Universidade Federal do Rio Grande – FURG

Instituto de Oceanografia

Programa de Pós-Graduação em Aquicultura

OTIMIZAÇÃO DAS CONDIÇÕES DE CULTIVO E PRODUÇÃO POR COLHEITAS MÚLTIPLAS DE *SALICORNIA NEEI*, *APIUM GRAVEOLENS* E *PASPALUM VAGINATUM* EM SISTEMA AQUAPÔNICO SALINO

Kennia Brum Doncato

Rio Grande – RS

Fevereiro – 2020

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Kennia Brum Doncato

Tese apresentada como parte dos requisitos para obtenção do grau de doutora no Programa de Pós-Graduação em Aquicultura da Universidade Federal do Rio Grande

Orientador: Prof. Dr. César Serra Bonifácio Costa

Rio Grande – RS

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AQUICULTURA

Universidade Federal do Rio Grande - FURG

ATA 01/2020

DE DEFESA DA 64ª TESE DE DOUTORADO EM AQUICULTURA

No dia dezenove de fevereiro de dois mil e vinte, às oito horas e trinta minutos, no Auditório da Estação Marinha de Aquacultura da FURG, reuniu-se a Banca Examinadora de Tese de Doutorado em Aquicultura, da **KENNIA BRUM DONCATO**, orientada pelo Prof. Dr. Cesar Serra Bonifácio Costa, composta pelos seguintes membros: Prof. Dr. Cesar Serra Bonifácio Costa (Orientador – IO/FURG), Prof. Dr. Luis Henrique da Silva Poersch (IO/FURG), Prof. Dr. Geraldo Kipper Fóes (IO/FURG), Profª. Drª. Marianna de Oliveira Lanari (PPGOB - FURG) e a Profª. Drª. Maria Betânia Galvão dos Santos Freire (UFRPE). Título da Tese: **"OTIMIZAÇÃO DAS CONDIÇÕES DE CULTIVO E PRODUÇÃO POR COLHEITAS MÚLTIPLAS DE *SALICORNIA NEEI*, *APIUM GRAVEOLENS* E *PASPALUM VAGINATUM* EM SISTEMA NFT AQUAPÔNICO SALINO ".** Dando início à defesa, o Coordenador Adjunto do PPGAq, Prof. Dr. Geraldo Kipper Fóes passou a presidência da sessão ao Prof. Dr. Cesar Serra Bonifácio Costa, que na qualidade de orientador, passou a palavra para a candidata apresentar a Tese. Após ampla discussão entre os membros da Banca e a candidata, a Banca se reuniu sob a presidência do Coordenador. Durante esse encontro ficou estabelecido que as sugestões dos membros da Banca Examinadora devem ser incorporadas na versão final, ficando a cargo do Orientador o cumprimento desta decisão. A candidata **KENNIA BRUM DONCATO** foi considerado **APROVADA**, devendo a versão definitiva de a Tese ser entregue na Secretaria do PPGAq, no prazo estabelecido nas Normas Complementares do Programa. Nada mais havendo a tratar, foi lavrada a presente ata, que após lida e aprovada, será assinada pela Banca Examinadora, pelo candidato e pelo Coordenador Adjunto do PPGAq.

PROF. DR. CESAR SERRA BONIFÁCIO COSTA (ORIENTADOR – IO/FURG)

PROF. DR. LUIS HENRIQUE DA SILVA POERSCH (IO/FURG)

PROF. DR. GERALDO KIPPER FÓES (IO/FURG)

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KENNIA BRUM DONCATO

PROF. DR. GERALDO KIPPER FÓES (Coordenador Adjunto do PPGAq)

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RESUMO

O cultivo de plantas vasculares em sistemas aquapônicos integrados a produção de animais marinhos esta crescendo no setor de alimentos. Com isto, o estabelecimento de melhores técnicas de propagação, o atendimento as preferências nutricionais e o uso de regime de poda apropriadas para as halófitas *Salicornia neei* Lag., *Apium graveolens* L. e *Paspalum vaginatum* Sw. irá contribuir para o aumento da produção vegetativa destas espécies comercializáveis. Esta tese teve como objetivo otimizar a produção das halófitas brasileiras *S. neei*, *A. graveolens* e *P. vaginatum* em aquaponia salina com água clarificada proveniente da produção de *Litopenaeus vannamei* B. em sistema de bioflocos (BFT). Para estabelecer um protocolo de germinação para a variedade selvagem de *A. graveolens*, ensaios com distintas temperaturas, concentrações e tipos de desinfetantes químicos foram testados, resultando na produção de mudas de aipo selvagem (90-100%) pela desinfecção das sementes com hipoclorito de sódio a 5-10% e incubadas sob um termoperíodo de 20/30 ºC (12h: 12h) (Capítulo 1). No Capítulo 2, foi verificado se as três halófitas estudadas podem absorver ambas as formas de nitrogênio fornecidas (amônio ou nitrato), e destacado que o nitrato é a forma preferida de nitrogênio absorvida por *S. neei* e *A. graveolens.* Níveis de nitrogênio acima de 10 mg L^{-1} melhoraram o crescimento e a produção de biomassa, mesmo em combinação de formas mistas de nitrogênio. O alto suprimento de amônio como forma exclusiva de nitrogênio afeta negativamente *S. neei* e *A. graveolens*, devido à acidificação da rizosfera, mas este estresse pode ser aliviado pelo aumento do pH da água. *Paspalum vaginatum* não foi sensível à alta concentração de amônio. O nível de fósforo não foi um problema para todas as espécies, mas pode limitar a produção de *S. neei* e *A. graveolens* sob alta concentração de nitrato na água de cultivo, condição de fósforo incomum nos sistemas de aquicultura atuais. Os requerimentos micronutricionais do crescimento e produção de biomassa de *S. neei* são atendidos pelo uso da água clarificada do sistema BFT de *L. vannamei*. Entretanto, a suplementação de micronutrientes na água (particularmente ferro, manganês e molibdênio) foi necessária para aumentar o crescimento de *P. vaginatum*. O fraco desenvolvimento de plantas de *A. graveolens* nas condições experimentais não permitiu avaliar suas respostas às adições de micronutrientes. A fertilização foliar de micronutrientes não foi eficaz para melhorar o crescimento das halófitas (Capítulo 3). As plantas foram submetidas a cortes consecutivos de suas estruturas aéreas a cada 14 e 28 dias (Capítulo 4), e *P. vaginatum* e *S. neei* mostraram capacidade de rebrotaram em todos os tratamentos aplicados. O tratamento de

um corte (a cada 28 dias) permitiu estruturas foliares com melhor valor comercializável, devido a prevenir ao acúmulo de matéria morta e ter abundante perfilhamento e folhas de *P. vaginatum*, assim como produzir *S. neei* com grande quantidade de ramos com tamanho comercializável e não lignificado. A prática de poda não modificou a capacidade das halófitas de assimilação de nitrogênio em sua biomassa a partir da água do sistema aquapônico. No geral, as halófitas brasileiras estudadas podem ser facilmente produzidas em aquaponia salina com águas do sistema BFT de *L. vannamei*, com as práticas divulgadas de melhor germinação, uso de necessidades nutricionais mínimas e manejo de poda estabelecido nesta tese.

ABSTRACT

The cultivation of vascular plants in aquaponic integrated systems to marine animal production is growing in the food sector. Thereby, the establishment of best propagation techniques, the attendance of nutritional requirements and appropriate use of cutting frequencies for the halophytes *Salicornia neei* Lag., *Apium graveolens* L. and *Paspalum vaginatum* Sw. will contribute to the increment of vegetative production of these marketable species. This thesis aimed to optimize the production of the Brazilian halophytes *S. neei*, *A. graveolens* and *P. vaginatum* in saline aquaponics with clarified water from the production of *Litopenaeus vannamei* B. in Biofloc Technology (BFT) system. In order to establish a protocol of germination for the wild variety of *A. graveolens*, trials with distinct temperatures, concentration and types of chemical disinfectants were tested, resulting in production of seedling of wild celery (90-100%) by the disinfection of the seeds with 5–10% sodium hypochlorite and incubating them under a 20/30 ºC thermoperiod (12h:12h) (Chapter 1). In Chapter 2, it was verified if the three studied halophytes can uptake both nitrogen forms supplied (ammonium or nitrate), and highlighted that nitrate is the preferred nitrogen form absorbed by *S. neei* and *A. graveolens*. Nitrogen level higher than 10 mg L^{-1} improved growth and biomass production, even in combination of mixed nitrogen forms. High supply of ammonium as sole nitrogen form affect negatively *S. neei* and *A. graveolens*, due to rhizosphere acidification, but this stress can be relieved by increasing water pH. *Paspalum vaginatum* was not sensitive to the high ammonium concentration. Phosphorus level was not an issue for all species, but may limit *S. neei* and *A. graveolens* production under a high nitrate concentration in the cultivation water, unusual phosphorus condition in current aquaculture systems. Micronutritional requirements of the *S. neei* growth and biomass production are attended by the use of the clarified water from BFT system of *L. vannamei*. However, micronutrient supplementation in the water (particularly iron, manganese and molybdenum) was necessary to increase *P. vaginatum* growth. Poor development of *A. graveolens* plants under the experimental conditions did not allow evaluation of their responses to micronutrient additions. Foliar fertilization of micronutrient was not effective to improve halophytes' growth (Chapter 3). Plants were submitted to consecutive cuttings of their aerial structures at every 14 and 28 days (Chapter 4), and *P. vaginatum* and *S. neei* showed ability to regrowth in all treatments applied. One cutting treatment (every 28 days) allowed foliar structures with better marketable value, due to

prevent the accumulation of dead matter and have plentiful tillering and leaves of *P. vaginatum*, as well as to produce *S. neei* with large amount of branches with marketable size and not lignified. Cutting practice did not modify halophytes capacity of nitrogen assimilation into their biomass from water of the aquaponic systems. Overall, the studied Brazilian halophytes can be easily produced in saline aquaponics with waters from BFT system of *L. vannamei* with the disclosed practices of best germination, use of minimum nutritional needs and cutting management established in this thesis.

Introdução Geral

Sistemas de recirculação de água são projetados para suportar uma produção intensiva de organismos aquáticos, volume menor de água que o convencional e com sistema fechado (Timmons & Ebeling 2010). O tratamento da água permite a remoção de resíduos que possam ser tóxicos aos organismos cultivados e a reutilização da mesma. Neste processo de reuso contínuo, há acúmulo de certos nutrientes inorgânicos e matéria orgânica (*i.e.* resíduos), que podem ser simplesmente removidos ou direcionados para outras produções, a fim de aumentar a variedade de produtos e o retorno financeiro ao empreendimento. Sistemas aquícolas que produzem culturas adicionais a partir dos subprodutos da produção principal são chamados de sistema integrados. Quando a integração se dá com plantas (aquáticas ou terrestres), se refere a este sistema integrado como aquaponia (Timmons & Ebeling 2010, Goddek *et al.* 2019).

A aquaponia consiste da associação do sistema hidropônico de produção de plantas com as águas ou efluentes da produção de organismos aquáticos (Rakocy 2006, Timmons & Ebeling 2010, Goddek *et al.* 2019), frequentemente da carcinicultura e piscicultura. A hidroponia (*hydro*= água e *ponos*= trabalho) é a produção de plantas sem solo, podendo ser fixadas em substrato inerte (*e.g.* areia, brita e perlita), de maneira que os nutrientes minerais essenciais sejam fornecidos em solução nutritiva (Neto & Barreto 2011, Resh 2012). Apesar da hidroponia não ser uma tecnologia atual, pois o primeiro registro de experimento aplicando este conceito é datado de 1840 (Hoagland & Arnon 1950), o termo "hidroponia" empregado para descrever este sistema foi modificado ao longo dos anos, passando pela denominação "nutricultura" (Hoagland & Arnon 1950) e mesmo "aquicultura" (Gericke 1937), sendo está última nomenclatura retificada pelo mesmo autor devido a já ser empregada para descrever a produção de qualquer organismo aquático e então estabelecida à terminologia atual pelo mesmo. A produção hidropônica pode ser realizada nos sistemas com uma camada de substrato em leitos vegetados (*i.e.* areia, pedrisco ou seixos; internacionalmente denominados *vegetated bed*), suportes flutuantes (DWS; *Deep Water System* ou *floating*) ou em sistema de fluxo laminar de nutrientes (NFT; *Nutrient Film Technique*). O sistema NFT é o mais popular na indústria hidropônica de vegetais comerciais em vários países, inclusive do Brasil (Resh 2012), consistindo em canais onde as plantas ficam com suas raízes parcialmente submersas na lâmina da solução nutritiva, a qual fica circulando e possibilita a respiração das raízes (Neto & Barreto 2011). Sistemas NFT são amplamente comercializados, devido ao seu

baixo custo e possibilidade de um retorno financeiro rápido. Podem ser utilizados para a aquaponia, sendo que as águas ou os efluentes gerados da produção aquícola animal passam a ser a solução nutritiva total ou parcial para as plantas.

A aquaponia permite uma considerável absorção de nutrientes dissolvidos na água do sistema aquícola. As plantas absorvem ativamente nutrientes, particularmente nitrato, nitrogênio amoniacal e fosfato, que têm as suas concentrações reduzidas proporcionalmente ao ganho de biomassa pelas plantas (Quintã *et al.* 2015, Pinheiro *et al.* 2017), que possui valor agregado devido ao seu uso alimentício, farmacêutico e/ou industrial (Ventura & Sagi 2013, Bertin *et al.* 2014, Doncato & Costa 2018a, 2018b, Souza *et al.* 2018). Micro-organismos heterotróficos associados à rizosfera das plantas ou superfícies no sistema aquapônico podem promover a mineralização da matéria orgânica dissolvida. Na rizosfera também se encontram bactérias autotróficas aeróbicas nitrificantes, que utilizam o nitrogênio amoniacal como uma fonte de energia formando nitrito, que é posteriormente oxidado a nitrato por outra classe de bactérias (Li *et al*. 2013, Goddek *et al.* 2019). A integração da produção de animais aquáticos e de plantas pode ser através de unidades funcionais (*i.e*. estruturas com animais e sistemas hidropônicos) conectadas ou separadas (Goddek *et al.* 2019) que são, respectivamente, designadas de sistemas aquapônicos acoplados (*coupled aquaponic system*) e sistemas aquapônicos desacoplados (*decoupled aquaponic system*). Este último tipo permite um controle independente sobre cada unidade do sistema, com a manutenção de condições ótimas para os animais e para as plantas, bem como possibilita eventuais desconexões para manutenções e prevenção de interferências em etapas específicas dos ciclos de produção.

Quanto mais intensivo o sistema aquícola, maior a densidade animal e o arraçoamento, consequentemente havendo maior a carga orgânica, material em suspensão e nutrientes gerados. Por exemplo, os sistemas de produção com mínima/"zero" troca de água (*i.e.* reposição ocasional de perdas por infiltração e/ou evaporação), como o sistema de bioflocos (BFT; *Biofloc Technology system*), podem utilizar a mesma água por vários ciclos de produção e com isso retém uma carga de nutrientes considerável. Gaona *et al*. (2011) trabalhando com clarificação (*i.e*. método para remoção de sólidos) do sistema BFT, com o camarão branco do pacífico (*Litopenaeus vannamei* B.), registrou concentrações mínima-máxima de 0,03-0,73 mg L^{-1} de amônia, 3,01-48,77 mg L^{-1} de nitrato e 2,20-7,84 mg L^{-1} de fosfato ao longo de 113 dias de produção. Tais concentrações geradas podem servir de fonte nutritiva para plantas que toleram altas salinidades (*e.g.*

halófitas) do cultivo marinho, e promover seu desenvolvimento e produção de biomassa vegetal, sem comprometer a produção animal. Ressaltando que o sistema de clarificação é um componente essencial da aquaponia, devido a remoção dos sólidos sedimentáveis da produção animal aquática intensiva, que poderiam acumular nas raízes das plantas e formar uma zona anaeróbica que impede a absorção de nutrientes pelo transporte ativo – processo que requer oxigênio (Rakocy 2006, Goddek *et al.* 2019).

Várias halófitas de marismas têm demonstrado serem candidatas promissoras para aplicação na aquaponia salina. Na Hungria, Hegedűs *et al.* (2010) trabalhando com leitos vegetados, observou que metade das halófitas testadas (*Phragmites australis, Typha angustifolia, Glyceria maxima* e *Scirpus lacustris*) exibiram tolerância ao estresse causado pelo sal e inundação com água de efluente salino aquícola, acumulando respectivamente 2,2 a 31,2 kg m^{-2} de biomassa fresca em 90 dias. Já as taxas de absorção de nitrogênio e fósforo na biomassa aérea destas plantas no mesmo período variaram, respectivamente, de 19,6-124,2 g N m^{-2} e 1,7-15,8 g P m^{-2}. Na Itália, 18 halófitas foram cultivadas em bandejas flutuantes (DWS/*floating*) com efluentes de um digestor anaeróbico e Pavan *et al*. (2014) verificaram que *Aster tripolium, Puccinellia palustris* e *Elytrigia atherica* produziram as maiores biomassas individuais (0,6-1,0 kg m^2) em 240 dias [8 meses em 2012] de cultivo comparadas as demais plantas (halófitas e não-halófitas), bem como demonstraram alta absorção de nitrogênio (12,8-21,6 g N m^2) e fósforo (1,6-3,7 g P m^2). Quintã *et al.* (2015) verificaram que a biomassa *A. tripolium* produzido em sistema aquapônico flutuante na Inglaterra não foi afetada pela forma fornecida de nitrogênio (amônio e nitrato) e que a taxa de absorção de nitrogênio em plantas com distintas concentrações de ^{15}N na solução chegou a 476-882 µg N (34-63 µmol N) por g raiz seca por hora.

Os estudos citados acima evidenciaram que halófitas têm potencialidade para remoção de nutrientes, particularmente nitrogênio e fósforo, de diferentes produções animais e em variados sistemas aquapônicos, como também podem alcançar uma grande produção vegetal. Estes estudos também mostraram que a biomassa das halófitas pode ser utilizada para diversos fins, como suplemento alimentar humano (fresco ou seco, vegetal gourmet) e animal (inserido na ração ou oferecido como forragem), produção de combustível (biodiesel, etanol e lenha), produção de óleos, produção de compostos bioativos (*e.g.* vitaminas, antioxidantes e anti-inflamatórios) (Panta *et al.* 2014, Ventura *et al.* 2015).

O grau de halofitismo das plantas varia conforme a espécie, porém como foi observado nos estudos anteriormente citados, a adoção de plantas distribuídas em marismas sujeitas a influência direta da água marinha é uma opção viável para sistemas aquapônicos salinos. Dentre cerca de setenta espécies que compõem a flora de marismas do estuário da Lagoa dos Patos (Costa 1997), a Amaranthaceae *Salicornia neei* Lag. (Figura 1A), a Apiaceae *Apium graveolens* L. (Figura 1B) e a Poaceae *Paspalum vaginatum* Sw. (Figura 1C) são espécies potenciais para o uso na aquaponia salina.

