al. 2017, Goldenberg et al. 2017, Manríquez et al. 2017, Schram et al. 2017).

Here, we examined the influence of OA and OW on the physiology and phytochemical composition (CaCO₃ and phenolic content) of a coralline alga (*Jania rubens*), oxygen consumption rates of a sea urchin species (*Echinometra lucunter*), and explore the implications of their responses in the context of the algal-herbivore interaction. This information will help to better understand the role of climate-change related stressors in structuring coastal marine trophic webs, beyond individual species.

2. Materials and Methods

2.1 Sample collection

Specimens of *J. rubens* and *E. lucunter* were collected in the rocky intertidal zone in Barra da Lagoa and Armação, Florianópolis, Brazil (27.573767° S, 48.420295° W and 27.749376° S, 48.502852° W) in November 2016. Algae and urchins were placed into coolers and immediately transported to the mesocosm facility at the Universidade Federal de Santa Catarina, where the algae were meticulously cleaned of epibionts and meiofauna before placing them into tanks, separately from the urchins. A three-day acclimatization period for both algae and sea urchins was used to slowly increase temperatures to treatment levels. After this acclimatization period, the mesocosm experiment was initiated.

2.2 Experimental setup

The study was performed in a closed, recirculating mesocosm system under natural light conditions for 21 days (see Fig. 1). A total of eight tanks (100 L) were used to maintain the temperature in smaller tanks (38 L), two of which were immersed in each 100-L tank, resulting in 16 treatment tanks. Water temperature was controlled using several 300 W aquarium heaters and a chiller (Chiller Radical, Brazil), set to the

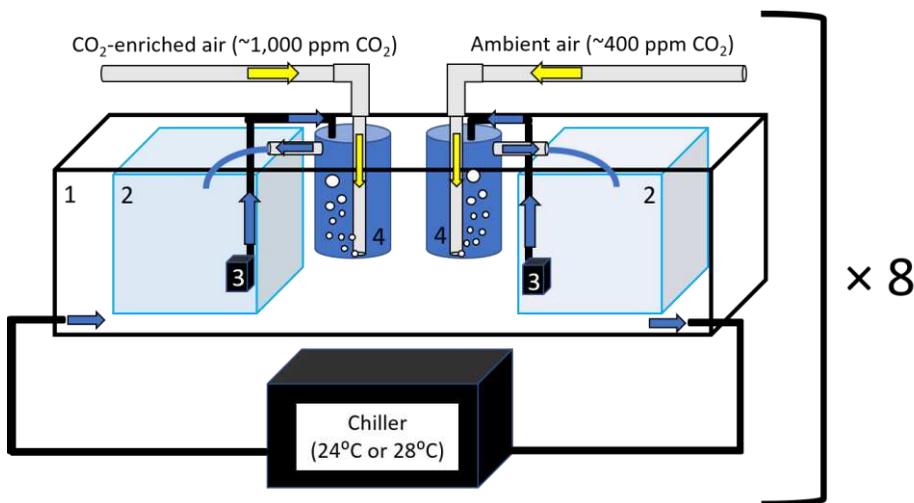
desired temperature. In each 100-L tank, one of the smaller tanks received ambient CO₂ treatment (~400 ppm) and the other received a “future” CO₂ treatment (~1,000 ppm). The concentration of CO₂ in the water was controlled by an IRGA (Infrared Gas Analyzer LIQUOR 7550) and monitored by an independent IRGA PP SystemEGM (EGM-4 Environmental Gas Monitor for CO₂) (described in Silva et al. 2008). The chosen temperatures were 24°C (temperature recorded in the field at time of sample collection) and 28°C (referring to the increase predicted by the IPCC RCP8.5 scenario). Air and a CO₂ mix prepared in a separate mixing tank, for control and the “future” CO₂ treatment, respectively, was injected into seawater within a separate container and pumped into the respective treatment tanks, and recirculated at a rate of 60 L·h⁻¹. A second pump was placed in each tank to ensure adequate within-tank circulation. A total of 10 L of seawater was replaced in each treatment tank every other day, resulting in a ~12.5% day⁻¹ water renovation. Water quality characteristics (temperature, pH, salinity, total alkalinity) were monitored throughout the experiment to calculate carbonate system parameters, using CO2Sys (Table 1). The design resulted in a fully factorial experiment with two temperature levels (24°C and 28°C) and two CO₂ levels (400 ppm and 1,000 ppm), with n=4 replications.

At the end of the experiment, determinations of photosynthesis and respiration of *J. rubens* and respiration of *E. lucunter* were performed and algal samples were dried and frozen for subsequent carbonate and biochemical analysis, respectively.