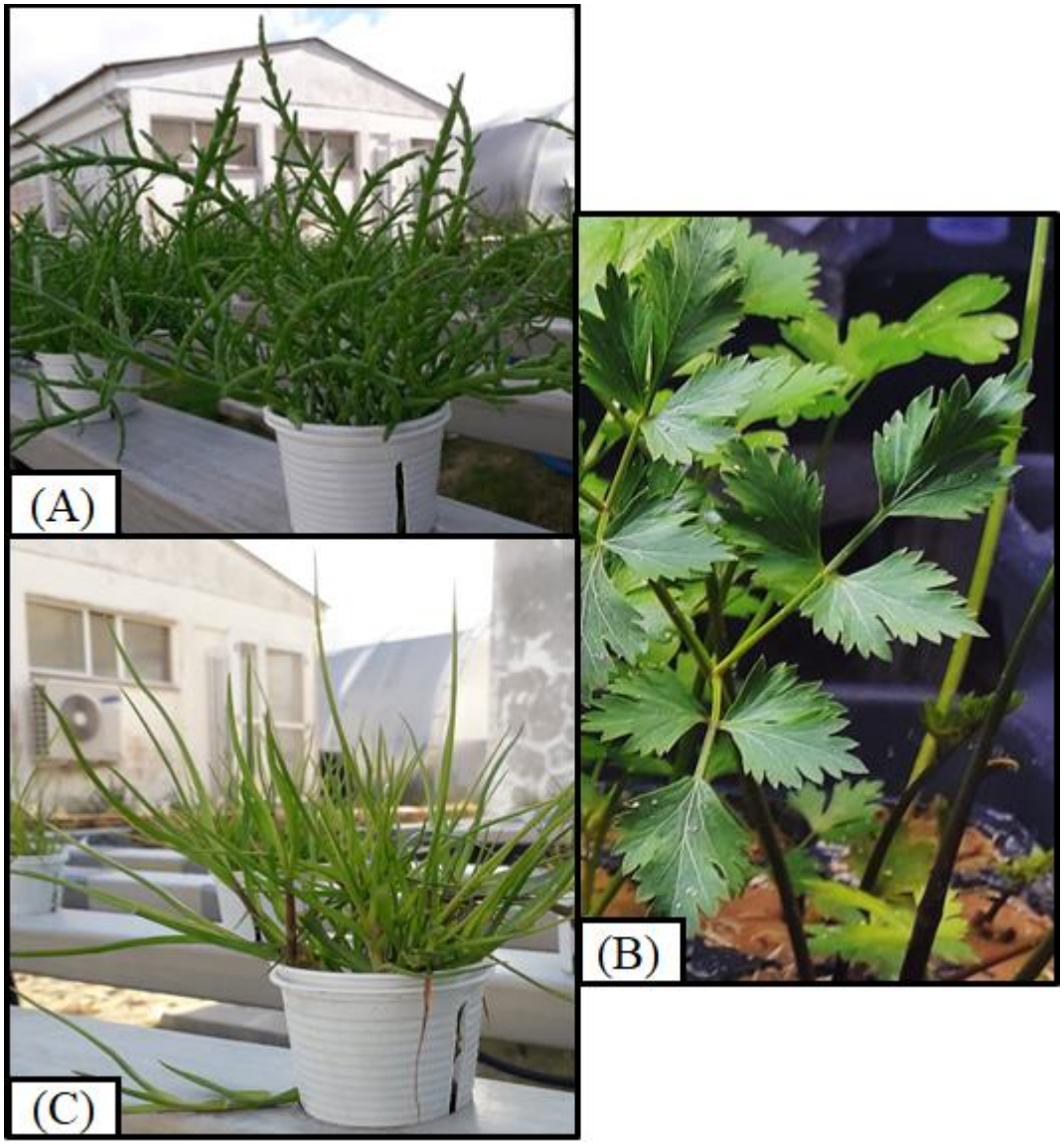

Figura 1. Fotografia das espécies halófitas estudadas nesta tese: *Salicornia neei* (A), *Apium graveolens* (B) e *Paspalum vaginatum* (C).

Salicornia neei Lag. [sin. *Salicornia gaudichaudiana* Moq. e *Sarcocornia ambigua* (Michx.) M.A. Alonso & M.B. Crespo] é um pequeno arbusto perene distribuído nas zonas entremarés das marismas ao longo da maior parte costa atlântica e pacífica da América do Sul (Costa & Herrera 2016, Costa *et al*. 2019). Na última década, cultivos de campo em canteiros abertos desta espécie sob irrigação salina atingiram produtividades por safra de 9 a 23 toneladas de peso fresco de caules por hectare (Costa 2006, 2011, Costa *et al.* 2014, Costa & Herrera 2016, Doncato & Costa 2018a). Estudos recentes têm mostrado uma ótima capacidade desta espécie para ser aclimatada ao cultivo NFT aquapônico (Pinheiro *et al.* 2017), bem como espécies deste gênero podem ser cultivadas com podas frequentes (Costa 2006, 2011, Ventura & Sagi 2013, Costa *et al.* 2014). Esta espécie pode ser cultivada em salinidades acima da concentração da água do mar (Costa 2006, Souza *et al*. 2018), apresenta biomassa de caules e raízes com alta qualidade nutricional, rica em macronutrientes e micronutrientes (particularmente ferro e manganês; Doncato & Costa 2018a, 2018b), além de produzir sementes ricas em ácidos graxos poliinsaturados, principalmente de ácido linoleico (ω-6) e ácido oleico (ω-9) (D'Oca *et al.* 2012). A biomassa dos caules pode ser utilizada na alimentação humana (Costa 2006, Bertin *et al*. 2014, Doncato & Costa 2018a, 2018b) e animal (D'Oca *et al.* 2012, Bertin *et al*. 2014, Costa *et al.* 2014b, Doncato & Costa 2018b), produção de biocombustível e na indústria farmacêutica (EPAGRI 2008, D'Oca *et al.* 2012, Bertin *et al*. 2014, Costa *et al.* 2014b, Souza *et al.* 2018).

Apium graveolens L. é uma herbácea bianual e nitrofila, distribuída em marismas da Europa Oriental, Ásia Menor, Norte da África e Américas (Browers & Orton 1986), que cresce naturalmente nas zonas de depósito de detritos nas marismas do sul do Brasil (Costa 1997). De acordo com Coolbear *et al.* (1993), *A. graveolens* é uma planta conhecida por ser difícil para germinação e no estabelecimento de plântulas. A temperatura e o fotoperíodo podem influenciar a germinação de *A. graveolens*, e há distintas respostas entre as variedades (Thompson 1974). A morfologia e a presença de tubos oleaginosos no pericarpo das sementes dificultam a limpeza das sementes, favorecendo o desenvolvimento de fungos durante a germinação. O estabelecimento de um procedimento de desinfecção adequado para sementes que possam melhorar a porcentagem de germinação e o estabelecimento de plântulas de *A. graveolens* ainda precisa ser realizado. Pardossi *et al.* (1999) cultivaram hidroponicamente *A. graveolens* cultivar Istar em um sistema NFT, em diferentes níveis de salinidade variando de 0,3 a

17,6 g NaCl L^{-1} (5-300 mM NaCl). Estes autores observaram uma ótima capacidade de crescimento em hidroponia desta espécie e um decréscimo de aproximadamente 78% no crescimento em biomassa entre a menor e a maior salinidade, sugerindo um maior potencial de cultivo desta espécie em águas ou efluentes com salinidades inferiores a metade do teor da água do mar. O cultivo de variedades de *A. graveolens* é difundido mundialmente por causa das suas raízes bulbosas, folhas verdes, pecíolos e sementes, consumidos frequentemente em saladas (Malhotra 2012, Rana 2016). Possui um aroma característico, devido à presença de óleos essenciais, composto por ftalidas incluindo 3-n-butiftalida e sedanolida; sendo utilizado principalmente como especiaria/condimento e cultura alimentar (Kokotkiewicz & Luczkiewicz 2016). O consumo de *A. graveolens* pode promover a suplementação de minerais (Ca, Mn, Mg, P, Fe e Zn), vitaminas (A, B_1, B_9, C, E e K) e fitoquímicos (antocianinas, carotenóides, ácido rosmarínico e principalmente ácidos fenólicos). A variedade selvagem encontrada nas marimas do sul do Brasil possui conteúdo de compostos fenólicos livres dez vezes maior que os valores encontrados em cultivares comerciais de aipo (Souza *et al.* 2018). Estes dados corroboram ao seu uso medicinal como anti-inflamatório, antiespasmódico e atividade antioxidante (Malhotra 2012, Helaly *et al*. 2015, Rana 2016).

Paspalum vaginatum Sw. é uma grama perene cosmopolita, que no sul do Brasil se distribui em marismas e áreas úmidas de dunas costeiras (Costa 1997). Pessarakli & Touchane (2006) e Uddin *et al*. (2012) observaram redução de 68% e 23% na biomassa de *P. vaginatum* cultivadas em altas salinidades (30-32 g NaCl L^{-1}) do que em água doce, respectivamente, em leitos vegetados e solos salinizados. Podas semanais foram realizadas satisfatoriamente por Pessarakli & Touchane (2006), porém ainda não há estudos avaliando o efeito da poda no crescimento e na produção de biomassa desta espécie. Devido ao seu crescimento vigoroso, mesmo quando irrigada com águas salobras, *P. vaginatum* é utilizada em áreas áridas na cobertura de campos para esportes como o golf (Brosnan & Deputy 2008, Lonard *et al*. 2015), paisagismo (Brosnan & Deputy 2008, Ntoulas & Nektarios 2015), forragem para cabras e gado (Shonubi & Okusanya 2007, Lonard *et al*. 2015) e fitorremediação de solos salinos ou com metais pesados tóxicos (Lonard *et al.* 2015). A biomassa de *P. vaginatum* é composta por 1,3% de ácidos graxos, 10,4% de proteínas e 77,1% de carboidratos (DesRochers *et al.* 2009), bem como apresenta alta concentração de compostos fenólicos (Souza *et al*., 2018).

As três halófitas nativas do sul do Brasil já citadas podem ser utilizadas como fonte alimentar animal e/ou humana. Entretanto, para produzi-las a necessidade de verificar quais as respostas de crescimento destas espécies frente às diferentes concentrações de nitrogênio e fósforo observadas em águas da aquicultura, que variam ao longo do período de produção. A forma do nitrogênio disponível na água de carcinicultura e piscicultura varia conforme o tipo de produção, manejo e o estágio de crescimento dos animais cultivados. Por exemplo, após a ativação de sistemas mais intensivos (*e.g.* BFT), há um período inicial para o desenvolvimento de bactérias nitrificantes, ocorrendo um ápice de nitrogênio amoniacal total (NAT) em aproximadamente 14 dias (Timmons & Ebeling 2010, Goddek *et al.* 2019). Com o posterior estabelecimento de uma flora microbiana quimioautotrófica, o processo de nitrificação é intensificado levando ao decréscimo do NAT, devido a este ser oxidado a nitrito (NO_2) pelas bactérias amônia-oxidantes (*e.g.* gênero *Nitrosomonas, Nitrosococcus, Nitrospira, Nitrosolobus* e *Nitrosovibrio*), e subsequentemente oxidação deste nitrito a nitrato (NO_3) pelas bactérias nitrito-oxidantes (*e.g.* gênero *Nitrobacter, Nitrococcus*, *Nitrospira* e *Nitrospina*). Em consequência deste processo de estabelecimento da comunidade microbiana em substrato como biofilme ou no biofloco, o acúmulo progressivo de nitrato na água é frequentemente observado a partir do 21º dia de cultivo (Timmons & Ebeling 2010, Goddek *et al.* 2019), podendo atingir níveis tóxicos para camarões (engorda de *L. vannamei*: >220 mg $N\text{-}NO_3$ L^{-1} em salinidade 11 g L^{-1}; Kuhn *et al.* 2011) e peixes (*Rachycentron canadum*: ≥ 1000 mg $N\text{-}NO_3$ L^{-1}; Rodrigues 2013) ao longo da produção.

Diferentemente do nitrogênio, o fósforo tem um ciclo sedimentar e sem uma rota de saída dos sistemas aquícolas tradicionais. Sua adição e acúmulo progressivo na água do cultivo ocorrem via fornecimento de rações ou mesmo bombeamento de águas subterrâneas ricas neste elemento, sendo necessária sua retirada a partir de métodos físicos, químicos ou biológicos (Timmons & Ebeling 2010, Silva *et al.* 2013). Altos teores de fósforo gerados por sistemas aquícolas podem causar impactos negativos no meio ambiente (*i.e.* eutrofização), como também o acúmulo deste elemento na água das estruturas de produção, o que pode propiciar ao desenvolvimento de cianobactérias nocivas aos camarões, quando a proporção de nitrogênio e fósforo esta baixa (Silva *et al*. 2013, Zarski *et al.* 2010). Como apontado anteriormente, espécies halófitas se diferenciam quanto suas preferências pela forma de nitrogênio suprida (Quintã *et al.* 2015), porém esta informação não é ainda conhecida para *S. neei*, *A. graveolens* e *P.*

vaginatum. Existe pouca informação sobre as concentrações de micronutrientes em águas da aquicultura. A disponibilidade de micronutrientes nas águas salinas pode limitar a assimilação de nitrogenados e a produção de biomassa das halófitas (Ventura *et al.* 2010, Ventura & Sagi 2013), sendo necessária a avaliação se as águas de sistemas aquícolas também podem suprir estas necessidades nutricionais específicas.

A técnica de manejo do cultivo aquapônico também tem que ser otimizada para cada planta cultivada conforme seus hábitos e velocidade de crescimento. Plantas anuais que somente crescem a partir de sementes e morrem após florescerem têm que ser replantadas a cada ciclo de cultivo. Contrastantemente, muitas plantas perenes são capazes de rebrotar após o corte de seus caules e folhas, podendo oferecer várias safras a partir de um único plantio. A técnica de poda em halófitas com colheitas repetidas (*e.g.* colheitas múltiplas) pode possibilitar um maior rendimento ao longo de um ciclo anual do que uma única colheita (Costa 2006, Costa *et al.* 2014, Ventura & Sagi 2013), bem como uma melhor qualidade nutricional da biomassa recém-formada (Ventura *et al.* 2011, Ventura & Sagi 2013). Entretanto, há poucos estudos quanto à resposta de crescimento ao regime de poda por plantas halófitas e isto afeta a qualidade da biomassa vegetal e a absorção de nutrientes da água de cultivo.

Hipóteses

As seguintes hipóteses foram testadas no desenvolvimento da Tese:

H1) Devido ao clima temperado quente do extremo sul do Brasil, uma maior geminação das sementes da variedade de *A. graveolens* encontrada no estuário da Lagoa dos Patos deve ser alcançada através da incubação das sementes em temperaturas alternadas durante o dia (termoperíodo) do que sob temperatura constante, bem como menores infestações das sementes por fungos poderão ser obtidas pela rotina de pré-lavagem das sementes com soluções concentradas de desinfetantes químicos;

H2) *S. neei, A. graveolens* e *P. vaginatum* apresentarão diferentes preferências quanto à forma de nitrogênio (amônio ou nitrato) para o seu desenvolvimento, podendo este aspecto ser limitante para o cultivo em determinadas fases de desenvolvimento dos micro-organismos quimioautotróficos. Entretanto, um melhor crescimento destas plantas ocorrerá quando submetidas a altos teores dissolvidos de nitrogênio e fósforo na água de cultivo;

H3) As águas de sistemas aquapônicos integrados ao cultivo de camarão não suprirão todos nutrientes essenciais para o desenvolvimento das plantas, inclusive os micronutrientes, havendo a necessidade de suplementação;

H4) A produção de biomassa e a assimilação do nitrogênio da água salina de sistemas aquapônicos com as halófitas perenes serão maiores em um manejo de podas múltiplas do que de poda única; e

H5) A qualidade nutricional para consumo da biomassa das três halófitas, em termos de teor de nitrogênio, será maior em cultivos com águas com maiores teores dissolvidos de nitrogênio e fósforo, bem como quando as plantas perenes forem submetidas a podas múltiplas no sistema aquapônico.

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Objetivos

Objetivo geral

O objetivo desta tese é otimizar a produção das halófitas *S. neei*, *A. graveolens* e *P. vaginatum* nativas da costa brasileira em sistema aquapônico salino com água clarificada do sistema BFT de *L. vannamei*, através do estabelecimento das melhores condições de produção de propágulos, qualidade nutricional e de manejo de poda para o cultivo.

Objetivos específicos

2.1. Testar aplicações de hipoclorito de sódio e ácido acético como desinfetantes e determinar a melhor temperatura para a germinação da variedade halófita selvagem de aipo (*A. graveolens*) encontrada no sul do Brasil (H1);

2.2. Avaliar o crescimento e a produção de biomassa das três halófitas em diferentes combinações de nitrogênio (amônio ou nitrato) ocorrentes em sistemas intensivos de produção de animais aquáticos, bem como níveis de fósforo dissolvido (H2);

2.3. Avaliar a ocorrência de limitação micronutricional (ferro, manganês, zinco, cobre, boro e molibdênio) para o crescimento das três halófitas em sistema aquapônico salino (H3);

2.4. Testar o efeito de diferentes regimes de poda na produção de biomassa das halófitas perenes *P. vaginatum* e *S. neei* em sistema aquapônico salino (H4); e

2.5. Verificar os efeitos na qualidade nutricional da biomassa das três halófitas do cultivo hidropônico com diferentes teores dissolvidos de nitrogênio, bem como diferentes regimes de poda no sistema aquapônico salino (H5).

Capítulo 1:
Germinação e infecção fúngica de sementes de aipo selvagem (*Apium graveolens* L.), do sul do Brasil, sob diferentes condições de temperatura e desinfecção

[**Germination and fungal infection of wild celery (*Apium graveolens* L.) seeds, from southern Brazil, under different temperature and disinfection conditions**]

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RESUMO EXPANDIDO

INTRODUÇÃO

Apium graveolens L. (Apiaceae) é uma planta herbácea de marismas, comumente denominada de aipo ou salsão, consumida desde a antiguidade devido ao seu sabor único, composição nutricional, teor de fibras e inúmeros usos farmacêuticos (Yao *et al*., 2010; Shad *et al*., 2011; Uddin *et al*., 2015). As variedades de aipo selvagem têm um interesse crescente no mercado e valor horticultural, devido ao seu comportamento de crescimento e sabor peculiar (Browers & Orton, 1986; Yao *et al*., 2010), visto que clientes de alimentos gourmet são abertos a novas variedades de vegetais comestíveis.

No sul do Brasil, Costa (1997) registrou uma variedade halofítica bianual de *A. graveolens* que ocorre em marismas do estuário da Lagoa dos Patos (RS). Esta variedade selvagem possui teor de compostos fenólicos livres (conhecidos como compostos bioativos) dez vezes maior do que de cultivares comercial de aipo (Souza *et al*., 2018). No entanto, estudos iniciais sobre a domesticação dessa variedade halofítica tiveram um grande entrave devido à intensa infestação de sementes por fungos durante a germinação. Além disso, a temperatura e o fotoperíodo podem influenciar a germinação de *A. graveolens*, e há respostas distintas entre as variedades (Thompson, 1974).

O estabelecimento de um procedimento de desinfecção adequado para sementes pode melhorar a porcentagem de germinação e o estabelecimento de plântulas de *A. graveolens*. O objetivo do presente estudo foi avaliar as aplicações de hipoclorito de sódio e ácido acético como desinfetantes e determinar a melhor temperatura para germinação da variedade selvagem halofítica de aipo (*A. graveolens*) encontrada no sul do Brasil.

MATERIAL E MÉTODOS

Sementes de aipo selvagem (*Apium graveolens* L.) do sul do Brasil foram obtidas na marisma da Ilha da Pólvora, localizada no estuário da Lagoa dos Patos (Rio Grande, RS; 32º01'S, 52º06'W), e desinfetadas superficialmente com diferentes soluções de hipoclorito de sódio (5 e 10%) e ácido acético (0,5, 1, 2, 4%). O sucesso da germinação e infecção por fungos foram avaliados sob os tratamentos de desinfecção previamente descritos em temperatura constante de 30 ºC e termoperíodo de 20/30 ºC (12h: 12h). A porcentagem total de germinação e a porcentagem de sementes com infecção por fungos foram comparadas entre as temperaturas e os tratamentos de desinfecção, usando os procedimentos de Análise de Variância (ANOVA).

RESULTADOS E DISCUSSÃO

Germinação total vigorosa (90 a 100%), velocidade de germinação mais rápida (1,8 a 2,5 sementes germinadas por dia) e infecção fúngica moderada (53,3 a 81,7%) de sementes de aipo selvagem foram obtidas com os tratamentos com hipoclorito de sódio (concentração de 5 a 10%) no termoperíodo de 20/30 ºC (Tabela 1). Da mesma forma, Morinaga (1926) encontrou uma germinação máxima para *A. graveolens* (cultivares Dreers Monarch e Columbia) entre 50 e 70% em um termoperíodo de 22/32 ºC. Thompson (1974) também relatou que um termoperíodo de 22/25 ºC foi mais eficaz para a germinação de outras variedades de *A. graveolens* (cultivares Golden Self-blanching, Avon Pearl, Lathom blanching, Giant Red e Solid White) do que incubações de sementes em temperatura constante. A germinação do aipo selvagem foi inibida à temperatura constante (30 ºC). De acordo com Biddington *et al*. (1980), uma alta temperatura (32 ºC) pode induzir dormência secundária de sementes de *A. graveolens* (cultivar Lathom Blanching), possivelmente impedindo o desenvolvimento embrionário e a ruptura do endosperma.

O tratamento com ácido acético a 4% foi muito eficaz na prevenção de infecções por fungos nas sementes (apenas 5% das sementes infestadas), mas reduziu a germinação total média para 60%. Anteriormente Doran (1929) já havia apontado que o ácido acético pode ter um efeito tóxico em plantas vasculares. Concentrações mais baixas de ácido acético (0,5% a 2%) resultaram em 100% de infecção por fungos nas sementes do aipo (Tabela 2). Semelhantemente, Van der Wolf *et al*. (2008) encontraram uma redução acentuada na eficiência de desinfecção do ácido acético em bactérias associadas a sementes com sua diluição. O tratamento com hipoclorito de sódio resultou em infecções por fungos intermediárias (53,3-81,7%) e alta taxa de germinação (90-100%). Taylor (1949) e Abdul-Baki & Moore (1979) destacaram que cultivares de *A. graveolens* respondem bem mesmo com soluções mais concentradas de hipoclorito de sódio.

CONCLUSÃO

Mudas de aipo selvagem do sul do Brasil podem ser efetivamente produzidas através da desinfecção das sementes com hipoclorito de sódio de 5 a 10% e incubação sob um termoperíodo de 20/30 ºC (12h: 12h).

ABSTRACT

Seeds of wild celery (*Apium graveolens* L.) from southern Brazil were surface disinfected with different solutions of sodium hypochlorite (5 and 10%) and acetic acid (0.5, 1, 2, 4%), and germination success and fungal infection were evaluated after 28 days of incubation at a constant temperature of 30 ºC and 20/30 ºC thermoperiod (12h:12h). Germination of wild celery was inhibited at the constant temperature (30 ºC). Vigorous total germination (90–100%), a faster germination velocity (1.8–2.5 germinated seeds per day) and moderate fungal infection (53.3–81.7%) of wild celery seeds were obtained with the sodium hypochlorite treatments (5–10% concentration) under the 20/30 ºC thermoperiod. The 4% treatment of acetic acid was very effective at preventing seed fungal infection (only 5% of the seeds) but it reduced the average total germination to 60%. Lower concentrations of acetic acid (0.5–2%) resulted in 100% fungal infection. In conclusion, seedlings of wild celery from southern Brazil can be effectively produced by disinfecting the seeds with 5–10% sodium hypochlorite and incubation under a 20/30 ºC thermoperiod (12h:12h).

Keywords: acetic acid; fungi; sodium hypochlorite; prophylaxis.

INTRODUCTION

Apium graveolens (Apiaceae) is an herbaceous marshland plant commonly used for consumption since antiquity, mainly due to its unique taste, nutritional composition, fiber content and innumerous pharmaceutical uses (Yao *et al*., 2010; Shad *et al*., 2011; Uddin *et al*., 2015). Browers & Orton (1986) stated that *A. graveolens* is distributed in coastal marshes of Eastern Europe, Asia Minor, North Africa and North America, and that three botanical varieties of celery (*i.e*., var. *dulce*, *rapaceum* and *secalinum*) were domesticated (also called smallage and marsh parsley). Nowadays, several cultivar varieties (cvs.) of celery are found worldwide (Yao *et al*., 2010; Uddin *et al*., 2015).

Wild celery varieties have an increasing market interest and horticultural value, due to their growth behavior (*i.e*., elongated) and peculiar flavor (*i.e*., pungent acrid) (Browers & Orton, 1986; Yao *et al*., 2010), since customers of gourmet foods are more open to new varieties of edible vegetables. Some *A. graveolens* varieties have a marked salt tolerance (*i.e*., halophytes) inherited from their ancestors that inhabited salt marshes (Everard, 1994), and cultivating these varieties with irrigated salt or brackish water can be marketed as environmentally friendly due to fresh water conservation, which increases the value of these vegetables. In southern Brazil, Costa (1997) recorded a biannual halophytic variety of *A. graveolens* that occurs in salt marshes of the Patos Lagoon estuary. This wild variety has free phenolic compounds content (known bioactive compounds) that is 10-fold greater than values found in commercial celery cultivars (Souza *et al*., 2018). However, initial studies about the domestication of this halophytic variety had a major setback due to intense seed infestation by fungi during germination. In Brazil, main fungal diseases recorded for commercial *A. graveolens* var. *dulce* are produced by seedborn fungi *Rhizoctonia solani*, *Pythium* spp., *Phytophthora nicotianae* and *Alternaria dauci* (Reis *et al*., 2018). According to Silveira (2012) *Rhizoctonia* sp. is one of most frequent fungi occurring on leaves of salt marsh plants of Patos Lagoon estuary during summer-autumn, but no proper identification of fungi on seeds of the halophytic variety of *A. graveolens* was done.

According to Coolbear *et al*. (1993), *A. graveolens* is known for being difficult in relation to germination and establishment. Temperature and photoperiod can influence the germination of *A. graveolens*, and there are different responses among the varieties (Thompson, 1974). Seed germination of this species usually takes a long time and there is some asynchrony under suboptimal temperatures (Van der Toorn & Karssen, 1992).

The pericarp of celery seeds has alternating longitudinal furrows (yellowish parts) and ridges (darkish parts), and schizogenous oil tubes run beneath the furrows (Hopkins, 1927). Volatile oil obtained from celery seeds is used in the perfume and pharmaceutical industries (Shad *et al*., 2011), but the presence of ridges and oil tubes on the seed pericarp make it difficult to clean the seeds, favoring the development of fungi during germination. Surface seed disinfection by germicide application is necessary to remove microorganisms that may interfere with germination (Abdul-Baki & Moore, 1979).

Among the many alternatives, sodium hypochlorite and acetic acid are the most affordable and easy to find, because they are used domestically as household bleach and vinegar, respectively. Sodium hypochlorite (NaOCl) is a chemical typically used as a sterilizing agent of seeds, since it does not affect seed germination and seedling growth (Abdul-Baki & Moore, 1979). Acetic acid (CH_3COOH) is an organic acid used as a seed disinfectant and adopted by organic agriculture, since it has low eco-toxicological risk (Van der Wolf *et al*., 2008). Besides the need to control seed infestation by microorganisms, exposure time and concentration of a disinfectant can affect seed germination and may lead to losses in viability. Thus, the establishment of a proper disinfectant procedure for seeds can improve germination percentage and the successful establishment of *A. graveolens* plantlets. The aim of the present study was to evaluate applications of sodium hypochlorite and acetic acid as disinfectants and to determine the best temperature for germination of the halophytic wild variety of celery (*A. graveolens*) found in southern Brazil.

MATERIAL AND METHODS

Material

Seeds of the halophytic wild variety of *A. graveolens* were collected in the Pólvora Island salt marsh located in the Patos Lagoon estuary, Rio Grande, RS (32º01'S; 52º06'W). The seeds were dried at room temperature (20–25 ºC) for 30 days and then stored at 5 ºC in the germplasm bank of the Laboratório de Biotecnologia de Halófitas (Instituto de Oceanografia – IO, Universidade Federal do Rio Grande – FURG) for six months before the experiments.

Experiment 1. Temperature effect on germination and fungal infestation

Seeds were surface sterilized by soaking them for 5 minutes using three disinfection solutions: 5% and 10% sodium hypochlorite, and 4% acetic acid. The concentrations of sodium hypochlorite were made from a dilution of 2.5% active chlorine (common concentration of Brazilian household bleach) and the concentration of acetic acid was made from Brazilian vinegar (4% acetic acid; Brazil, 2000); they were prepared with pure chemicals. After disinfection, the seeds were rinsed with distilled water and placed in autoclaved Petri dishes with filter paper dampened with 6 mL of distilled water. The Petri dishes were placed in germination chambers at a constant temperature of 30 ºC and thermoperiod of 20/30 ºC. Seed incubation lasted for 28 days and both chambers had a photoperiod of 12 h light/12 h dark (40 µmol photons $m^{-2}s^{-1}$, 400–700 nm; provided by cold white fluorescent light). Three Petri dishes with 20 seeds were used as replicates of each treatment. Seed germination (radicle protrusion) was recorded every week and the percentage of seeds with fungal infestation after one week of incubation was used as a proxy of disinfection efficiency. For this procedure individual seeds were graded according to a 2 – digit pathogenicity scale (0 and 1); whereby 0 indicates without fungi, 1 = with fungi. Due to the high fungal infestation at the end of first week, the disinfection treatments were once repeated.

Experiment 2. Disinfection procedure effect on germination and fungal infestation

Due to the strong inhibition of seed germination by the 4% acetic acid solution, three additional disinfection treatments with lower concentrations of acetic acid (0.5%, 1% and 2%) were tested in a second 28-day trial using only the 20/30 ºC thermoperiod. This second experiment used the same photoperiod, number of seeds per Petri dish and number of replicates as the first experiment.

Statistical analysis

Germination speed index (GSI) was calculated as described in Maguire (1962) and expressed in germinated seeds per day. Average values of total germination percentage and percentage of seeds with fungal infection were compared between temperatures (20/30 ºC and 30 ºC) and among disinfection treatments (5% and 10% sodium hypochlorite, and 4% acetic acid) using a bifactorial ANOVA. Data for the disinfection treatments of both germination trials exposed to the thermoperiod were combined and the total germination percentage, GSI and percentage of seeds with fungal

infection were compared with a one-way analysis of variance (ANOVA). Germination speed index values for the constant temperature (30 ºC) were not calculated due to the absence of germination under this experimental condition. The requirements of normality and homoscedasticity for the ANOVA procedures (Zar, 2010) were evaluated using Shapiro-Wilk's and Levene's tests, respectively. Significant differences in the ANOVA ($p < 0.05$) were followed by Fisher's least difference (LSD) test at 5% significance.

RESULTS AND DISCUSSION

The germination and seed fungal infection data are in Tables 1 and 2. At a constant temperature of 30 ºC (Table 1), no germination of the wild celery seeds occurred after 28 days of incubation. In contrast, at the 20/30 ºC thermoperiod up to 100% of the seeds germinated when they were disinfected with sodium hypochlorite. Previously, Morinaga (1926) found a maximum germination for *A. graveolens* (cvs. Dreers Monarch and Columbia) between 50–70% at a 22/32 ºC thermoperiod. Thompson (1974) also reported that a thermoperiod of 22/25 ºC was most effective for the germination of other cultivar varieties of *A. graveolens* (cvs. Golden Self-blanching, Avon Pearl, Lathom blanching, Giant Red and Solid White), but he noted that germination velocity response to temperature can be different for each celery variety. Concerning the effect of constant temperatures, Hopkins (1927) worked with cultivar varieties of celery and Parera *et al*. (1993) studied non-primed *A. graveolens* seeds (cv. M-68-29-5) and found overall seed germination percentages of 28% and 2% at 30 ºC, respectively. Coolbear *et al*. (1992), working with pre-imbibed seeds of two cultivars of *A. graveolens* (cvs. Tall Utah 52-70 and Green Giant Hybrid), recorded only an average of 6% germination of seeds exposed to 25 ºC for 34 days. Morinaga (1926) observed no germination for varieties of *A. graveolens* at 32 ºC for 30 days. According to Biddington *et al*. (1980), a high temperature (32 ºC) may induce secondary dormancy of *A. graveolens* seeds (cv. Lathom Blanching), possibly preventing embryo development and endosperm breakdown, making the seed deal directly or indirectly (pre-imbibed and dried seeds) with desiccation.

Table 1: Mean ± (standard error) of the total germination and fungal infection of the wild *A. graveolens* under thermoperiod and constant temperature among disinfection treatments. Summary of two-way ANOVA results for all parameters among disinfection levels and seed incubation temperatures are presented.

Treatment	Total germination (%)				Fungal infection (%)			
	20/30 °C [$]		30 °C		20/30 °C		30 °C	
5% NaOCl	90.0 (5.8)	c	0.0 (0.0)	a	53.3 (8.8)	b	68.2 (5.6)	bc
10% NaOCl	100.0 (0.0)	d	0.0 (0.0)	a	81.7 (10.9)	c	65.2 (2.2)	bc
4% CH_3COOH	60.0 (2.9)	b	0.0 (0.0)	a	5.0 (2.9)	a	6.1 (1.5)	a
F Disinfection	31.2 (***)				46.1 (***)			
F Temperature	1500.0 (***)				0.00 (ns)			
F Disinfection x Temperature	31.2 (***)				1.2 (ns)			

[$] Different lowercase letters represent significant differences between temperatures and disinfection treatments, according to Fisher's least difference (LSD) test at 5% significance. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant ($p > 0.05$).

Fungal disinfection of wild *A. graveolens* seeds was statistically better for the 4% acetic acid treatment (lowest fungal infection = 5% of the seeds), but this procedure strongly inhibits the average total germination (60% at the 20/30 ºC thermoperiod after 28 days). Lowering the concentration of acetic acid (0.5–2%) led to fungal infection of 100% of the seeds, lower germination velocities and smaller final total germination than seeds disinfected with sodium hypochlorite (Table 2). Similarly, Van der Wolf *et al*. (2008) found a marked decrease in disinfection efficiency of acetic acid on seed-associated bacteria with this dilution. According to Doran (1929), acetic acid may have a toxic effect on vascular plants. For the sodium hypochlorite treatments, 53.3–81.7% of seeds were infected by fungi after one week of incubation, but the seeds showed high germination velocities (average GSI =1.8–2.5 germinated seeds per day) and final total germination values above 90%. Pathogenicity scale did not distinguish several levels of infestation (only infected and not infected seeds), but the results suggest that sodium

hypochlorite treatments were effective to inhibit fungi seed damage, and the most concentrated solution allowed the highest total germination. Taylor (1949) and Abdul-Baki & Moore (1979) pointed out that *A. graveolens* cultivar varieties responded well to concentrated sodium hypochlorite solutions, being the total germination of *A. graveolens* cv. Detroit Golden reduced somewhat by the solutions of 1.5% and 2% "active chlorine" tolerated by cv. Tall Utah (Taylor, 1949).

Table 2: Mean ± (standard error) of the total germination, germination speed index (GSI) and fungal infection of the wild *A. graveolens* under thermoperiod among disinfection treatments. Summary of one-way ANOVA results for all parameters among disinfection treatments are presented.

Treatment	**Total germination (%)** [$]		**GSI (Germinated seeds per day)**		**Fungal infection (%)**	
5% NaOCl	90.0	c	1.8	ab	53.3	b
	(5.8)		(0.2)		(8.8)	
10% NaOCl	100.0	c	2.5	c	81.7	c
	(0.0)		(0.0)		(10.9)	
0.5% CH_3COOH	75.0	b	2.1	bc	100.0	d
	(2.9)		(0.1)		(0.0)	
1% CH_3COOH	68.3	ab	1.7	ab	100.0	d
	(1.7)		(0.2)		(0.0)	
2% CH_3COOH	70.0	ab	1.4	a	100.0	d
	(7.6)		(0.2)		(0.0)	
4% CH_3COOH	60.0	a	1.6	a	5.0	a
	(2.9)		(0.2)		(2.9)	
F (p-value)	12.0 (***)		5.2 (**)		42.5 (***)	

[$] Different lowercase letters (within a column) represent significant differences between disinfection treatments, according to Fisher's least difference (LSD) test at 5% significance. *p < 0.05; **p < 0.01; ***p < 0.001; ns: non-significant (p > 0.05).

CONCLUSIONS

Seedlings of wild celery can be effectively produced by disinfecting the seeds with 5–10% sodium hypochlorite and incubating them under a 20/30 ºC thermoperiod (12h:12h).

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Capítulo 2:
Requerimentos de nitrogênio e fósforo de halófitas brasileiras para aquaponia salina

[**Nitrogen and phosphorus requirements of Brazilian halophytes for saline aquaponics**]

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RESUMO EXPANDIDO

INTRODUÇÃO

A aquicultura é o setor de produção de alimentos que mais cresce no mundo, mas suas atividades geram águas e efluentes ricos em partículas orgânicas, micro-organismos e macronutrientes nas águas estuarinas e costeiras, o que pode vim a causar poluição, perda de habitats e doenças (Hargreaves, 2013; Buhmann & Papenbrock, 2013). Aquaponia (combinação da hidroponia e aquicultura animal) é uma técnica de cultivo de plantas já usada na antiguidade, mas atualmente vista como uma alternativa para reciclar e reutilizar nutrientes acumulados, particularmente em sistemas intensivos de peixes e crustáceos (Webb et al., 2012; Buhmann & Papenbrock, 2013; Quintã, Santos, Thomas, & Le Vay, 2015; Pinheiro et al., 2017). Dentre os minerais inorgânicos na água de produção animal, o nitrogênio e o fósforo são os macronutrientes mais abundantes em sistemas intensivos, porque são produtos residuais derivados da alimentação e tendem a se acumular no sistema (Timmons & Ebeling, 2010).

As práticas aquapônicas tradicionais foram baseadas no equilíbrio das necessidades de plantas e animais aquáticos produzidos. No entanto, a maioria das publicações sobre aquaponia salina se concentra nas taxas de absorção e remoção de nitrogênio e fósforo por filtros vegetados. Pouco ainda é conhecido sobre os requisitos de macronutrientes das halófitas (plantas tolerantes a altas salinidades), a fim de suprir as necessidades dessas plantas quando integradas a aquicultura.

Plantas halófitas têm múltiplos usos econômicos. Suas biomassas podem ser usadas para suplementar dietas humanas e de animais, na produção de biocombustíveis ou para extração de compostos bioativos (Panta et al., 2014; Ventura, Eshel, Pasternak, & Sagi, 2015; Souza, Mendes, Doncato, Badiale-Furlong, & Costa, 2018). Quanto aos seus usos, algumas halófitas nativas do Brasil se destacam. *Salicornia neei* Lag. é altamente nutritiva (Costa & Herrera, 2016; Doncato & Costa, 2018a,b), rica em ácidos fenólicos e flavonóides, exibindo alta atividade antioxidante (Pinheiro et al., 2017; Souza et al., 2018). O aipo, *Apium graveolens* L., é amplamente utilizado para consumo em saladas frescas e na indústria nutracêutica, sendo que uma variedade selvagem, encontrada em marismas do sul do Brasil, mostrou uma concentração de compostos fenólicos dez vezes maior do que cultivares comerciais (Souza et al., 2018). *Paspalum vaginatum* Sw. é uma gramínea perene halófita, abundante em marismas, cujas as populações são frequentemente pastadas por gado e cavalos (Costa, 1997), sendo sua

biomassa rica em potássio, magnésio, prolina e compostos antioxidantes (Hegazi & Khatab, 2016).

O objetivo do presente estudo foi avaliar as exigências nutricionais de nitrogênio e fósforo de *S. neei*, *A. graveolens* e *P. vaginatum* visando seu uso na aquaponia salina. Plantas das três espécies foram cultivadas hidroponicamente em sala de cultivo, utilizando diferentes formas de nitrogenadas (amônio e nitrato), como fonte única e mista encontradas na aquacultura intensiva, e expostas a diferentes concentrações de fósforo.

MATERIAIS E MÉTODOS

Três experimentos foram realizados com plantas de *S. neei*, *A. graveolens* e *P. vaginatum* cultivadas hidroponicamente em frascos de vidro (frascos de DBO, 300 ml) com soluções Hoagland (Hoagland & Arnon, 1950) modificadas. Todos os experimentos foram realizados em sala de cultivo, sob condições controladas de temperatura (mínima= 23,1 ± 0,3 °C; máxima= 26,6 ± 0,3 °C; média ± erro padrão) e luz (fotoperíodo de 14h de luz, radiação fotossinteticamente ativa de 115,0 ± 8,0 µmol m^{-2} s^{-1}). As soluções nutritivas foram trocadas semanalmente para garantir as condições de cada tratamento avaliado. No primeiro experimento se estabeleceu a preferência por forma nitrogenada como fonte única. Para tal, cada espécie foi cultivada em duas formas nitrogenadas (amônio e nitrato), bem como dois níveis de nitrogênio e fósforo (baixo= 0,2 mg/L e alto= 10 mg/L). Visto que algumas plantas tiveram inibição de seus crescimentos em alto suprimento de amônio (ver Resultados e Discussão), foi realizado uma avaliação da acidificação do pH da solução de cultivo na última semana do experimento.

No segundo experimento foi avaliado se o tamponamento do pH poderia aliviar o estresse observado do amônio sobre as plantas. Plantas das três espécies halófitas foram cultivadas em alto suprimento de amônio e fosforo (10 mg/L) em dois níveis de pH: 5,5 (usual na solução Hoagland) e 7,5 (usual em aquicultura marinha), ajustados com hidróxido de sódio e ácido sulfúrico.