Table 1: Carbonate system parameters. Values calculated using CO2Calc with total alkalinity and pH_{tot} as input parameters.

Treatment	Temp. (°C)	TA ($\mu\text{mol kg}^{-1}$)	pH	$p\text{CO}_2$ (ppm)
24°C, 400 ppm	23.7 ± 0.08	3,037 ± 124.77	8.23 ± 0.01	293 ± 13.11
24°C, 1,000 ppm	23.7 ± 0.08	3,657 ± 171.10	7.75 ± 0.01	1,371 ± 58.28
28°C, 400 ppm	27.4 ± 0.33	3,196 ± 105.00	8.28 ± 0.01	271 ± 14.99
28°C, 1,000 ppm	27.4 ± 0.33	3,625 ± 73.44	7.78 ± 0.01	1,284 ± 43.28

Figure 1: Schematic representation of the mesocosm system used for the study. A large tank acted as a water bath (1) in which two treatment tanks (2) were immersed. Temperature of the water bath was maintained by 300W heaters and pumping the water through chillers. Water within treatment tanks was constantly circulated via water pumps (3) into separate mixing tanks (4), where either CO₂-enriched air or ambient air was bubbled to attain the desired $p\text{CO}_2$ concentrations. A total of eight water bath tanks was used for the experiment, with four set to 24°C and four set to 28°C.



2.3 Oxygen production and consumption measurements

The algae were incubated with filtered seawater from the respective treatment tanks in 960 mL gas-tight incubation chambers with magnetic stirring to ensure adequate water circulation. Chambers were immersed in a water bath with 50 W aquarium heaters to maintain the same temperature as their respective experimental conditions (24°C or 28°C). Initial oxygen concentration of the seawater was measured with a benchtop oximeter (YSI 5000, Ohio, USA) before organisms were placed in the chambers and quickly sealed. *Jania rubens* were incubated for one hour under saturating light conditions (650 $\mu\text{mol photons m}^{-1} \text{s}^{-1}$) before oxygen concentrations were re-measured. The same protocol was used for the 20-minute dark incubations, which immediately followed the light incubations. For both light and dark incubations, blank chambers with only seawater from the respective treatment tank were incubated to monitor and correct for any background microbial oxygen production/consumption. Oxygen production/consumption was measured as the difference in oxygen concentration from initial and final incubation times, normalized for wet weight of the individual algae. Respiration of *E. lucunter* was measured during dark incubations in the same manner as described above for *J. rubens*, but respiration rates were normalized for sea urchin test diameter.

2.4 Biochemical analyses of the algae

Biochemical composition of *J. rubens* from the different treatments was evaluated at the end of the experiment. Extraction of phenolic compounds followed Schiavon et al. (2012) with some modifications. Samples (~300 mg fresh weight) were ground with liquid nitrogen and 8 ml 80% methanol solution was added. After a 1-h dark incubation, samples were centrifuged for 10 min at 4,400 rpm. 200 μl of the supernatant was pipetted to a separate glass tube; then, 150 μl of folin and 1,650 μl of a 2% NaCO_3 solution were added. Following a 1-h dark

incubation, absorbance of samples was read in a spectrophotometer at 750 nm.

To assess carbonate content, samples of *J. rubens* were dried at 60°C for 24 h, weighed, and afterwards submerged in a 10% hydrochloric acid solution for 30 min. Samples were then gently rinsed with distilled water, dried again at 60°C for 24 h and re-weighed. The CaCO₃ content was derived from the difference in dry weight before and after decalcification of the tissue.

2.5 Determination of feeding rates

Herbivory rates were measured weekly by placing a known quantity of *J. rubens* with each sea urchin from their respective treatment tanks. *J. rubens* samples were weighed prior to being placed with sea urchins, and the remaining quantity of the algae after one week was weighed again. *Jania rubens* growth rates were also monitored weekly, so that herbivory was calculated as the difference between initial and final algal biomass corrected for algal growth during the same period. However, weekly changes in *J. rubens* biomass were minimal.

2.6 Statistical analysis

Statistical analyses were performed using the software Statistica. Interactive and isolated effects between temperature and pCO₂ concentrations were evaluated for algal photosynthesis, respiration, carbonate content, phenolics and sugar content, and for sea urchin respiration and feeding rates at the end of the experiment, using two-way ANOVA. For weekly feeding rates, three-way ANOVA was used to determine isolated and interactive effects between temperature, pCO₂ and time. Newman-Keuls Significant Difference post hoc tests were used to identify the statistically different groups. Homogeneity of variance was tested a priori using Cochran's test.