O terceiro experimento investigou o crescimento das halófitas em suprimento com diferentes concentrações e proporções de formas nitrogenadas encontradas na aquicultura intensiva, cujos valores foram baseados no processo de nitrificação. Foram testados: nível de nitrogênio 1 (NL1; 0,2 mg NO_3-N/L e 1,0 mg NH_4-N/L), característico no início da produção, quando há dominância de amônia como forma nitrogenada; o nível de nitrogênio 2 (NL2; 10 mg NO_3-N/L e 0,6 mg NH_4-N/L), apresentando concentrações

intermediárias de amônia e com aumento da concentração de nitrato, devido a conversão de amônia a formas menos tóxicas como nitrito e subsequentemente a nitrato pelo processo de nitrificação; e o nível de nitrogênio 3 (NL3; 50 mg NO_3-N/L e 0,2 mg NH_4-N/L), em que o processo de nitrificação está totalmente ativo e o nitrato se acumula na água. Todas as combinações tinham dois níveis de fósforo (baixo= 0,2 mg/L e alto= 10 mg/L), exceto para *A. graveolens* que foi cultivado apenas com alta concentração de fósforo.

Parâmetros biométricos foram medidos no início e no final de cada experimento, e a biomassa fresca foi pesada e depois seca na estuda a 60 °C por 48 h, para então ser quantificada a biomassa seca. A diferença entre a biomassa fresca e seca estima o conteúdo de água das plantas. Adicionalmente, o teor de nitrogênio dos tecidos foi analisado de acordo com o protocolo da AOAC (1990).

Todos os experimentos foram analisados por Análises de Variância (ANOVA), precedidas pelos testes de normalidade (Shapiro-Wilk) e homoscedasticidade (Levene). Quando houveram diferenças significativas (p-valor 0,05) foi utilizado o teste de Tukey.

RESULTADOS E DISCUSSÃO

A baixa concentração de nitrogênio (0,2 mg/L) causou inibição de muitos parâmetros de crescimento. Todas as espécies podem absorver ambas as formas de nitrogênio, mas *S. neei* e *A. graveolens* mostraram preferir o nitrato (Figura 1 e 2; Tabela 1, 2 e 3). Resultado semelhante foi relatado para várias espécies anuais de *Salicornia* (Davy, Bishop, & Costa, 2001; Quintã et al., 2015) e *A. graveolens* (Santamaria, Elia, Serio, Gonnella, & Parente, 1999; Abd-Elkader & Alkharpotly, 2016). A alta concentração de amônio como única forma nitrogenada fornecida (10 mg/L) afetou negativamente *S. neei* e *A. graveolens*, mas principalmente a sobrevivência e o sistema radicular de *A. graveolens*. De acordo com Britto & Kronzucker (2002) e Liu & Von Wirén (2017), a inibição do alongamento radicular é um sintoma comum de toxicidade do amônio, principalmente quando o amônio é a única forma de nitrogênio. Essa toxicidade pode ser parcialmente explicada pela acidificação da rizosfera (Figura 3; Tabela 4), uma vez que pode ser aliviada pelo aumento do pH para *A. graveolens* (Tabela 5). *Paspalum vaginatum* não foi sensível a altas concentrações de amônio, provavelmente devido à sua preferência por um ambiente de pH baixo (Tabela 5).

As formas mistas de nitrogênio resultaram em crescimento abundante de todas as espécies, o que aumentou acentuadamente em relação ao tratamento rico em nitrato (Figura 4; Tabela 6, 7 e 8), devido à suplementação de formas mistas de nitrogênio poder atuar como um tampão de pH (Britto & Kronzucker, 2002; Li et al., 2013). O fósforo não foi um problema, mas pode limitar a produção de *S. neei* e *A. graveolens* sob uma condição altamente nitrificante na água, o que é uma situação improvável que ocorra nos atuais sistemas intensivos da aquicultura.

Abstract

Most publications about saline aquaponics have concentrated on the uptake and removal rates of nitrogen and phosphorus by vegetated filters. Yet little is known about macronutrient requirements for halophytes, in order to provide the needs of plants in aquaculture systems. To evaluate nitrogen (form and level) and phosphorus (level) requirements of *Salicornia neei* Lag., *Apium graveolens* L. and *Paspalum vaginatum* Sw. for use in saline aquaponics, plants were hydroponically cultivated with distinct concentrations of sole and mixed nitrogen forms, under low and high phosphorus concentrations. Low nitrogen concentration (0.2 mg/L) caused inhibition of many growth parameters. All species can uptake both nitrogen forms, but *S. neei* and *A. graveolens* preferred nitrate. High ammonium concentration as sole nitrogen form (10 mg/L) negatively affected *S. neei* and *A. graveolens*, and toxicity may be partially explained by rhizosphere acidification, since it can be relieved by increasing the pH. *Paspalum vaginatum* was not sensitive to high ammonium concentration. Mixed nitrogen forms resulted in plentiful growth of all species, which increased markedly towards the nitrate rich treatment. Phosphorus was not an issue in the current scenario of aquaculture systems.

KEYWORDS: ammonium, phosphate, marine aquaculture, nitrate.

1 INTRODUCTION

Aquaculture is the fastest growing food production sector, but among its footsteps is the release of waters and effluents rich in organic particulate matter, microorganisms and macronutrients into estuarine and coastal waters, which might potentially cause pollution, loss of habitats and diseases (Hargreaves, 2013; Buhmann & Papenbrock, 2013). Aquaponics (combination of hydroponics and animal aquaculture) is an ancient plant cultivation technique, which is seen as a new alternative to recycle and reuse harmful accumulated nutrients, particularly in inland-based intensive marine systems of fishes and crustaceans (Webb et al., 2012; Buhmann & Papenbrock, 2013; Quintã, Santos, Thomas, & Le Vay, 2015; Pinheiro et al., 2017).

Among the inorganic minerals in cultivation water, nitrogen and phosphorus are the most abundant macronutrients in intensive systems because they are waste products derived from animal feeding and tend to accumulate in the system (Timmons & Ebeling, 2010). For instance, the superintensive production of aquatic organisms using a Biofloc Technology (BFT) system is considered an eco-friendly approach, allowing low water exchange and simultaneously reducing ammonia-nitrogen and nitrite due to the action of chemoautotrophic bacteria, heterotrophic bacteria and microalgae; however, nitrate may accumulate up to hundreds of mg N/L, reaching toxic levels because of ongoing nitrification (Timmons & Ebeling, 2010; Avnimelech, 2012; Hargreaves, 2013). Although phosphorus is not frequently toxic to aquatic animals, excessive concentrations of phosphate when there is a low nitrogen and phosphorus ratio can lead to cyanobacteria dominated blooms, which can produce compounds that are toxic to aquatic animals. Yusoff, Matias-Peralta, & Shariff (2010) reported a 2/3 drop in shrimp production (*Penaeus monodon*) in ponds with eutrophic water with a phosphate concentration about 1.0 mg PO_4-P/L and phytoplankton community dominated by cyanobacteria in relation to ponds with a low phosphorus level. Further, phosphate concentrations accumulated during shrimp cultivation in intensive BFT systems and average levels ranged between 4.8–5.5 mg PO_4-P/L (Gaona, Serra, Furtado, Poersch, & Wasielesky Jr., 2016; Pinheiro et al., 2017).

Aquaponics integration with BFT systems can be an alternative to handle high concentrations of nitrogen and phosphorus in the water. Plants can uptake inorganic nitrogen as ammonium (ionized ammonia-nitrogen; NH_4-N), ammonia (non-ionized ammonia-nitrogen; NH_3-N) and nitrate (NO_3-N; Li, Wang, & Stewart, 2013; Liu & Von

Wirén, 2017), and the presence of ammonia-nitrogen forms is highly controlled by local pH, temperature and salinity (Timmons & Ebeling, 2010; Liu & Von Wirén, 2017). Cerozi & Flitzsimmons (2016) pointed out that orthophosphate (PO_4-P) is the most common inorganic phosphorus form in aquaponic systems. Although nitrogen and phosphorus need to be supplied in proper amounts for plants, little is known about the requirements of halophytes (*i.e*., plants adapted to grow in saline environments) used in saline aquaponics (but see Quintã et al., 2015). Traditional aquaponic practices have been based on balancing the needs of plants and aquatic animals produced. However most recent publications about aquaponics with marine aquaculture have concentrated on the uptake and removal rates of nitrogen and phosphorus by vegetated filters applied to intensive systems, with the goal of maintaining nutrient concentrations below toxic levels and/or preventing unwanted changes in the phytoplanktonic community (Webb et al., 2012; Buhmann & Papenbrock, 2013; Pinheiro et al., 2017).

There are multiple economic uses of halophytes produced by saline aquaponics. For example, halophyte biomass can be used to supplement human and animal diets, as a biofuel, or in the production of bioactive compounds (Panta et al., 2014; Ventura, Eshel, Pasternak, & Sagi, 2015; Souza, Mendes, Doncato, Badiale-Furlong, & Costa, 2018). The diversity of Brazilian halophytes has been evaluated and research on biotechnological and biomass production (Costa & Herrera, 2016; Pinheiro et al., 2017; Doncato & Costa, 2018a,b; Souza et al., 2018) has pointed out highly promising native species for saline aquaponics. For instance, the perennial sea asparagus, *Salicornia neei* Lag. [family Amaranthaceae; previously named *Salicornia gaudichaudiana* Moq. and *Sarcocornia ambigua* (Michx.) M.A. Alonso & M.B. Crespo], has been introduced as a new halophytic crop under irrigation with saline water and shrimp farm effluents in different climatic regions in Brazil (Costa & Herrera, 2016; Pinheiro et al., 2017; Doncato & Costa, 2018a,b). *Salicornia neei* is highly nutritious (Costa & Herrera, 2016; Doncato & Costa, 2018a,b), with shoots rich in phenolic acids and flavonoids, and exhibits high antioxidant and free radical scavenger activities (Pinheiro et al., 2017; Souza et al., 2018). Celery, *Apium graveolens* L. (family Apiaceae), is widely used by humans as a vegetable and an accession from a southern Brazilian salt marsh showed a tenfold higher concentration of phenolics than found in commercial celery cultivars (Souza et al., 2018). The seashore paspalum, *Paspalum vaginatum* Sw. (family Poeceae), is a halophytic perennial grass species used as a turfgrass in salinity-affected areas, particularly abundant in salt marshes

strongly affected by cattle and horse grazing (Costa, 1997), and rich in potassium, magnesium, proline and radical scavenging compounds (Hegazi & Khatab, 2016).

This study assessed the effect of nitrogen form and concentration on the growth of *S. neei*, *A. graveolens* and *P. vaginatum*, with the goal of using these species in saline aquaponics. Plants were evaluated through laboratory experiments, where they were exposed to concentrations of sole and mixed forms of nitrogen typical of the process of nitrification occurring in aquaculture systems. We also evaluated the effect of low and high phosphate availabilities and water pH on the above cited processes.

2 MATERIALS AND METHODS

2.1 Acquisition and production of plants

Vegetative propagules of *S. neei* and *P. vaginatum* were obtained from an active germplasm bank at the Laboratório de Biotecnologia de Halófitas, Instituto de Oceanografia-IO, Universidade Federal do Rio Grande-FURG. Plants of *S. neei* were the BTH2 lineage that originated from a natural population in the Patos Lagoon estuary (Doncato & Costa, 2018a). *Paspalum vaginatum* was originally from a salt flat in the tropical estuary of Caravelas, in Bahia State (17º45'03"S; 39º13'04"W). Seeds of *A. graveolens* were collected in the Pólvora Island salt marsh, in Rio Grande (RS, Brazil, 32º01'S; 52º06'W). Seeds were germinated under a thermoperiod of 20/30 °C and 12 h photoperiod (Doncato & Costa, 2019). Plants of the three species were planted in 50 cm^3 plugs filled with washed fine beach sand placed in polyethylene trays with 10% Hoagland solution (Hoagland & Arnon, 1950), and cultivated in an unheated greenhouse. Solutions were changed weekly and the sand plugs were kept close to the maximum holding capacity. Before the experiments, the plant roots were cleaned with distilled water to remove the substrate and sterilized with 5.0% sodium hypochlorite (commercial bleach). Uprooted plants of the halophytes used in the experiments had different initial ages: *S. neei*= 20 weeks old, *A. graveolens*= 27 weeks old, and *P. vaginatum*= 6 weeks old.

2.2 Experimental features

Three experiments were performed in a heated growth room at the Laboratório de Biotecnologia de Halófitas, in the Estação Marinha de Aquacultura-EMA, FURG (Rio Grande, RS). The growth room was kept under stable conditions: photoperiod (14 h of light), temperature (minimum= 23.1 ± 0.3 °C; maximum= 26.6 ± 0.3 °C; mean ± standard

error), and radiation (115.0 ± 8.0 µmol m^{-2} s^{-1} of photosynthetically active radiation-PAR). The species (n= 4 plants per treatment) were cultivated hydroponically in glass bottles (*i.e.*, BOD bottles) filled with 300 ml of full-strength Hoagland nutrient solution (Hoagland & Arnon, 1950), made with distilled water and modified by the addition of sodium chloride to achieve 5.0 g/L and changes in the concentrations of nitrogen and phosphorus, supplied as $NaNO_3$, NH_4Cl and NaH_2PO_4 (pH= 5.5 ± 0.1). The bottles were covered with black plastic to avoid a light inhibition effect on the roots and to prevent microalgae growth. During all experiments, nutrient solutions were replaced every seven days, together with the replacement of cultivation bottles by new ones, previously cleaned with hydrochloric acid (10%). Once per day, the bottles were shaken to homogenize the solution and then randomly placed on shelves.

The first experimental trial aimed to establish the preference of *S. neei*, *A. graveolens* and *P. vaginatum* for a nitrogen form (ammonium or nitrate) as the sole form, during 47, 33 and 28 days of hydroponic cultivation, respectively. The distinct experimental length of each species occurred because of their different growth rates and size development. The end of experiments occurred before any physical limitation of plant growth were observed. All the species were tested with two levels of nitrogen (*i.e.*, low vs. high), which were 0.2 and 10 mg N/L of ammonium or nitrate as sole nitrogen form, and two phosphorus concentration levels (0.2 and 10 mg PO_4-P/L). In the first weeks of the experiment, some plants showed growth inhibition when cultivated with ammonium as the sole nitrogen form. The amount of ammonium uptake by a plant is proportional to the released protons, leading to rhizosphere acidification and may induce growth inhibition or even mortality (Britto & Kronzucker, 2002; Li et al., 2013). To test the acidification hypothesis for each species, one selected bottle of each treatment level was randomly chosen and its pH value was measured at the beginning and at the end of the last week of cultivation.

A second experimental trial tested if pH buffering can release plant ammonium stress occurred in the first experiment (see Results). Plants of the three halophytes were cultivated under a high supply of ammonium and phosphorus (*i.e.*, 10 mg/L) during 30 days at two pH levels: 5.5 (usual in Hoagland solution) and 7.5 (usual in saline aquaculture). The pH solutions were adjusted using sodium hydroxide and sulfuric acid.

The third experiment was conducted to investigate the halophytes' growth when supplied with different concentrations and proportions of nitrogen forms found in

intensive aquaculture conditions. *Salicornia neei*, *A. graveolens* and *P. vaginatum* were cultivated for 47, 33 and 28 days, respectively. The experimental conditions replicated typical nitrogen forms and concentrations associated with microorganism changes in the water during intensive aquaculture cultivation, based on the nitrification process (Timmons & Ebeling, 2010; Avnimelech, 2012; Hargreaves, 2013; Pinheiro et al., 2017). Three treatments were tested: nitrogen level 1 (NL1; 0.2 mg NO_3-N/L and 1.0 mg NH_4-N/L), characteristic when production begins and dominance of ammonia-nitrogen; nitrogen level 2 (NL2; 10 mg NO_3-N/L and 0.6 mg NH_4-N/L), intermediary ammonia-nitrogen concentration with increased nitrate, which occurs due to the conversion of ammonia-nitrogen and nitrite to a less toxic form of nitrate by nitrifying bacteria; and nitrogen level 3 (NL3; 50 mg NO_3-N/L and 0.2 mg NH_4-N/L), nitrification process fully activated with nitrate accumulated in the water. Except for *A. graveolens* that was cultivated only with 10 mg PO_4-P/L, all combinations of nitrogen were tested under phosphorus concentrations of 0.2 and 10 mg PO_4-P/L.

For each individual plant, biometric analyses were made before transferring it to the glass bottle and at the end of each experimental trial. In all experiments, shoot height and root length of the plants were measured. Additionally, other shoot parameters specific of *S. neei* (*i.e.*, branch number and branch length), *A. graveolens* (*i.e.*, leaf area, number of petioles and number of leaves) and *P. vaginatum* (*i.e.*, number of tillers, number of leaves and leaf length) were quantified. After the cultivation period, shoot and root components of the three species were separated and weighed on a precision scale to determine the fresh biomass and then oven dried at 60 °C for 48 h to quantify the dry biomass. The difference between fresh and dry biomass estimates the water content in shoot tissue. Nitrogen tissue content in shoots and roots was measured by Kjeldahl digestion following the protocol of the Association of Official Analytical Chemists-AOAC (1990).

2.3 Data analysis

In the first experiment that evaluated the plant responses to sole nitrogen forms, factorial analyses of variance (ANOVA) were carried out to compare vegetative growth, biomass and nitrogen tissue content for each one of the halophytes, considering nitrogen form (ammonium and nitrate), nitrogen level (high and low concentrations) and phosphorus level (high and low phosphate concentrations). The experimental design of

the ANOVA considered only two-way interactions and Tukey's HSD post-hoc multiple comparison test following a significant effect of the experimental treatments (Zar, 2010). A two-way repeated measures ANOVA was used to compare the pH in the solutions of high and low concentrations of ammonium and nitrate source bottles, at the beginning and at the end of the last week of cultivation, where the repeated (within-subject) factor was time (day 0 and day 7 of the last week) and the fixed between-subject factors were nitrogen form and nitrogen level. The latter analysis was made with combined data of the low and high phosphorus levels and the three halophytes (n= 6 for each nitrogen form and level). The repeated measures ANOVA was followed by Tukey's HSD post-hoc multiple comparison test. Before all ANOVA of experiment 1, data normality and homoscedasticity were tested using the Shapiro-Wilk and Levene tests, respectively. In order to meet the assumptions of the ANOVA, some parameters of *S. neei* (*i.e.*, branch number and branch length), *A. graveolens* (*i.e.*, shoot height, leaf area, number of petioles and leaves) and *P. vaginatum* (*i.e.*, root length, leaf length, number of tillers and leaves) were transformed by square root (x). Shoot, root and total biomass of all three species (except *P. vaginatum* root biomass), as well as shoot, root and total nitrogen tissue content of *A. graveolens*, were transformed by $\log_{10}(x)$.

In the second experimental trial, vegetative growth and biomass of each species were compared between two pH treatments (*i.e.*, 5.5 and 7.5) under ammonium supply using t-tests (Zar 2010). To meet the assumptions for the t-test, shoot and total biomass of *P. vaginatum* were transformed by $\log_{10}(x)$.

In the third experimental trial, vegetative growth, biomass and nitrogen tissue content of *S. neei* and *P. vaginatum* were compared among combinations of nitrogen forms typical of the nitrification process in aquaculture systems and phosphorus levels using two-way ANOVA. The results for *A. graveolens* were analysed with one-way ANOVA, since this species was cultivated with only 10 mg PO_4-P/L. Number of branches and branch length of *S. neei*, as well as root length and leaf area of *A. graveolens*, were transformed by square root (x). Shoot and total biomass of *S. neei* and *A. graveolens*, and root length of *P. vaginatum*, were transformed by $\log_{10}(x)$. The ANOVA were followed by Tukey's HSD multiple comparisons (Zar 2010). All the values were reported as mean ± standard error and a $p < 0.05$ significance level was considered for all statistical analyses. The Appendix provides the results of the factorial ANOVA of the experiment that evaluated plant responses to sole nitrogen forms (Table A1) and of the experiment

with mixed nitrogen forms typical of the nitrification process in aquaculture systems (Table A2).

3 RESULTS

3.1 Cultivation of halophytes with sole nitrogen forms (ammonium and nitrate)

Only *A. graveolens* plants exposed to high concentration levels of ammonium and phosphorus suffered mortality and half of the individuals died. Except for *P. vaginatum* that was indifferent to the nitrogen form ($p > 0.05$), all species had better growth when supplied with nitrate rather than ammonium (Figure 1, a-c; Table 1-3). This nitrate preference was evident for shoot height and root length of *S. neei* and *A. graveolens* ($p < 0.01$) and, considering global averages of all nitrogen and phosphorus levels, nitrate nutrition resulted in 23–76% taller shoots and 40–101% longer roots for these two species compared to ammonium nutrition. Nitrate fed alone also resulted in longer branches of *S. neei* and a considerably higher number of leaves with larger leaf area for *A. graveolens* ($p < 0.05$) than when ammonium was supplied.

Most growth parameters were positively affected by nitrogen level (Figure 1, a-c, Table 1-3). Shoots of *S. neei* and *P. vaginatum* ($p < 0.01$) were taller under the high nitrogen level compared to the low one. The average values of branch number and length for *S. neei* ($p < 0.001$) were 4–5-fold higher and *A. graveolens* leaf area was larger (ninefold; due to an augment in the number of petioles and leaves) under the high nitrogen level compared to the low one ($p < 0.001$). *Paspalum vaginatum* had a marked improvement in tiller number (fourfold) and increased leaf length and number of leaves for the high nitrogen level in relation to the low nitrogen level plants ($p < 0.001$). In contrast, root length of *A. graveolens* ($p < 0.001$) was longer for the low nitrogen level than the high level. There were significant interactions between nitrogen form and nitrogen level, which were related to high ammonium supply inhibition of shoot height, root length, number of leaves and leaf area of *A. graveolens* ($p < 0.01$), as well as of branch number and length of *S. neei* ($p < 0.05$) (Figure 1, a-c, Table 1-2).

At the end of the sole nitrogen form experiment, the averages for shoot tissue water content of *S. neei*, *A. graveolens* and *P. vaginatum* shoots were 82.5 ± 0.8%, 82.2 ± 1.3% and 82.9 ± 0.8%, respectively. Statistical results for the fresh and dry weights of the biomass components (shoot, root and total) were similar; only dry biomass data are presented below (Figure 2, a-c; Table 1-3). Shoot and total biomass of *S. neei* and *A.*

graveolens, and root biomass of *A. graveolens*, were significantly ($p < 0.01$) higher under nitrate nutrition than under ammonium nutrition. *Apium graveolens* roots had higher nitrogen content when supplied with ammonium compared to nitrate (Table 2). Shoot and total biomass, shoot allocation and nitrogen content in shoots and roots of the three species rose with the increment of the nitrogen level. There were significant interactions between nitrogen level and nitrogen form for shoot, root and total biomass of *S. neei* and root biomass of *A. graveolens* ($p < 0.01$; Table 1-2), being plant weights reduced in high level of ammonium. Additionally, *S. neei* shoot allocation was stimulated by high nitrate concentration, whereas ammonium level did not affect this parameter (Table 1). Root nitrogen content of *S. neei* had the lowest average for the low nitrate supply and *A. graveolens* had the highest root nitrogen content when supplied with the high amount of ammonium (Table 1-2).

Concerning phosphorus concentrations (Figure 1, a-c; Table 1-3), longer *S. neei* branch lengths occurred under the high phosphorus level ($p < 0.05$). Root length and leaf parameters (number and area) of *A. graveolens* were significantly longer and smaller under the low phosphorus concentration compared to the high concentration ($p < 0.05$), respectively. Phosphorus concentration only significantly affected biomass components of *A. graveolens* (Figure 2, a-c), which had higher shoot and total biomass for the high phosphorus level ($p < 0.01$; Table 2). Significant interactions of the phosphorus level and nitrogen form were associated with high ammonium exposure. Branch length ($p < 0.01$) of *S. neei* markedly decreased under the high phosphorus level when supplied with ammonium. *Apium graveolens* had a striking tiny leaf area and low number of leaves ($p < 0.05$) under the low phosphorus level and high level of ammonium. Only *A. graveolens* had significant interaction of nitrogen and phosphorus levels ($p < 0.05$) for leaf area, number of petioles and number of leaves, due to growth limitations under high nitrogen availability combined with low phosphorus level. Phosphorus and nitrogen levels also showed significant interactions for nitrogen content of shoots and roots of *S. neei* and, for both tissues, under low and high nitrogen availability in the nutrient solution the increment of phosphorus supply decreased and increased tissue nitrogen content, respectively.

The two-way repeated measures ANOVA of solution pH showed an overall reduction in this parameter after a week of hydroponic halophyte cultivation, but a markedly and significant difference (Tukey test $p < 0.05$) in pH average occurred only at

day 7 for the high ammonium level (Figure 3). For this nutritional condition, pH dropped from 5.46 ± 0.09 to 3.90 ± 0.31, whereas in the other experimental treatments the average pH values ranged between 4.97–5.54 during the week. Due to this result, significant effects of the factors nitrogen form and nitrogen level, as well as interactions between these two factors and between them with cultivation time, were found (Table 4).

3.2 The pH effect under ammonium supply on halophytes

No plants died in the pH experiment. Averages of water tissue were 90.5 ± 0.8%, 83.8 ± 0.7% and 79.4 ± 1.6% for *S. neei*, *A. graveolens* and *P. vaginatum*, respectively. The growth and biomass of *S. neei* was not affected by pH (Table 5, a). The best growth of *A. graveolens* was in pH 7.5, where the highest values for shoot height, leaf area and number of leaves were observed ($p < 0.05$; Table 5, b). Fresh and dry matter results were statistically similar for all species, and cultivation of *A. graveolens* in pH 7.5 resulted in larger shoot, root and total biomass than in pH 5.5. In contrast, *P. vaginatum* showed the highest averages of root and total biomass in pH 5.5 (Table 5, c).

3.3 Halophyte cultivated with mixed nitrogen forms typical in aquaculture

Mortality was not recorded for the three species. The nitrate-rich condition, typical of aquaculture water with an active nitrification process, improved growth parameters of all three species (Figure 4, a-c; Table 6-8). Shoot height of *S. neei* and *P. vaginatum* ($p < 0.05$) increased between the NL1 and NL3 treatments. Under the highest nitrate concentration solution, *S. neei* also showed a significantly higher branch number (28–49%) and length (47–109%; $p < 0.001$) than the NL1-NL2 treatments, and *A. graveolens* had a higher number of petioles on its shoots with considerably larger (5–8-fold) and more numerous leaves in the NL3 treatment (twofold; $p < 0.05$). *Paspalum vaginatum* had a significant longest root length ($p < 0.05$) in the NL1 treatment. The high phosphorus level positively affected shoot height, root length and branch length of *S. neei* ($p < 0.05$).

There was no statistical difference between the results for fresh and dry weights, so only dry weight results are presented, and the averages of water tissue content were 84.7 ± 0.7%, 85.8 ± 0.6% and 85.3 ± 0.4% for *S. neei*, *A. graveolens* and *P. vaginatum*. Some biomass parameters showed significant differences among the mixed nitrogen form treatments (Figure 2, d-f; Table 6-8). *Salicornia neei* and *A. graveolens* had a progressive increment in shoot biomass, being 1–6-fold heavier in the NL3 than in the NL1 treatment

(Figure 2, d-e; $p < 0.001$). Shoot allocation of the three halophytes increased with the amount of nitrate in the solution (Table 6-8), but only shoot, root and total biomass significantly ($p < 0.05$) increased in *S. neei* with high phosphorus exposure. Shoot nitrogen content of *S. neei*, *A. graveolens* and *P. vaginatum* had averages ranging between 1.8–1.9%, 1.5–2.9% and 3.1–3.3% in NL2-NL3, respectively, and they were higher than NL1 treatment values. Root nitrogen content of all species increased from the NL1 to NL3 treatments (Table 6-8). Shoot nitrogen content of *P. vaginatum* slightly increased under exposure to the high phosphorus level in relation to the low level (Table 8). Finally, all biomass components and nitrogen content of *S. neei* shoots showed their values limited by the phosphorus level under the NL3, nitrate-rich condition (Table 6).

4 DISCUSSION

4.1 Nitrogen forms and concentration effects on halophytes

The three studied halophytic species were able to uptake both nitrogen forms supplied, but *S. neei* and *A. graveolens* preferred the nitrate nutrition. For *S. neei*, there was no previous information about preference for a nitrogen form and nitrate supply improves its growth parameters (*i.e.*, shoot height, root length, leaf length, shoot biomass and total biomass). Similarly, Davy, Bishop, & Costa (2001) and more recently Quintã et al. (2015) reported that several species of annual *Salicornia* studied in Britain and Canada prefer nitrate; however, ammonium is more favorable for the growth of *Salicornia bigelovii* (concentrations ≤ 42 mg N/L) in saline conditions (11.7 g NaCl/L and pH= 5.5; Kudo & Fujiyama, 2010). According to Li et al. (2013), the preference of plants for a nitrogen form depends on the species and, in general, it is a combination of plant demand and adaptability to the local environment. *Salicornia neei* is typically distributed in salt marshes at middle of the intertidal zone (Marangoni & Costa, 2009) and plants growing in less flooded tidal levels may evolve in more oxygenated soils and prefer nitrate nutrition, where the nitrification process allows the oxidation of ammonia to nitrite and subsequently to nitrate (Li et al., 2013).

Elsewhere, *A. graveolens* has been documented as a nitrate-fed plant (Santamaria, Elia, Serio, Gonnella, & Parente, 1999; Abd-Elkader & Alkharpotly, 2016). The southern Brazilian *A. graveolens* stands out as a nitrophilous plant, which colonizes the drift line zone between mid-high salt marshes (Costa, 1997; Doncato & Costa, 2019). Hybrid varieties of *A. graveolens* can be cultivated in higher concentrations of nitrate and showed

optimum vegetative growth, in hydroponics, at nitrate concentrations higher than 180 mg NO_3-N/L (salinity= 0; Abd-Elkader & Alkharpotly, 2016). Additionally, in our experiment, *A. graveolens* showed it can improve its nitrogen use efficiency by modulating root growth and architecture (*sensu* Kiba & Krapp, 2016), since plants under the low nitrogen level elongated roots, probably to increase the root surface in the growth-limiting concentration of this element.

A low concentration of sole nitrogen forms (0.2 mg N/L) caused inhibition of a great number of growth parameters of *S. neei*, *A. graveolens* and *P. vaginatum*, but these species did not show any visible nitrogen deficiency symptoms (*e.g.*, chlorosis). Davy et al. (2001) reported field and laboratory nitrogen limiting conditions for different species of *Salicornia*, which positively responded to increasing nitrogen concentration treatments, particularly those that had received nitrate. After 20 days growing in a greenhouse, *S. bigelovii* doubled its shoot dry biomass with an increasing nitrate concentration from 14 to 56 mg/L (Kudo & Fujiyama, 2010), a response similar to *S. neei* (63% increase with an increment from 0.2 and 10 mg NO_3-N/L). Noaman (2004) found increased shoot growth of *P. vaginatum* in plots fertilized with 120 Kg N/ha compared to other plots that received 40 and 80 Kg N/ha (irrigated with 22 g NaCl/L). In our experiment, low nitrogen concentrations limited plant growth under both nitrogen forms but *S. neei* and *A. graveolens* plants showed asymmetrically larger growth in high nitrate-fed medium than when exposed to the high ammonium concentration. This distinctive growth was responsible for significant nitrogen form and nitrogen level interactions for several shoot parameters of these species. Thus, a high ammonium concentration may restrict the growth of these two halophytes.

The high concentration of ammonium supplied as the sole nitrogen form increased plant mortality of *A. graveolens* and had a significant negative effect on the root system of this plant; root biomass of *A. graveolens* for the high ammonium concentration was threefold smaller than for the high nitrate concentration, and root length was significantly lower under this nutrition than for the other treatments. Since root nitrogen content of *A. graveolens* was also higher under ammonium than under nitrate nutrition, halophyte growth inhibition may be related to ammonium toxicity. Inhibition of root elongation is a commonly observed symptom of ammonium toxicity, especially when ammonium is the sole nitrogen form (Britto & Kronzucker, 2002; Liu & Von Wirén, 2017). According to Britto & Kronzucker (2002), plants exposed to a toxic ammonium concentration lack

the regulation of ammonium in the plasma membrane, producing high bidirectional fluxes (*i.e.*, influx and efflux) with an increase in nitrogen content. These fluxes can exceed ammonium assimilation capacity, tissue accumulation of ammonium and/or increase efflux of ammonium from the cytosol to the external medium by an energy-demanding process (Britto & Kronzucker, 2002).

Ammonium toxicity may also be partially explained by rhizosphere acidification, since it can be relieved by increasing the pH of the growth medium, and ammonium-fed *A. graveolens* growing in pH 7.5 solutions showed as good performance as nitrate-fed plants in pH 5.5 solutions, even under the high supply of ammonium. Ammonium is transported into the cell by ion transporters in the cytoplasmic membrane, and protons are pumping out decreasing extracellular pH (Liu & Von Wirén, 2017). Since extracellular pH controls the availability of several elements, such as Mn, Cu, Zn, B, P and Fe, acidification can affect plant growth (Cometti, Furlani, Ruiz, & Fernandes Filho, 2006).

Contrasting with the above cited species, *P. vaginatum* had no preference for a nitrogen form and showed 50% more root and total biomass under the acidic solution (*i.e.*, pH 5.5) than the pH 7.5 solution. Since root length of this species was not affected by pH, the increase in root biomass indicates that plants possibly directed their growth to lateral roots, improving nutrient uptake that enables larger plants. Similarly, Kaminski, Rheinheimer, Santos, Santos, & Oshe (1998) described an increase in shoot biomass production of *Paspalum notatum* biotype Eldorado at a pH near 5.0 in relation to plants growing at pH 6.0. Thus, *P. vaginatum* can be characterized as an acid-tolerant plant, although several authors highlight that plants adapted to low redox potential (waterlogged soils/wetlands) can also show acid-tolerance responses (Runge, 1983; Britto & Kronzucker, 2002; Li et al., 2013). The lack of ammonium growth impairment/toxicity in *P. vaginatum* is probably associated with its preference for a low pH environment.

4.2 Halophyte responses to nutrient conditions typical in aquaculture

Under mixed nitrogen form conditions typical of the nitrification process in aquaculture systems, *A. graveolens* and *S. neei* growth increased markedly towards the nitrate rich NL3 treatment (*e.g.*, 50 mg NO_3-N/L and 0.2 mg NH_4-N/L) that are usual in mature BFT systems, leading to large vegetative structures, allocation to shoot biomass and high nitrogen content in the whole plant (*i.e.*, shoot and root). Similarly, Santamaria et al. (1999) showed large biomass production of *A. graveolens* var. *dulce* under the

supply of mixed nitrogen forms (28 mg NO_3-N/L and 28 mg NH_4-N/L). The acidification problem for *A. graveolens* detected at the high ammonium concentration, in the sole nitrogen form experiment, seems not to have been an issue at pH 5.5 in the experiment of the mixed nitrogen form. Supplementation with mixed nitrogen forms may act as a pH buffer, so the released proton from ammonium assimilation, that would increase the acidification of roots, can be used for nitrate reduction, facilitating the stability of intercellular pH (Britto & Kronzucker, 2002; Li et al., 2013). The large vegetative development of *S. neei* under the NL2-NL3 treatments was quite similar to that observed in plants growing under the high nitrate sole form. Similarly, *S. europaea* had no significant difference in biomass production between nitrate as a sole nitrogen form and a mixed ammonium and nitrate solution (14 mg N/L; Quintã et al., 2015). Concerning the nitrogen tissue content of *S. neei*, mixed forms NL2-NLl3 even seem to improve the nitrogen uptake, contrasting with nitrate supplied alone. According to Marschner (1995) and Li et al. (2013), supplementation of mixed nitrogen forms allows plants to regulate intracellular pH easily and accumulate nitrogen at low energetic expenses. Additionally, *P. vaginatum* plants were taller, allocating more biomass in shoots with higher nitrogen content under high proportions of nitrate, but in the poor nitrate mixed solution (NL1; 0.2 mg NO_3-N/L and 1.0 mg NH_4-N/L) the roots had less nitrogen content and were longer. Our result shows that nutrition of the three halophytes tested with high ammonium concentration (usual in aquaculture) might be applied, once the nitrate concentration is equal or higher than 10 mg/L. The BFT system begin is characterized by time lags in peak concentrations of ammonia and then nitrite as the different populations of bacteria develop. Nitrification rates of 3.0 mg/L per day have been obtained in BFT systems (Hargreaves, 2003), and nitrate accumulated to 50–100 NO_3-N L^{-1} over a few weeks of operation. Thus, the use of water with mixed nitrogen forms, a common feature of nitrification process occurring in intensive aquaculture, for integrated cultivation (*i.e.*, aquaponics) of the three tested halophyte species is very promising.

4.3 Phosphorus availability effect on halophyte response to nitrogen forms

S. neei and *P. vaginatum* growth parameters were little affected by the phosphorus level (0.2 to 10 mg PO_4-P/L) under sole or mixed forms of nitrogen. Slightly poor development for the low phosphorus level of *S. neei* plants contrasts with *S. bigelovii*, which showed higher phosphorus demand and severe phosphorus deficiency, such as

necroses and purplish coloration, and retarded growth under 1.0 mg PO_4-P/L nutrition (Alsaeedi & Elprince, 2000). *Salicornia neei* biomass components and shoot nitrogen content grow higher with an increment of nitrate and phosphorus concentrations in mixed forms of nitrogen. Concerning *P. vaginatum*, under the low phosphorus level in the mixed nitrogen form experiment, shoots had a slightly smaller nitrogen content than other treatments, but shoot nitrogen content in a similar range of that described by Andrew & Robins (1971), who worked with *Paspalum dilatatum* under different phosphorus fertilization rates in soil.

On the other hand, *A. graveolens* was the most sensitive species to the phosphorus level, reducing leaf area, number of leaves, shoot and total biomass and elongating root length for 0.2 mg PO_4-P/L and sole nitrogen forms. Previously, Rice, Datnoff, Raid, & Sanchez (2002) pointed out that for commercial *A. graveolens* a high fertilization of phosphorus is recommended, partially because of the long growing season (*i.e.*, 165 to 180 days). In severe cases of phosphorus deficiency, there is limitation of total plant growth, including root growth (Grant, Flaten, Tomasiewicz, & Sheppard, 2001; Wissuwa, 2003), but *A. graveolens* invested in root elongation under low phosphate availability in order to expand root area and uptake more phosphorus.

Significant phosphorus level and nitrogen form interactions in the sole nitrogen experiment observed for *S. neei* (*i.e.*, branch length) and *A. graveolens* (*i.e.*, leaf number and area) are explained by growth impairment at high phosphorus-high ammonium supply. Ammonium toxicity and/or extracellular acidification due to ammonium uptake, explained above, seems to be aggravated under high phosphorus availability that stimulates further nitrogen absorption. Miller, Mamaril, & Blair (1970) suggested that extracellular acidification created by ammonium uptake reduces the precipitation of calcium phosphates at the root surface.

Under the mixed nitrogen form experiment, *S. neei* did not show growth impairment for the high phosphorus level with ammonium dominated nutrition (NL1), but phosphorus limited biomass production (all components) under low phosphorus supply and the nitrate rich NL3 treatment, which is unlikely to happen in current aquaculture systems. Similarly, growth of aerial structures of *A. graveolens* (*i.e.*, number of petioles and leaves and leaf area) was clearly limited under the high nitrate concentration by low phosphorus supply in the sole nitrogen form experiment. Thus, phosphorus may limit *S. neei* and *A. graveolens* production under the highly nitrifying

condition occurring in the water of a mature BFT system if systems could keep low phosphorus supply. Further studies are necessary to clarify these phosphorus-nitrogen interactions.

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FIGURE 1 Mean (± standard error) of shoot heights, root length and size of photosynthetic structures (gray line; secondary axis) of *Salicornia neei* (a), *Apium graveolens* (b) and *Paspalum vaginatum* (c) after hydroponic cultivation under sole nitrogen forms (NH_4 and NO_3; low and high concentrations of 0.2 to 10 mg N/L, respectively) and two concentrations of phosphorus (low and high concentrations of 0.2 to 10 mg PO_4-P/L, respectively). Different lowercase letters indicate significant differences ($p < 0.05$) according Tukey test, capital letters correspond to secondary axis.