3. Results

3.1 Algal physiological and biochemical response

Gross photosynthesis and respiration rates of *J. rubens* were not significantly affected by temperature, pCO₂, or the interaction between these factors (Fig. 2a, Table 2). On the other hand, the carbonate content of *J. rubens* decreased slightly but significantly in treatments with increased pCO₂ (6-7% lower compared to control treatment), but was not affected by temperature or the interaction between temperature × pCO₂ (Fig. 2b, Table 2).

Phenolic concentrations ranged from 0.07-0.12 mg g FW⁻¹ algae, but were unaffected by the different treatments (Fig. 2c, Table 2).

3.2 Sea urchin physiological and feeding response

Respiration of *E. lucunter* was significantly affected by pCO₂, with significantly higher rates in the high pCO₂, ambient temperature treatment; in addition, there was an interactive antagonistic effect of pCO₂ and temperature (Fig. 3a, Table 2).

Feeding rates of *E. lucunter* were significantly affected by both time and the interaction between temperature and pCO₂ (Table 2). The general trend showed an increase in feeding over time in all treatments, reaching the highest rates during the third week of the experiment (Fig. 3b). This effect was more pronounced in the high pCO₂ treatment under ambient temperature conditions, compared to the other treatments, with an 87% increase in feeding from the first to the third experimental week (Fig. 3b). The highest overall feeding rates were also found in this treatment (Fig. 3b).

Figure 2: Response of *Jania rubens* to different temperature and pCO₂ treatments. (a) Photosynthesis and respiration, (b) carbonate content, and (c) phenolic content. Data present mean±SE (n=4) and different letters indicate significant statistical differences (ANOVA, p<0.05, Newman Keuls).

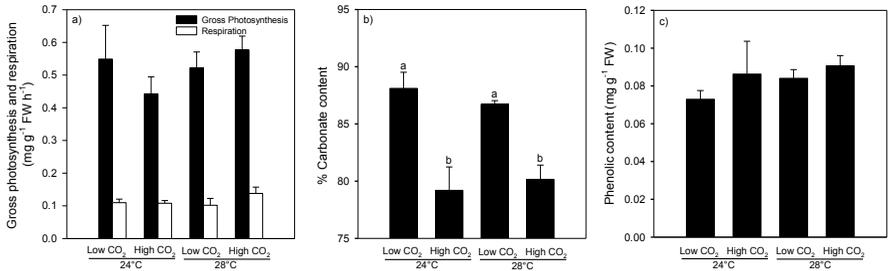


Figure 3: Sea urchin responses to different temperature and pCO₂ treatments. (a) Sea urchin respiration and (b) weekly feeding rates during the experiment. Sea urchin respiration and feeding are standardized by test diameter of individuals. Data present mean±SE (n=4) and different letters indicate significant statistical differences (ANOVA, p<0.05, Newman Keuls).

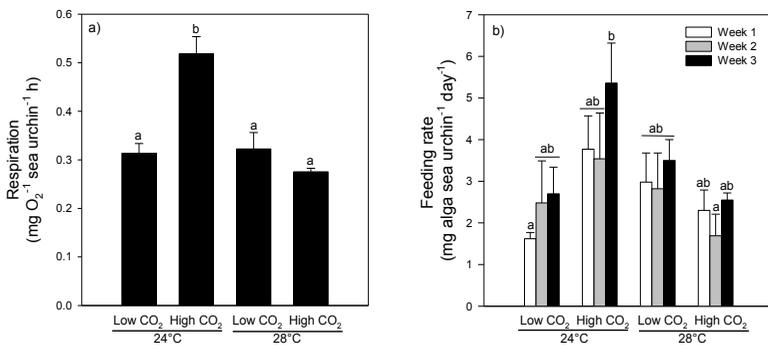


Table 2: Summary of two- and three-way ANOVAs examining the influence of temperature and pCO₂ (and time, in case of the feeding rates) on measured parameters.