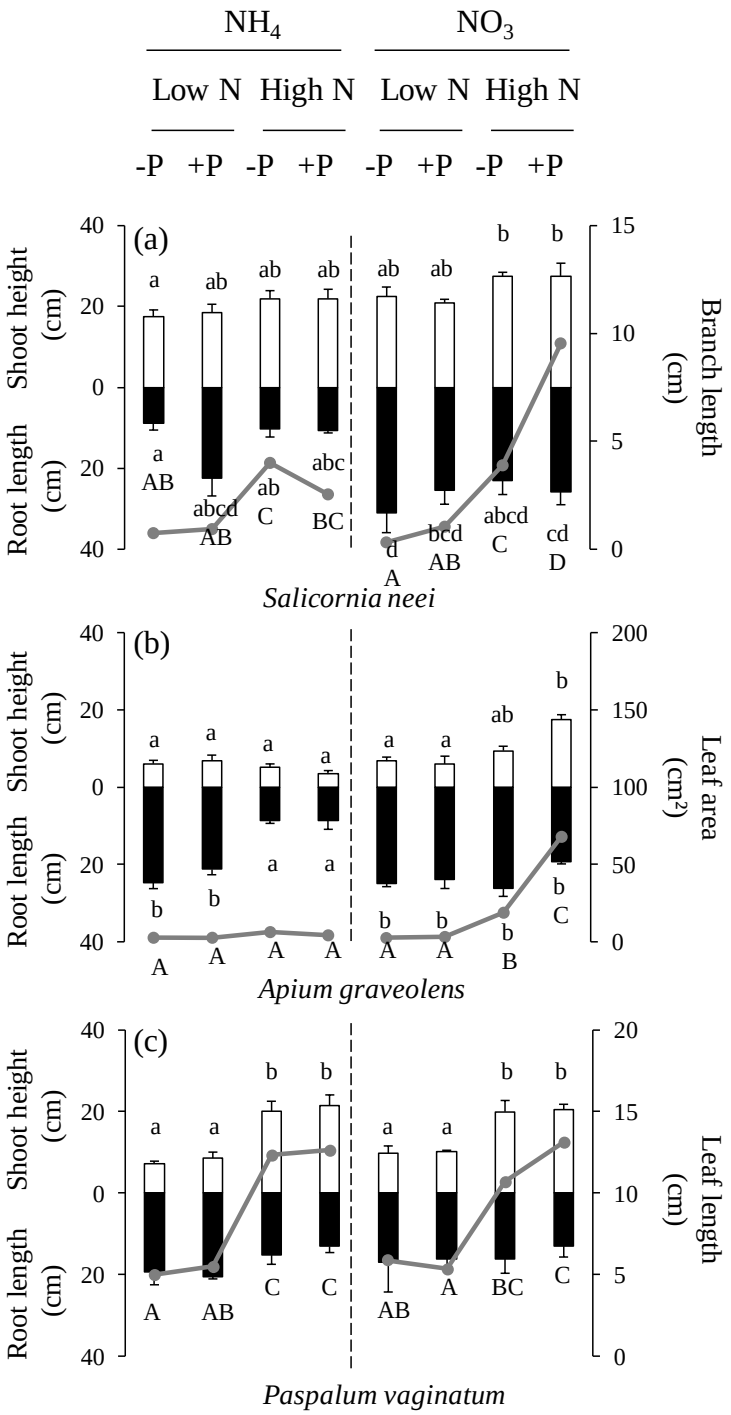
NH4
NO3
Low N
High N
Low N
High N
-P +P -P +P -P +P -P +P
(a)
Shoot height (cm)
Root length (cm)
Branch length (cm)
Salicornia neei
(b)
Shoot height (cm)
Root length (cm)
Leaf area (cm2)
Apium graveolens
(c)
Shoot height (cm)
Root length (cm)
Leaf length (cm)
Paspalum vaginatum

FIGURE 2 Mean (± standard error) of shoot and root biomass of *Salicornia neei* (a), *Apium graveolens* (b) and *Paspalum vaginatum* (c) after hydroponic cultivation under sole nitrogen forms (NH_4 and NO_3; low and high concentrations of 0.2 to 10 mg N/L, respectively) and with mixed nitrogen forms found in intensive aquaculture (NL1, NL2 and NL3). All sole nitrogen form treatments were applied under low and high phosphorus concentrations (0.2 to 10 mg PO_4-P/L, respectively). All nitrogen level treatments were applied under low and high phosphorus concentrations (0.2 to 10 mg PO_4-P/L, respectively), except for *A. graveolens*. Different lowercase letters indicate significant differences ($p < 0.05$) according Tukey test.

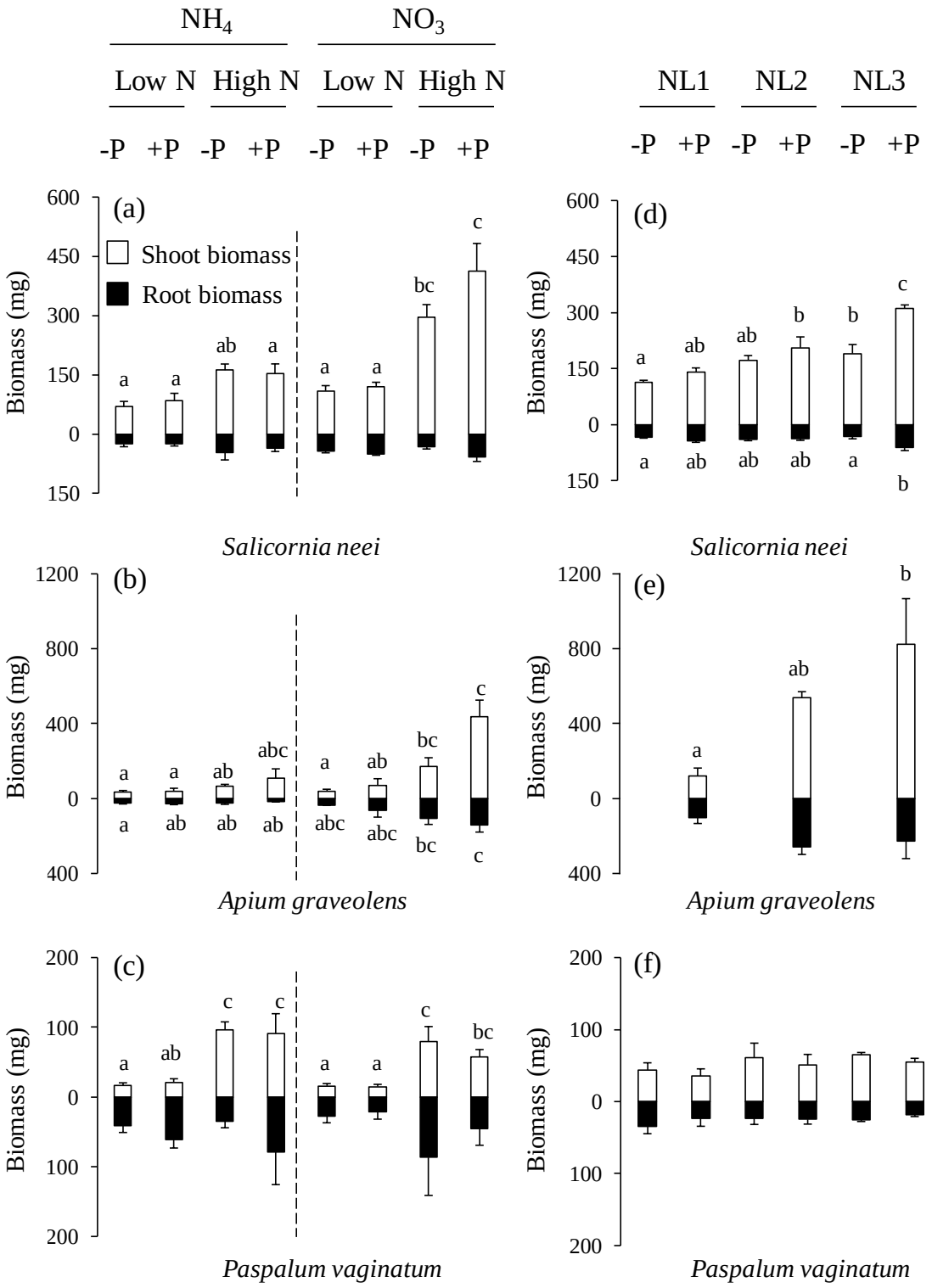
NH4
NO3
Low N
High N
Low N
High N
NL1
NL2
NL3
-P +P -P +P -P +P -P +P
-P +P -P +P -P +P
(a)
Shoot biomass
Root biomass
Biomass (mg)
600
450
300
150
0
150
a a ab a a a bc c
(d)
a ab ab b b c
a ab ab ab a b
Salicornia neei
Salicornia neei
(b)
1200
800
400
0
400
a a ab abc a ab bc c
a ab ab ab abc abc bc c
(e)
a ab b
Apium graveolens
Apium graveolens
(c)
200
100
0
100
200
a ab c c a a c bc
(f)
Paspalum vaginatum
Paspalum vaginatum

FIGURE 3 Mean (± standard error) of pH in nutrient solutions with high and low concentrations (0.2 to 10 mg N/L, respectively) of ammonium and nitrate in the beginning (day 0) and in the end (day 7) of the last week of hydroponic cultivation of halophytes. Each average represents the combined data of low and high phosphorus concentrations (0.2 to 10 mg PO_4-P/L, respectively), and *Salicornia neei*, *Apium graveolens* and *Paspalum vaginatum* (n= 6 for each nitrogen form and level). Different lowercase letters indicate significant differences ($p < 0.05$) according Tukey test.

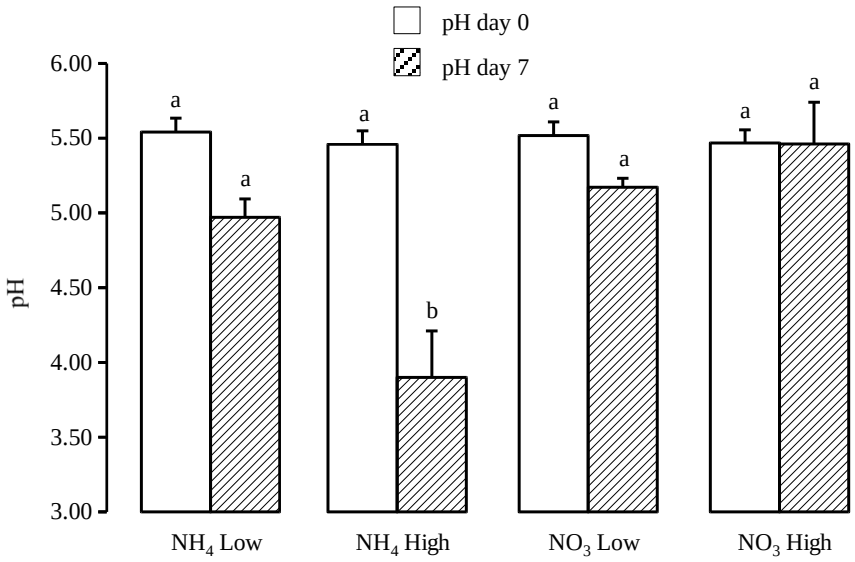
pH day 0
pH day 7
pH
6.00
5.50
5.00
4.50
4.00
3.50
3.00
a
a
a
b
a
a
a
a
NH4 Low
NH4 High
NO3 Low
NO3 High

FIGURE 4 Mean (± standard error) of shoot heights, root length and size of photosynthetic structures (gray line; secondary axis) of *Salicornia neei* (a), *Apium graveolens* (b) and *Paspalum vaginatum* (c) after hydroponic cultivation with mixed nitrogen forms found in intensive aquaculture (NL1, NL2 and NL3). All nitrogen level treatments were applied under low and high phosphorus concentrations (0.2 to 10 mg PO_4-P/L, respectively). Different lowercase letters indicate significant differences ($p < 0.05$) according Tukey test, capital letters correspond to secondary axis.

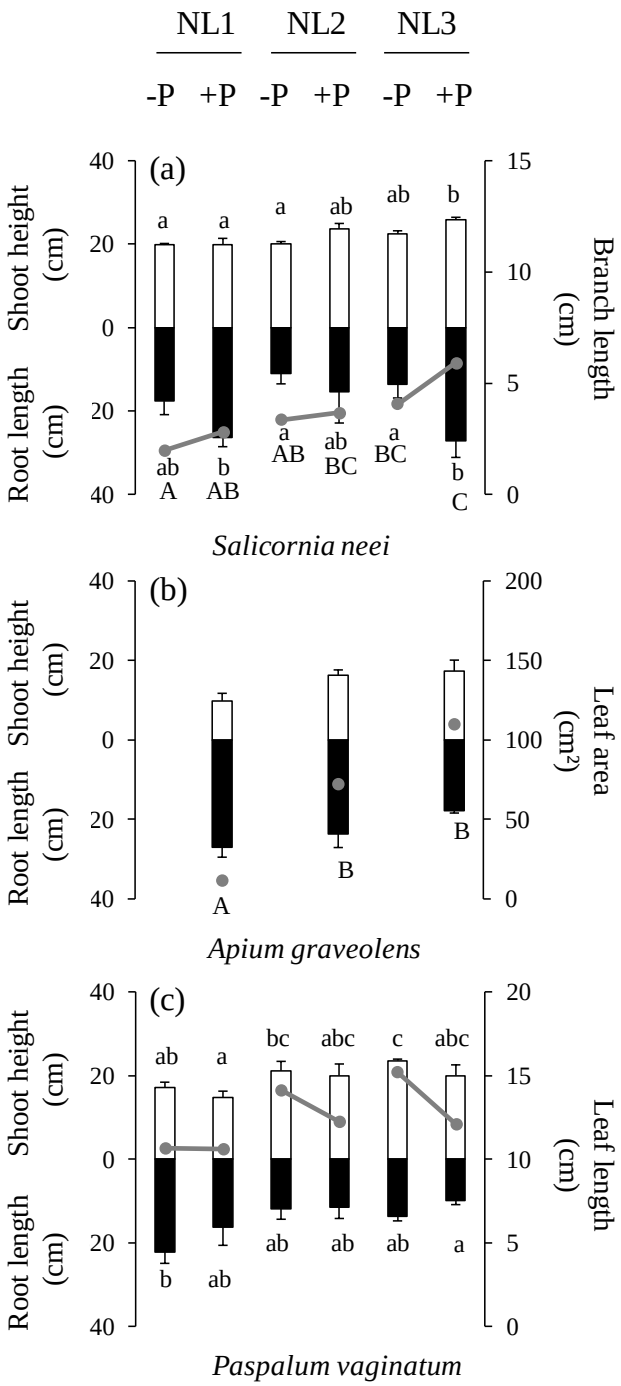
NL1
NL2
NL3
-P
+P
-P
+P
-P
+P
(a)
Shoot height (cm)
Root length (cm)
Branch length (cm)
40
20
0
20
40
15
10
5
0
a
a
a
ab
ab
b
ab
b
a
AB
ab
BC
a
BC
b
C
A
AB
Salicornia neei
(b)
Shoot height (cm)
Root length (cm)
Leaf area (cm²)
40
20
0
20
40
200
150
100
50
0
A
B
B
Apium graveolens
(c)
Shoot height (cm)
Root length (cm)
Leaf length (cm)
40
20
0
20
40
20
15
10
5
0
ab
a
bc
abc
c
abc
b
ab
ab
ab
ab
a
Paspalum vaginatum

TABLE 1 Mean ± standard error of branch number, total biomass, shoot biomass allocation and nitrogen tissue content (shoot and root) of *Salicornia neei* after hydroponic cultivation under sole nitrogen forms (NH_4 and NO_3; low and high concentrations of 0.2 to 10 mg N/L, respectively) and two concentrations of phosphorus (low and high concentrations of 0.2 to 10 mg PO_4-P/L, respectively).

N form	N level	P level	Branch number	Total biomass (mg)	Shoot allocation (%)	Shoot N (%)	Root N (%)
NH_4	Low N	Low P	6.75 ± 3.94 ab	94.57 ± 19.42 a	73.04 ± 2.53 ab	1.11 ± 0.19 ab	1.49 ± 0.13 bc
		High P	6.50 ± 1.66 abc	108.85 ± 17.99 a	75.47 ± 6.94 abc	0.70 ± 0.01 a	1.34 ± 0.05 ab
	High N	Low P	18.00 ± 1.58 bcd	208.95 ± 28.09 ab	78.84 ± 5.33 abc	1.63 ± 0.02 bc	1.84 ± 0.08 cd
		High P	18.75 ± 2.06 cd	188.65 ± 32.63 ab	81.31 ± 1.80 abc	1.79 ± 0.30 c	1.89 ± 0.10 d
NO_3	Low N	Low P	2.75 ± 1.03 a	152.50 ± 11.43 a	70.85 ± 3.99 ab	0.69 ± 0.06 a	1.24 ± 0.06 ab
		High P	3.50 ± 1.32 a	169.63 ± 14.98 ab	69.80 ± 1.10 a	0.61 ± 0.05 a	1.05 ± 0.04 a
	High N	Low P	29.75 ± 1.65 d	328.35 ± 34.56 bc	90.31 ± 1.70 c	1.67 ± 0.06 bc	1.89 ± 0.04 d
		High P	27.50 ± 5.95 d	470.30 ± 80.03 c	87.60 ± 1.15 bc	1.81 ± 0.07 c	2.00 ± 0.06 d

Factors	Branch number		Total biomass (mg)		Shoot allocation (%)		Shoot N (%)		Root N (%)	
	F	p	F	p	F	p	F	p	F	p
N form	0.51	ns	26.91	***	0.95	ns	1.43	ns	3.30	ns
N level	81.97	***	45.88	***	23.12	***	103.72	***	141.21	***
P level	0.03	ns	1.89	ns	0.01	ns	0.27	ns	0.72	ns
N form x N level	7.43	*	6.30	*	6.35	*	2.42	ns	11.15	**
N form x P level	0.18	ns	2.20	ns	0.72	ns	0.69	ns	0.01	ns
N level x P level	0.30	ns	0.45	ns	0.03	ns	4.67	*	5.65	*

Different lowercase letters indicate significant differences ($p < 0.05$) according Tukey test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant ($p > 0.05$).

TABLE 2 Mean ± standard error of petioles and leaves number, total biomass, shoot biomass allocation and nitrogen tissue content (shoot and root) of *Apium graveolens* after hydroponic cultivation under sole nitrogen forms (NH_4 and NO_3; low and high concentrations of 0.2 to 10 mg N/L, respectively) and two concentrations of phosphorus (low and high concentrations of 0.2 to 10 mg PO_4-P/L, respectively).

N form	N level	P level	Number of petioles	Number of leaves	Total biomass (mg)	Shoot allocation (%)	Shoot N (%)	Root N (%)
NH_4	Low N	Low P	3.50 ± 0.29 a	3.50 ± 0.29 a	59.15 ± 12.16 a	57.11 ± 4.40 ab	0.71 ± 0.06 a	0.84 ± 0.09 a
		High P	3.75 ± 0.25 a	3.25 ± 0.25 a	68.45 ± 13.88 a	50.98 ± 11.47 a	0.79 ± 0.08 a	0.88 ± 0.02 a
	High N	Low P	4.25 ± 0.85 ab	7.00 ± 1.47 ab	96.10 ± 18.49 ab	73.75 ± 0.95 bcd	2.92 ± 0.69 c	2.73 ± 0.34 cd
		High P	6.00 ± 1.00 ab	6.50 ± 2.50 ab	127.20 ± 49.20 ab	83.22 ± 6.69 d	3.59 ± $ c	3.42 ± $ d
NO_3	Low N	Low P	4.00 ± 0.41 a	3.50 ± 0.29 a	83.60 ± 8.55 ab	55.78 ± 5.50 ab	0.77 ± $ ab	0.99 ± 0.08 ab
		High P	3.50 ± 0.65 a	3.75 ± 0.75 a	135.07 ± 69.53 ab	57.18 ± 8.51 abc	0.83 ± 0.01 a	1.11 ± 0.30 ab
	High N	Low P	5.00 ± 0.71 ab	8.75 ± 0.48 b	275.38 ± 78.86 bc	63.05 ± 4.33 abcd	2.26 ± 0.01 bc	1.85 ± 0.05 bc
		High P	7.00 ± 0.41 b	18.25 ± 2.39 c	577.50 ± 125.75 c	76.23 ± 1.54 cd	1.78 ± 0.28 abc	1.75 ± 0.08 ab

Factors	F	p	F	p	F	p	F	p	F	p	F	p
N form	1.04	ns	11.12	**	14.56	**	0.46	ns	2.33	ns	9.52	ns
N level	18.13	***	64.72	***	16.41	***	16.22	***	32.73	***	92.61	***
P level	3.77	ns	4.46	*	5.10	*	0.88	ns	0.01	ns	0.86	ns
N form x N level	0.69	ns	8.97	**	7.25	*	1.44	ns	3.28	ns	19.86	***
N form x P level	0.33	ns	4.70	*	2.07	ns	0.38	ns	0.81	ns	1.15	ns
N level x P level	5.46	*	4.86	*	1.84	ns	2.13	ns	0.00	ns	0.16	ns

Different lowercase letters indicate significant differences ($p < 0.05$) according Tukey test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant ($p > 0.05$). $ Insufficient biomass to replicate the nitrogen analysis.

TABLE 3 Mean ± standard error of tillers and leaves number, total biomass, shoot biomass allocation and nitrogen tissue content (shoot and root) of *Paspalum vaginatum* after hydroponic cultivation under sole nitrogen forms (NH_4 and NO_3; low and high concentrations of 0.2 to 10 mg N/L, respectively) and two concentrations of phosphorus (low and high concentrations of 0.2 to 10 mg PO_4-P/L, respectively).

N form	N level	P level	Number of tillers			Number of leaves			Total biomass (mg)			Shoot allocation (%)			Shoot N (%)			Root N (%)		
NH_4	Low N	Low P	0.00 ± 0.00	a		3.50 ± 0.29	a		57.62 ± 12.62	ab		30.08 ± 4.12	a		1.36 ± $	ab		0.56 ± $	a	
		High P	0.25 ± 0.25	ab		3.25 ± 0.48	a		82.55 ± 12.35	ab		27.25 ± 6.43	a		1.12 ± $	a		0.55 ± $	a	
	High N	Low P	1.25 ± 0.48	bcd		7.75 ± 0.48	bc		131.40 ± 15.63	b		73.87 ± 4.58	b		3.01 ± 0.19	abc		1.40 ± 0.07	b	
		High P	1.75 ± 0.48	d		7.75 ± 1.65	bc		169.78 ± 67.29	b		63.14 ± 9.25	ab		3.55 ± 0.29	c		1.18 ± 0.02	ab	
NO_3	Low N	Low P	0.25 ± 0.25	ab		3.25 ± 0.63	a		43.35 ± 9.86	ab		43.28 ± 10.45	ab		1.28 ± $	ab		0.55 ± $	a	
		High P	0.50 ± 0.29	abc		4.25 ± 0.25	ab		35.93 ± 11.55	a		47.37 ± 10.22	ab		1.26 ± $	ab		0.55 ± $	a	
	High N	Low P	1.50 ± 0.65	cd		10.25 ± 1.18	c		166.53 ± 73.84	b		57.43 ± 9.81	ab		3.21 ± 0.14	bc		0.53 ± $	a	
		High P	0.75 ± 0.48	abcd		8.00 ± 1.29	bc		102.35 ± 14.53	ab		61.80 ± 14.59	ab		3.57 ± 0.10	c		1.34 ± $	ab	
Factors			F		p	F		p	F		p	F		p	F		p	F		p
N form			0.04		ns	1.67		ns	1.01		ns	0.36		ns	0.19		ns	1.38		ns
N level			14.04		***	62.59		***	12.71		**	17.67		***	180.55		***	13.49		*
P level			0.10		ns	0.20		ns	0.01		ns	0.04		ns	1.09		ns	0.51		ns
N form x N level			1.48		ns	0.31		ns	0.11		ns	3.94		ns	0.07		ns	1.31		ns
N form x P level			1.08		ns	0.00		ns	1.63		ns	0.73		ns	0.03		ns	3.96		ns
N level x P level			0.50		ns	1.55		ns	0.20		ns	0.09		ns	3.54		ns	0.55		ns

Different lowercase letters indicate significant differences ($p < 0.05$) according Tukey test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant ($p > 0.05$). $ Insufficient biomass to replicate the nitrogen analysis.

TABLE 4 Two-way repeated measures ANOVA for sole nitrogen forms (NH_4 and NO_3) and concentration (low and high levels of 0.2 to 10 mg N/L, respectively), and cultivation time effects on pH of hydroponic solutions used in the experiment of sole nitrogen forms. Cultivation time evaluates pH values measured in the beginning (day 0) and in the end (day 7) of the last week of cultivation.

Factors	df	F	p	Repeated factor	df	F	p
N form	1	15.99	***	Time	1	32.93	***
N level	1	4.37	*	Time X N form	1	16.90	***
N form X N level	1	10.13	**	Time X N form	1	2.26	ns
Residual	20			Time X N form X N level	1	9.42	**
				Residual	20		

*p < 0.05; **p < 0.01; ***p < 0.001; ns: non-significant (p > 0.05).

TABLE 5 Mean ± standard error of growth parameters and biomass of *Salicornia neei* (a), *Apium graveolens* (b) and *Paspalum vaginatum* (c) under two pH treatments.

(a) *Salicornia neei*	pH 5.5			pH 7.5			t
Shoot height (cm)	25.68	±	3.15	27.13	±	4.44	0.27 ns
Root length (cm)	17.03	±	3.31	17.88	±	2.21	0.21 ns
Branch number	18.00	±	4.32	25.25	±	5.07	1.09 ns
Longest branch	7.08	±	2.94	10.13	±	2.94	0.73 ns
Shoot biomass (mg)	177.45	±	41.31	188.65	±	84.81	0.12 ns
Root biomass (mg)	48.23	±	5.67	69.55	±	10.49	1.79 ns
Total biomass (mg)	268.28	±	53.94	348.58	±	92.74	0.75 ns
Shoot allocation (%)	80.88	±	2.05	76.95	±	4.42	0.81 ns
(b) *Apium graveolens*	pH 5.5			pH 7.5			t
Shoot height (cm)	6.15	±	0.40	8.53	±	0.74	2.81 *
Root length (cm)	9.73	±	2.14	13.25	±	0.70	1.57 ns
Number of petioles	6.00	±	0.41	7.25	±	0.48	1.99 ns
Number of leaves	8.50	±	0.87	15.00	±	1.22	4.33 **
Leaf area (cm^2)	8.60	±	0.75	25.76	±	1.84	8.66 ***
Shoot biomass (mg)	99.90	±	14.45	255.43	±	24.31	5.50 ***
Root biomass (mg)	19.48	±	5.87	48.57	±	2.59	4.53 **
Total biomass (mg)	119.38	±	19.78	304.00	±	21.98	6.24 ***
Shoot allocation (%)	84.70	±	2.86	83.59	±	2.08	0.32 ns
(c) *Paspalum vaginatum*	pH 5.5			pH 7.5			t
Shoot height (cm)	21.33	±	2.39	17.83	±	2.91	0.93 ns
Root length (cm)	24.63	±	1.39	17.50	±	3.61	1.84 ns
Number of tillers	5.00	±	1.22	3.50	±	1.26	0.85 ns
Number of leaves	5.25	±	0.48	4.25	±	0.25	1.85 ns
Leaf length (cm)	11.95	±	1.13	10.68	±	1.44	0.70 ns
Shoot biomass (mg)	242.33	±	49.76	121.83	±	3.90	2.39 *
Root biomass (mg)	85.40	±	12.19	49.35	±	4.49	2.77 *
Total biomass (mg)	327.73	±	59.52	171.18	±	8.23	2.58 *
Shoot allocation (%)	72.49	±	3.09	71.35	±	1.33	0.34 ns

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant ($p > 0.05$).

TABLE 6 Mean ± standard error of branch number, total biomass, shoot allocation and nitrogen tissue content (shoot and root) of *Salicornia neei* after hydroponic cultivation with mixed nitrogen forms found in intensive aquaculture (NL1, NL2 and NL3). All nitrogen level treatments were applied under low and high phosphorus concentrations (0.2 to 10 mg PO_4-P/L, respectively).

N level	P level	Branch number	Total biomass (mg)	Shoot allocation (%)	Shoot N (%)	Root N (%)
NL1	Low P	17.25 ± 0.63 a	146.10 ± 8.30 a	77.03 ± 1.08 ab	1.41 ± 0.07 a	1.65 ± 0.12 a
	High P	17.25 ± 0.75 a	185.73 ± 12.76 ab	75.93 ± 1.09 a	1.30 ± 0.03 a	1.70 ± 0.03 ab
NL2	Low P	20.75 ± 1.38 ab	210.63 ± 16.52 ab	81.51 ± 1.11 abc	1.72 ± 0.08 b	2.09 ± 0.07 bc
	High P	23.25 ± 2.29 ab	242.63 ± 32.71 b	84.52 ± 1.84 bc	1.81 ± 0.09 bc	2.09 ± 0.08 bc
NL3	Low P	23.00 ± 3.34 ab	222.35 ± 26.26 ab	85.46 ± 2.49 c	1.69 ± 0.02 b	2.04 ± 0.11 abc
	High P	28.25 ± 2.02 b	371.10 ± 6.06 c	83.68 ± 2.40 abc	2.01 ± 0.02 c	2.41 ± 0.03 c

Factors	Branch number F	Branch number p	Total biomass (mg) F	Total biomass (mg) p	Shoot allocation (%) F	Shoot allocation (%) p	Shoot N (%) F	Shoot N (%) p	Root N (%) F	Root N (%) p
N level	11.09	***	22.12	***	11.70	***	41.10	***	22.60	***
P level	2.81	ns	20.57	***	0.001	ns	4.18	ns	3.93	ns
Interaction	0.99	ns	5.00	*	1.07	ns	6.58	*	2.53	ns

Different lowercase letters indicate significant differences ($p < 0.05$) according Tukey test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant ($p > 0.05$).

TABLE 7 Mean ± standard error of petioles and leaves number, total biomass, shoot allocation and nitrogen tissue content (shoot and root) of *Apium graveolens* after hydroponic cultivation with mixed nitrogen forms found in intensive aquaculture (NL1, NL2 and NL3). All nitrogen level treatments were applied under low and high phosphorus concentrations (0.2 to 10 mg PO_4-P/L, respectively).

N level	Number of petioles				Number of leaves				Total biomass (mg)				Shoot allocation (%)				Shoot N (%)				Root N (%)			
NL1	5.00	±	0.00	a	7.00	±	0.41	a	221.28	±	74.60	a	54.04	±	2.11	a	0.89	±	0.12	a	1.24	±	0.10	a
NL2	6.00	±	0.41	ab	19.75	±	2.06	b	798.38	±	67.72	b	67.95	±	2.37	b	1.51	±	0.17	a	1.49	±	0.12	a
NL3	6.50	±	0.29	b	22.75	±	4.68	b	1051.83	±	332.44	b	80.07	±	2.08	c	2.91	±	0.11	b	2.51	±	0.09	b
Factor	F		p		F		p		F		p		F		p		F		p		F		p	
N level	7.00		*		7.98		*		6.32		*		35.29		***		51.69		***		41.04		***	

Different lowercase letters indicate significant differences ($p < 0.05$) according Tukey test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant ($p > 0.05$).

TABLE 8 Mean ± standard error of tillers and leaves number, total biomass, shoot allocation and nitrogen tissue content (shoot and root) of *Paspalum vaginatum* after hydroponic cultivation with mixed nitrogen forms found in intensive aquaculture (NL1, NL2 and NL3). All nitrogen level treatments were applied under low and high phosphorus concentrations (0.2 to 10 mg PO_4-P/L, respectively).

N level	P level	Number of tillers	Number of leaves	Total biomass (mg)	Shoot allocation (%)	Shoot N (%)	Root N (%)
NL1	Low P	0.75 ± 0.25	10.68 ± 1.16	78.00 ± 20.14	58.08 ± 3.83 a	1.84 ± 0.09 a	0.91 ± 0.06 ab
	High P	0.50 ± 0.29	10.60 ± 0.94	58.75 ± 20.19	65.69 ± 7.34 ab	2.08 ± 0.02 a	0.88 ± 0.01 a
NL2	Low P	0.75 ± 0.25	14.13 ± 1.60	83.65 ± 29.08	73.31 ± 2.10 b	2.88 ± 0.06 b	1.33 ± 0.14 bc
	High P	0.50 ± 0.29	12.25 ± 1.45	75.85 ± 20.91	67.31 ± 0.50 ab	3.38 ± 0.00 c	1.25 ± $ abc
NL3	Low P	0.25 ± 0.25	15.18 ± 0.55	90.38 ± 3.63	71.66 ± 2.08 b	3.19 ± 0.05 bc	1.45 ± $ c
	High P	0.75 ± 0.48	12.08 ± 1.63	73.35 ± 5.73	74.86 ± 2.92 b	3.37 ± 0.08 c	1.47 ± 0.15 c

Factors	F	p	F	p	F	p	F	p	F	p	F	p
N level	0.11	ns	0.30	ns	0.31	ns	4.86	*	308.95	***	12.47	*
P level	0.00	ns	0.23	ns	0.90	ns	0.27	ns	42.18	***	0.09	ns
Interaction	0.96	ns	0.01	ns	0.06	ns	1.68	ns	4.13	ns	0.08	ns

Different lowercase letters indicate significant differences ($p < 0.05$) according Tukey test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant ($p > 0.05$). $ Insufficient biomass to replicate the nitrogen analysis.

Table A1. Analyses of variance (ANOVA) of growth parameters and biomass of *Salicornia neei* (a), *Apium graveolens* (b) and *Paspalum vaginatum* (c) after hydroponic cultivation under sole nitrogen forms (NH_4 and NO_3; low and high concentrations of 0.2 to 10 mg N/L, respectively) and two concentrations of phosphorus (low and high concentrations of 0.2 to 10 mg PO_4-P/L, respectively). Branch number *S. neei*= gl of 30; shoot and root biomass *A. graveolens*= gl of 28. *p < 0.05; **p < 0.01; ***p < 0.001; ns: non-significant (p > 0.05).

(a) *Salicornia neei*

Factor	gl	Shoot height			Root length			Branch length			Shoot biomass			Root biomass		
		SS	F	p	SS	F	p	SS	F	p	SS	F	p	SS	F	p
Nitrogen form	1	171.13	10.57	**	1396.56	27.60	***	0.60	5.92	*	0.01	30.07	***	0.00	3.79	ns
Nitrogen level	1	192.08	11.86	**	160.21	3.17	ns	8.78	86.46	***	0.03	58.97	***	0.00	1.14	ns
Phosphorus level	1	0.13	0.01	ns	61.61	1.22	ns	0.44	4.36	*	0.00	2.00	ns	0.00	0.64	ns
N form x N level	1	7.41	0.46	ns	3.65	0.07	ns	0.91	8.95	**	0.01	12.19	**	0.00	2.03	ns
N form x P level	1	3.00	0.19	ns	142.81	2.82	ns	1.07	10.50	**	0.00	1.55	ns	0.00	2.93	ns
N level x P level	1	0.21	0.01	ns	10.35	0.20	ns	0.04	0.36	ns	0.00	0.57	ns	0.00	0.10	ns
Total	31	778.70			3040.20			13.29			0.06			0.00		

(b) *Apium graveolens*

Factor	gl	Shoot height			Root length			Leaf area			Shoot biomass			Root biomass		
		SS	F	p	SS	F	p	SS	F	p	SS	F	p	SS	F	p
Nitrogen form	1	3.70	12.71	**	459.04	44.11	***	27.43	34.24	***	0.79	12.66	**	1.21	21.57	**
Nitrogen level	1	1.10	3.78	ns	497.17	47.77	***	51.05	63.72	***	2.25	36.14	***	0.20	3.48	ns
Phosphorus level	1	0.30	1.04	ns	70.19	6.74	*	6.58	8.21	**	0.28	4.45	*	0.02	0.38	ns
N form x N level	1	4.19	14.42	***	315.75	30.34	***	27.41	34.21	***	0.20	3.28	ns	0.43	7.71	*
N form x P level	1	0.42	1.44	ns	5.08	0.49	ns	7.44	9.29	**	0.09	1.38	ns	0.02	0.37	ns
N level x P level	1	0.47	1.60	ns	5.96	0.57	ns	6.93	8.65	**	0.07	1.17	ns	0.01	0.01	ns
Total	29	17.59			1379.37			159.30			5.59			3.24		

(c) *Paspalum vaginatum*

Factor	gl	Shoot height			Root length			Leaf length			Shoot biomass			Root biomass		
		SS	F	p	SS	F	p	SS	F	p	SS	F	p	SS	F	p
Nitrogen form	1	4.96	0.37	ns	0.52	0.79	ns	0.00	0.01	ns	0.10	2.14	ns	0.00	0.21	ns
Nitrogen level	1	1076.48	79.62	***	1.60	2.45	ns	10.59	68.38	***	3.55	78.77	***	0.00	1.48	ns
Phosphorus level	1	7.03	0.52	ns	0.07	0.11	ns	0.08	0.51	ns	0.01	0.31	ns	0.00	0.04	ns
N form x N level	1	13.78	1.02	ns	0.58	0.89	ns	0.05	0.31	ns	0.01	0.15	ns	0.00	0.84	ns
N form x P level	1	1.45	0.11	ns	0.02	0.02	ns	0.01	0.05	ns	0.01	0.33	ns	0.01	2.04	ns
N level x P level	1	0.00	0.00	ns	0.43	0.66	ns	0.06	0.41	ns	0.04	0.82	ns	0.00	0.02	ns
Total	31	1441.72			19.55			14.66			4.84			0.09		

Table A2. Analyses of variance (ANOVA) of growth parameters and biomass of *Salicornia neei* (a), *Apium graveolens* (b) and *Paspalum vaginatum* (c) after hydroponic cultivation with mixed nitrogen forms found in intensive aquaculture (NL1, NL2 and NL3). All nitrogen level treatments were applied under low and high phosphorus concentrations (0.2 to 10 mg PO_4-P/L, respectively), except for *A. graveolens*. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant ($p > 0.05$).

(a) *Salicornia neei*

Factor	gl	Shoot height			Root length			Branch length			Shoot biomass			Root biomass		
		SS	F	p	SS	F	p	SS	F	p	SS	F	p	SS	F	p
N level		73.19	10.31	**	348.75	2.51	ns	0.41	16.41	***	0.01	25.40	***	0.00	1.31	ns
P level		33.61	9.47	**	480.62	6.92	**	0.08	6.71	*	0.00	18.08	***	0.00	7.38	*
Interaction		15.13	2.13	ns	83.99	0.60	ns	0.03	1.15	ns	0.00	3.96	*	0.00	3.68	*
Total	23	185.81			2164.35			0.74			0.01			0.00		

(b) *Apium graveolens*

Factor	gl	Shoot height			Root length			Leaf area			Shoot biomass			Root biomass		
		SS	F	p	SS	F	p	SS	F	p	SS	F	p	SS	F	p
N level	2	133.04	3.87	ns	1.87	3.65	ns	122.57	22.58	***	0.08	8.45	**	0.06	1.85	ns
Total	11	287.80			4.18			122.57			0.13			0.19		

(c) *Paspalum vaginatum*

Factor	gl	Shoot height			Root length			Leaf length			Shoot biomass			Root biomass		
		SS	F	p	SS	F	p	SS	F	p	SS	F	p	SS	F	p
N level		151.38	4.92	*	0.22	4.82	*	41.65	3.17	ns	0.00	1.62	ns	0.00	0.43	ns
P level		33.37	2.17	ns	0.07	3.24	ns	17.00	2.59	ns	0.00	0.87	ns	0.00	0.78	ns
Interaction		5.88	0.19	ns	0.02	0.55	ns	9.26	0.70	ns	0.00	0.00	ns	0.00	0.37	ns
Total	23	467.55			0.72			186.25			0.01			0.00		

Capítulo 3:
Suplementação de micronutrientes para halófitas brasileiras crescendo em aquaponia salina

[**Micronutrients supplementation for Brazilian halophytes growing in saline aquaponics]**

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RESUMO EXPANDIDO

INTRODUÇÃO

Os nutrientes se acumulam na água dos sistemas de aquicultura, de acordo com a intensidade do sistema, o volume de troca de água e a duração do ciclo de produção. Mais atenção tem sido dada aos papéis dos macronutrientes na aquicultura, como nitrogênio e fósforo, devido as questões econômicas e ambientais. A fim de minimizar seu impacto negativo e gerar outra fonte de renda, os nutrientes da aquicultura animal podem ser direcionados à produção comercial de plantas, e este tipo de integração é denominado de aquaponia (Rakocy et al., 2006). A maioria dos trabalhos realizados avaliando cultivos integrados de plantas e animais têm se concentrado no uso de plantas como biofiltro de macronutrientes, sendo pouca atenção dada aos micronutrientes nas águas de cultivo e ao seu potencial nutricional para manter a produção comercial de vegetais (Quintã et al., 2015).