Model Source	F-value	P-value
<i>Jania</i> gross photosynthesis		
pCO ₂	0.178	0.681
Temperature	0.772	0.398
pCO ₂ × Temperature	1.74	0.214
<i>Jania</i> respiration		
pCO ₂	1.55	0.239
Temperature	0.637	0.442
pCO ₂ × Temperature	1.86	0.200
<i>Jania</i> carbonate content		
pCO ₂	7.84	0.017
Temperature	0.010	0.920
pCO ₂ × Temperature	0.047	0.831
<i>Jania</i> phenolic content		
pCO ₂	1.22	0.298
Temperature	0.73	0.414
pCO ₂ × Temperature	0.136	0.721
<i>Echinometra</i> respiration		
pCO ₂	6.386	0.032
Temperature	14.678	0.004
pCO ₂ × Temperature	13.288	0.005
<i>Echinometra</i> feeding (by week)		
Time	2.19	0.128
pCO ₂	1.69	0.203
Temperature	2.29	0.141
pCO ₂ × Temperature	12.94	0.001
Time × pCO ₂	0.49	0.619
Time × Temperature	0.50	0.611
Time × pCO ₂ × Temperature	0.26	0.77

4. Discussion

Effects of climate change on species interactions, such as herbivory, will determine ecosystem-level responses under future conditions (Harley et al. 2012). Here, we demonstrate that feeding rates on algae by a marine herbivore are affected by climate change stressors (OW and OA), which seem to be driven more by direct effects on herbivore metabolism than indirect effects to algal palatability.

Sea urchin feeding rates on coralline algae tended to increase over time in all treatments, but the highest feeding rate occurred under control temperature and increased pCO₂ conditions. These higher feeding rates compliment higher respiration rates of the sea urchin in this same treatment, and is best explained by increased metabolic demand under these conditions. Generally, metabolic rates of marine invertebrates increase with increasing ambient temperature (Newell & Branch 1980). However, in this study the respiration rates of sea urchins were found to be unaffected by temperature (see Fig. 3a). This result runs contrary to many other studies investigating metabolic rates of herbivores under warming (Uthicke et al. 2014, Manriquez et al. 2017). In contrast, studies have shown mixed results regarding acidification, with some finding no effect on respiration rates of sea urchins (Stumpp et al. 2012, Moulin et al. 2015, Uthicke et al. 2016) and others showing an increase in respiration (Stumpp et al. 2011, Jager et al. 2016). A lack of response to warming in the current study may be due to the organisms' natural acclimatory ability (e.g., Newell & Branch 1980, Vargas et al. 2017). Both *J. rubens* and *E. lucunter* inhabit shallow intertidal coastal areas (<0.5m), where they are subject to variable temperatures. Thus, these organisms were most likely accustomed to temperatures within the range used in the current study. A similar pattern of higher feeding rates under high pCO₂ conditions has been demonstrated with the sea urchin *Strongylocentrotus purpuratus*, in which *S. purpuratus* consumed the calcareous red alga *Lithothrix* sp. at the highest rates under ambient temperature and high pCO₂ (Briggs 2017).

Photosynthesis and respiration rates of *J. rubens* at the end of the experiment were unaffected by treatments (Fig. 2a). Algal photosynthetic rates have shown to be highly variable to warming and acidification (Ji et al. 2016). For example, though mild warming usually increases photosynthetic rates in algae (Ji et al. 2016), some studies have found no effect of increased temperature on photosynthesis for both fleshy algae (Gutow et al. 2016) and calcareous algae (Martin et al. 2013), while others reported a decrease in photosynthesis in coralline algae (e.g., Tait 2014, Kram et al. 2016). As mentioned above, the studied coralline algae inhabit intertidal rocky shores and are therefore most likely acclimated to a wide range in temperature.

Jania rubens did, however, suffer reduced calcium carbonate content in high pCO₂ treatments, regardless of temperature (Fig. 2b). Acidification has been found to reduce calcium carbonate content in other studies of calcified algae (Johnson & Carpenter 2012, Campbell et al. 2012) that may be due to either increased metabolic demand for calcification, or actual dissolution of calcium carbonate structures (Kroeker et al. 2013a). Calcium carbonate is incorporated into the cell walls of red coralline algae and together with chemical defenses, such as phenolic compounds, serves as a deterrent to herbivory (Hay & Fenical 1988). In contrast to the carbonate content of the algal thallus, phenolic compounds were unaffected by different temperature and acidification treatments (see Fig. 2c). Previous studies of phenolic compounds in macroalgae under climate change conditions have found conflicting results, including decreases (Endo et al. 2015, Yildiz & Dere 2015), increases (Celis-Plá et al. 2015, 2017, Hargrave et al. 2016, Gouvêa et al. 2017), and no changes (Endo et al. 2013, Campbell et al. 2014, Vizzini et al. 2017). Despite reduced calcium carbonate content in the acidified treatments, sea urchins only increased feeding rates in the ambient temperature, high pCO₂ treatment (see Fig. 3b), thus suggesting that feeding rates by the sea urchin were driven by increased metabolism rather than changes to algal palatability.

Several experiments with other algal and herbivore species have found that warming and/or acidification can affect herbivory rates indirectly.

Two species of sea urchins, *Lytechinus variegatus* and *Diadema antillarum*, both increased feeding rates on the calcified green alga *Halimeda optunia* under acidified conditions, which was correlated with reduced algal carbonate content; as with *J. rubens* in the current study, *H. optunia* also did not experience changes in phenolic content (Campbell et al. 2014). Similarly, the crustose coralline alga *Porolithon onkodes* suffered reduced calcification rates under warming and acidification, and the sea urchin *Echinothrix diadema* increased consumption of algae in these treatments (Johnson & Carpenter 2012). These studies lend support to the hypothesis that decreased carbonate content increases the palatability of algae (i.e., Hay & Fenical 1988). However, in the aforementioned cases the sea urchins were not exposed to the same treatment conditions as the algae, therefore did not exhibit any changes in metabolic rates, as observed in the present study.

Many studies have found interactive relationships between primary producers, herbivores, and effects of warming and acidification on the strength of such interactions. Predicting larger community-level responses are more difficult, as many effects of climate change are taxon-specific and may scale differently with different species. For example, feeding rates of an amphipod scaled faster with warming than growth of a brown algae food source (Gutow et al. 2016), but turf algal primary production scaled faster with warming than consumption by an herbivorous gastropod (Mertens et al. 2015). Although some experiments have assessed effects of climate change on sea urchin herbivory rates and algal growth rates simultaneously (Brown et al. 2014, Briggs 2017), there is a lack of long-term studies at the community level. As sea urchins are one of the most important herbivores in many marine ecosystems, such studies are needed.

5. Conclusion

Direct and indirect effects of acidification and warming will shape future marine ecosystems. The results presented here suggest that, at

least in the short-term, direct effects of acidification appear to be driving increased metabolism and feeding rates of the herbivore *E. lucunter*. However, warming and acidification also had complex interactive effects on algal chemical composition, which in the long-term may lead to other impacts on this algal-herbivore interaction. More studies are needed to further investigate the long-term physiological and chemical changes in both algae and sea urchins that will affect patterns of herbivory. Furthermore, other species of sea urchins or herbivores may respond differently to climate change conditions and herbivory rates may be affected differently (Kroeker et al. 2013a).

6. Acknowledgements

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing a master's scholarship to W.A.R. We are grateful to Alessandra Carneiro (Universidade Federal de Santa Catarina) for helping set up the mesocosm system for the current experiment. We also thank Lídiane Gouvêa, Giulia Costa, Gabrielle Koerich, and Fernanda Ramlov (Universidade Federal de Santa Catarina) for assisting with phenolic analyses, and João Silva (Universidade de Algarve) for fruitful discussions.

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CONCLUSÃO GERAL

As interações entre espécies são importantes para determinar a estrutura e a função dos ecossistemas marinhos, mas podem ser impactadas por mudanças globais antropogênicas. Os resultados apresentados aqui sugerem que as macroalgas intermareais afetam a fisiologia e a composição bioquímica das espécies vizinhas, que conseqüentemente, afetam a palatabilidade a um herbívoro. Estudos adicionais em condições mais controladas são necessários para compreender de que forma as macroalgas podem influenciar a fisiologia das espécies vizinhas. Além disso, embora não tenham sido investigados neste estudo, OW e OA são susceptíveis de mudar o equilíbrio competitivo entre as espécies de macroalgas. Assim, estudos de mesocosmos que utilizem diferentes tratamentos de temperatura e acidificação são necessários para elucidar as respostas das espécies em condições futuras. Esses experimentos podem auxiliar na compreensão de como as interações bióticas irão moldar comunidades futuras das macroalgas intermareais sob condições abióticas alteradas. Embora as interações entre macroalgas em condições futuras não tenham sido avaliadas neste estudo, a interação algas-herbívoros entre *Jania rubens* e *Echinometra lucunter* parece ser afetada por mudanças globais. O ouriço-do-mar *E. lucunter* aumentou as taxas de alimentação em condições acidificadas, aparentemente devido aos efeitos diretos da OA no metabolismo do ouriço do mar, mas o aquecimento concomitante parecia negar esse efeito. A alga coralina *J. rubens* também sofreu redução do conteúdo de carbonato em ambos tratamentos acidificados, independentemente da temperatura, indicando que um custo metabólico para a calcificação surgiu sob maior concentração de CO₂. Mais estudos são necessários para investigar os efeitos a longo prazo de OW e OA nessa interação. Além disso, estudos de escala mais ampla, que incluam maior número de espécies, ajudarão a esclarecer as respostas a nível da comunidade frente as mudanças globais.