No caso da aquaponia salina, é necessário o uso de plantas com mecanismos para tolerar a salinidade (halófitas). *Salicornia neei* Lag., *Apium graveolens* L. e *Paspalum vaginatum* Sw. são halófitas nativas da costa brasileira, que são utilizadas para produção de alimentos para humanos (Doncato e Costa, 2018b; Rana, 2016) e animais (isto é, *P. vaginatum*; Lonard et al., 2015), previamente testadas em condições aquapônicas salinas com sucesso (Capítulo 2).

Para evitar níveis críticos de elementos que levam a deficiências nutricionais, a suplementação de micronutrientes tem sido fornecida como fertilizante para a produção intensiva de culturas, que pode ser direta nas raízes (ou seja, fertilização do solo ou da água) ou por aplicação na superfície foliar (absorção pelas folhas) (Fageria et al., 2002; Fernández et al., 2013). Pouca informação está disponível sobre o efeito da suplementação de micronutrientes na produção de halófitas em aquaponia.

Com a finalidade de avaliar se a quantidade de microelementos na água clarificada de sistemas de bioflocos (BFT- *Biofloc Technology* system) do estoque de reprodutores de *Litopenaeus vannamei* B. pode garantir as necessidades nutricionais para o desenvolvimento de halófitas, este estudo testou os efeitos da suplementação de micronutrientes diretamente na água e por fertilização foliar no crescimento e produção de biomassa de *S. neei*, *A. graveolens* e *P. vaginatum* em aquaponia salina.

MÉTODOS

As plantas foram dispostas em unidades hidropônicas do tipo NFT (*Nutrient Film Technique*) e a água clarificada dos tanques de estoque de reprodutores de *L. vannamei*, crescendo em sistema BFT, foi recirculada e substituída semanalmente durante 30 dias. Os três tratamentos testados foram: T1 = controle sem suplementação de micronutrientes na água; T2 = adição de micronutrientes na água; e T3 = adição de micronutrientes nas folhas (fertilização foliar). A suplementação de micronutrientes foi feita com ¼ da solução de micronutrientes e força total de ferro de Hoagland (Hoagland e Arnon, 1950), que foram aplicados semanalmente na água. O tratamento com fertilização foliar consistiu na aplicação duas vezes por semana na superfície das folhas da mesma solução de micronutrientes com a adição de surfactante (0,1% Tween® 20).

Durante o experimento foram realizados monitoramentos da qualidade da água duas vezes na semana, correspondente a: pH (pHmetro FEP20 Mettler Toledo®), condutividade elétrica (condutivímetro HI9835 Hanna®), temperatura da água (termômetro TDU-300 Unity®), sólidos em suspensão totais (Strickland e Parsons, 1972), total de nitrogênio amoniacal (UNESCO, 1983), nitrito (Bendschneider e Robinson, 1952), assim como nitrato e ortofosfato foram medidos de acordo com Aminot e Chaussepied (1983). As análises de água de potássio, cálcio, magnésio, sulfato, ferro, manganês, zinco, cobre, boro e molibdênio seguiram a metodologia da EPA (1994). Adicionalmente, análises biometrias de todas as plantas foram realizadas no início e no final do experimento, e a biomassa fresca foi pesada e depois seca na estuda a 60 °C por 48 h, para então ser quantificada a biomassa seca. A diferença entre a biomassa fresca e seca estima o conteúdo de água das plantas.

Todos os dados foram analisados por Análises de Variância (ANOVA), precedidas pelos testes de normalidade (Shapiro-Wilk) e homoscedasticidade (Levene). Quando houveram diferenças significativas (5%) foi utilizado o teste de Tukey.

RESULTADOS E DISCUSSÃO

A suplementação com micronutrientes na água aumentou significativamente as concentrações de ferro, manganês e molibdênio, mas os outros parâmetros de qualidade da água de rotina e os macronutrientes não foram modificados (Tabela 1 e 2). A adição de micronutrientes à água do sistema BFT clarificada aumentou o crescimento de *P. vaginatum* (altura e número de folhas) e a produção de biomassa em 20-30% em relação às plantas não suplementadas, mostrando uma alta necessidade micronutricional dessa

espécie (Figura 1). Provavelmente associada ao ferro, o qual é um elemento chave para o gênero *Paspalum*, tendo sido reportado melhora do crescimento vegetativo com o aumento das concentrações de ferro para *Paspalum densum* (Siqueira-Silva et al., 2018), *Paspalum urvillei* (Araújo et al., 2014) e *P. vaginatum* cultivar Sea Isle 2000 (Pessarakli e Kopec, 2004). Em condições aeróbicas e alcalinas (típicas na aquicultura marinha) o ferro tende a se tornar insolúvel (Weinberg, 1989), não sendo esta uma condição ideal para espécies ácido-tolerantes como o *P. vaginatum* (Capítulo 2).

Plantas de *S. neei* crescendo com suprimento extra de micronutrientes na água não mostraram benefícios em relação ao controle, porém ocorreu uma redução de 73% da biomassa aérea sob fertilização foliar em relação ao tratamento controle (Figura 1). Diferente do observado por Ventura et al. (2010), em que *Salicornia europaea* aumentou a biomassa com fertilização foliar com molibdênio (287,82 µg Mo L^{-1}). Adicionalmente, plantas de *A. graveolens* apresentaram um pequeno desenvolvimento, que foi relacionado ao estresse térmico ocorrido durante o período do cultivo (máximas diárias de 30 ºC em metade dos dias de cultivo). Esta sensibilidade térmica de já havia sido apontada por Watts et al. (1984), não permitindo avaliar as respostas de crescimento e biomassa do *A. graveolens* as adições de micronutrientes.

CONCLUSÕES

A aquaponia com água clarificada do sistema BFT do estoque de reprodutores de *L. vannamei* atende aos requisitos micronutricionais de crescimento vegetativo e produção de biomassa de *S. neei*. A suplementação da água com micronutrientes afetou as concentrações de ferro, manganês e molibdênio e aumentou o crescimento de *P. vaginatum*, mostrando uma alta necessidade micronutricional dessa espécie, provavelmente relacionada ao ferro. Adicionalmente, o pequeno desenvolvimento das plantas de *A. graveolens* durante o estudo não permitiu avaliar suas respostas às adições de micronutrientes. A fertilização foliar de micronutrientes com 0,1% de surfactante não foi eficaz para melhorar o crescimento das halófitas.

ABSTRACT

Saline aquaponics is a fast growing research field, meanwhile little attention has been paid on micronutrients in the water from aquaculture and their nutritional potential to sustain commercial vegetable production. This study evaluated the effects of micronutrient supplementation, direct in the water and by foliar spray, on growth and biomass production of Brazilian halophytes *Salicornia neei* Lag., *Apium graveolens* L. and *Paspalum vaginatum* Sw. in saline aquaponic with water from a BFT system of *Litopenaeus vannamei* B.. Plants were established in NFT hydroponic units and clarified water from marine shrimp breeding stock tanks was recirculated and replaced weekly during 30 days. Micronutrient supplementation of the water significantly increased the concentrations of iron, manganese and molybdenum, but water quality parameters and macronutrients were not modified. Micronutrient addition to clarified BFT system water increased *P. vaginatum* growth (shoot height and leaf number) and biomass production 20-30% in relation to non-supplemented plants, showing a high micronutritional requirement of this species, most probably for iron. *Salicornia neei* plants growing with extra supply of micronutrient in water showed no benefits, but a 73% reduction of shoot biomass occurred under foliar fertilization in relation to control treatment. Poor development of *A. graveolens* plants did not allow evaluation of their responses to micronutrient additions. Clarified BFT system water can supply micronutritional requirements for halophytes, but supplementation may be necessary for species with high demands and/or more sensitive to neutral-alkaline condition of marine aquaculture, like *P. vaginatum*.

Keywords: BFT; foliar fertilization; green technology; integrated system; *Litopenaeus vannamei*; water fertilization.

1. Introduction

Nutrients accumulate in the water of aquaculture systems, according to the system intensity, volume of water exchange and duration of the production cycle. Nitrogen compounds and phosphorus are the main economic and environmental issues. They represent a great part of the material used for animal feeding, thus important production costs and are present in high concentration in the waters and effluents of aquaculture. These high concentrations can be toxic and/or stimulate eutrophication of undesirable planktonic communities inside the cultivation tanks or adjacent aquatic environments (Silva et al., 2013). However, nutrients from animal aquaculture can be directed to commercial production of plants. For instance, aquaponics consists in plants cultivation with water from animal production (Rakocy et al., 2006), recirculating in closed cycled (i.e., coupled) or not (i.e., decoupled); the latter has the advantages of better control of optimal parameters for both animal and plant production (Goddek et al., 2019). Integration of vascular plants with marine aquaculture (saline aquaponics) is a fast growing research field, meanwhile most work done has focus on the use of plants as a biofilter of macronutrients and little attention has been paid on micronutrients in the water of aquatic animal production and their nutritional potential to sustain commercial vegetable production (Quintã et al., 2015).

Macronutrients from the production of marine shrimp *Litopenaeus vannamei* B. under Biofloc Technology (BFT) system can be a nutritive source for halophytes (i.e., plants that tolerate salinity) grown in aquaponics (Pinheiro et al., 2017). Halophytes comprise to a vast amount of species that can be distributed along coastal areas. *Salicornia neei* Lag. [syn. *Salicornia gaudichaudiana* Moq. and *Sarcocornia ambigua* (Michx.) M.A. Alonso & M.B. Crespo], *Apium graveolens* L. and *Paspalum vaginatum* Sw. are all species used for food production (Doncato and Costa, 2018b; Rana, 2016) and animal feeding (i.e., *P. vaginatum*; Lonard et al., 2015) and previously tested with saline aquaponic condition with success (see Chapter 2).

Micronutrients demand for plants growing in environments rich in macronutrients is high. In order to prevent critical elements' levels that lead to nutritional deficiencies, micronutrients supplementation has been supplied as fertilizers to intensive crop production, which can be direct to the roots (i.e., soil or water fertilization) or by foliar spray (leaf absorption) (Fageria et al., 2002; Fernández et al., 2013). Micronutrient supplementation by addition to the water and foliar fertilization are acceptable practices for certified organic aquaponics, since the micronutritional deficiency is documented and

the use of appropriate products is applied (Kasozi et al., 2018; Treadwell et al., 2019), as well as problems of toxicity and environmental pollution are addressed. Little information is available on the effect of micronutrient supplementation on the production of halophytes in aquaponics. Ventura et al. (2010) recorded an improvement in *Salicornia europaea* biomass under foliar supplementation of molybdenum. *Salicornia dolichostachya* (Singh et al., 2014), *Paspalum* sp. (Pessarakli and Kopec, 2004; Araújo et al., 2014) and *Aster tripolium* (Ventura et al., 2013) cultivated in hydroponics were very responsive to iron supplementation in the water and *A. graveolens* as foliar spray (Sbai and Haouala, 2018).

In order to find out if the amount of micronutrients of one-year-old water from a Biofloc Technology (BFT) system with breeding stock of *L. vannamei* can secure the nutritional requirements for development of halophytes, this study evaluated the effects of micronutrient supplementation direct in the water and by foliar spray on growth and biomass production of *S. neei, A. graveolens* and *P. vaginatum* in saline aquaponics.

2. Methods

2.1. Plants acquisition

Salicornia neei BTH2 lineage with background described in Doncato and Costa (2018a) and *P. vaginatum* originally from NE Brazil (17º45'03"S; 39º13'04"W) were vegetatively propagated from active germplasm bank of the Laboratório de Biotecnologia de Halófitas, Instituto de Oceanografia-IO, Universidade Federal do Rio Grande-FURG. *Apium graveolens* was propagated through germination according to Doncato and Costa (2019) and seeds were collected in salt marshes of Pólvora Island, Patos Lagoon estuary (southern Brazil; 32º01'S; 52º06'W). Plants were planted into 50 cm^3 plugs filled with fine sand beach, placed within polyethylene trays with 10% of Hoagland solution (Hoagland and Arnon, 1950). Plants of *S. neei, A. graveolens* and *P. vaginatum* were cultivated for 12, 28 and 6 weeks in an unheated greenhouse, respectively. Previously to the aquaponic cultivation, all plants were uprooted and roots cleaned with water to remove the substrate.

2.2. Animal unit (water origin)

Water used for aquaponics came from tanks of *L. vannamei* breeding stock in BFT system of the Estação Marinha de Aquacultura (EMA, FURG). Two tanks were used as source of water and nutrients for plants in decoupled aquaponic systems. Both tanks (40 m^3 each) had shrimps (Tank 1: 555 and Tank 2: 520 individuals per tank) with an average

of 50 g and the animals were fed twice a day (morning and afternoon) with 300 g of commercial extruded feed per tank (Poti Evolution 35 Guabi®, 1.6 mm, containing 35% of crude protein, 15-30 g of calcium and a minimum of 15 g of phosphorus, 10 mg of manganese, 75 mg of zinc and 34 mg of copper per kg). Residence time of both tanks was of one year with occasional water reposition with seawater or dechlorinate tap water.

Water from both tanks of shrimp breeding stock was clarified (described in Gaona et al., 2011; conical cylinder tank of 500 L) and filtered (BP-420-50 Pentair® filter bags; 50 microns) before stored in a reservoir (3,000 L). This stored water was then pumped to the hydroponic units, each one with its on 500 L reservoir, previously clean with sodium hypochlorite, which were filled up till 450 L of active volume. This procedure was repeated every week, as well as the cleaning of clarifier tank, filter bags, pipes and 3,000 L storage reservoir.

2.3. Decoupled aquaponic system and experiment setup

The experiment was performed during 30 days in the summer of 2019 (January-February). All soilless plants were transferred to 150 cm^3 plastic net pots fulfilled with small gravel. Net pots with plants were randomly placed in Nutrient Film Technique (NFT) hydroponic benches, each with irrigation channels formed by six 10 cm X 5 cm PVC pipes and 3.0 m length, arranged side by side. Water was recirculated from the reservoir of the hydroponic unit at a rate of 13.1 L^{-1} min^{-1} every other 15 minutes (393 L^{-1} h^{-1}).

Three treatments were tested using clarified BFT system water from tanks of shrimp breeding stock and each treatment was setup in one aquaponic system: T1= control without micronutrients supplementation, T2= addition of micronutrients in the water, and T3= addition of micronutrients in the leaves (foliar spray). Twenty and two plants of each halophyte species were used per treatment, except by *A. graveolens* that had 12 plants. In the water addition treatment, except by full amount of iron, micronutrients of Hoagland solution (Hoagland and Arnon, 1950) were set to ¼ of full-strength in a total volume of 1.0 L and added to the water reservoir, during the weekly water exchange. The foliar spray treatment consisted of the application in the leaves surface, twice a week, of 100 mL of the same micronutrient solution of treatment T2 with 0.1% of the surfactant Tween® 20. Similar surfactant type and concentration were applied for foliar spraying of *S. europaea* (Lv et al., 2015), Apiaceae species (Elhindi et al., 2016) and *P. vaginatum* (Hegazi and Metwaly, 2016). The surfactant was applied to stimulate nutrient uptake through leaf structures and foliar spraying was performed with a hand

pressure sprayer (Export, Guarani®) until leaf surface saturation and starting run off of the applied solution. During the experiment, the automatic station of Instituto Nacional de Meteorologia-INMET (32°04'43" S; 52°10'03" W) recorded average values (± standard error) of temperature of 24.6 ± 0.5 °C and daily solar radiation of 19.2 ± 1.3 MJ m^{-2} day^{-1}.

2.4. Physical and chemical water parameters

Physical-chemical parameters of water were monitored in the aquaponic systems twice a week, before and after the weekly water exchange. The following parameters were measured *in situ*: pH, electrical conductivity and water temperature by FEP20 Mettler Toledo® pHmeter, HI9835 Hanna® condutivimeter and TDU-300 Unity® thermometer, respectively. Total suspended solids were measured according to Strickland and Parsons (1972). In each water sampling, fifty mL of water was collected in each aquaponic system to determine nitrogen ammoniacal total according to the described at UNESCO (1983), nitrite by the methodology of Bendschneider and Robinson (1952), as well as nitrate and orthophosphate were measured according to Aminot and Chaussepied (1983). Water analyses of potassium, calcium, magnesium, sulfate, iron, manganese, zinc, copper, boron and molybdenum followed the methodology of EPA (1994). All the sample bottles were cleaned with chloridric acid 10% and rinsed with distilled water three times.

2.5. Halophytes growth parameters and biomass

The biometry of all plants from the three species was quantified in the beginning and at the end of the experimental trial. For all species, shoot height (cm) and biomass (mg of dry matter) was quantified. Branch number and the length of all *S. neei* branches (cm) were measured. *S. neei* foliar index was estimated by the sum of shoot heights and lengths of the branches (cm). Each leaf width and length (cm) of *A. graveolens* plants was measured to estimate leaf area (cm^2) by the formula of an ellipse area, and the number of petioles of this species was also quantified. The numbers of tillers and leaves, as well as longest leaf length (cm) of all *P. vaginatum* plants were quantified. Additionally, after the initial measurement of *S. neei* and *P. vaginatum*, shoots were cut at the top of the net pot (i.e., 4 cm above the gravel level), thus responses were evaluated from their regrowth. *Apium graveolens* plants obtained from seedlings were cut only at the final harvest. At the end of the experiment, all plants species were again cut at the same height. Initial and final shoot biomass were weighted on a precision scale to determine the fresh biomass and then oven dried at 60 °C for 48 h to quantify dry biomass. The difference between fresh and dry matter estimates the water content in shoot tissue.

2.6. Statistics

The effect of micronutrient treatments on water physical-chemical parameters and vegetative growth and biomass production of halophytes was determined by one-way Analyses of Variance (ANOVA). In order to achieve normality (Shapiro-Wilk test) and homoscedasticity (Levene test) some parameters needed to be transformed before ANOVA (Zar, 2010). Nitrite, calcium, magnesium, iron, manganese, copper, boron and molybdenum were transformed by $\log_{10}(x)$. Square root (x) was used for foliar index and shoot biomass of *S. neei*, as well as for number of tillers, number of leaves and shoot biomass of *P. vaginatum*, while $\log_{10}(x)$ was used for branch number and longest branch of *S. neei*, number of petioles of *A. graveolens* and shoot height of *P. vaginatum*. When significant differences among treatments were found Tukey post-hoc test was performed. All the values were reported as mean ± standard error and 5% significance level was considered for all statistical analyses.

3. Results

3.1. Physical and chemical water parameters

The clarified BFT system water recirculating in the hydroponic units subjected to different micronutrient treatments had no statistical differences among their averages of pH (7.82 ± 0.04), electrical conductivity (20.66 ± 1.57 mS cm^{-1}), temperature (25.12 ± 0.38 °C), total suspended solids (global average = 104.90 ± 0.70 mg L^{-1}) and macronutrients (nitrogen ammoniacal total, nitrite, nitrate, phosphate, potassium, calcium, magnesium and sulfate; Table 1). Water micronutrient concentrations were presented at Table 2. Micronutrient supplementation of the water of shrimp breeding stock (T2) significantly increased the concentrations of iron, manganese and molybdenum, being their content 97-152 folds, 18 folds and 2 folds higher than T1 and T3 treatments, respectively.

3.2. Initial growth and biomass parameters

Except for *P. vaginatum* shoot height ($F= 4.77$; $p < 0.05$) that was taller in T1 (15.56 ± 1.67 cm) than T2 (10.74 ± 1.28 cm) and T3 treatment (10.21 ± 1.03 cm), there were no statistical differences ($p > 0.05$) in the initial growth parameters of halophytes among treatments. The average values of shoot height and shoot dry biomass of *S. neei* were 9.40 ± 0.63 cm and 153.58 ± 21.80 mg, respectively. *Apium graveolens* shoot height was 9.34 ± 1.05 cm and *P. vaginatum* shoot biomass was 633.61 ± 40.14 mg. Other specific parameters quantified showed the following averages: *S. neei* foliar index= 37.25

± 4.89 cm, branch number= 6.50 ± 0.81 and longest branch= 6.10 ± 0.56 cm; *A. graveolens* leaf area= 10.69 ± 1.30 cm^2, number of petioles= 5.14 ± 0.68 and number of leaves= 24.86 ± 3.21; and *P. vaginatum* number of tillers= 5.20 ± 0.34, number of leaves= 32.11 ± 1.71 and longest leaf= 11.19 ± 0.30 cm.

3.3. Growth and biomass after different supplementation of micronutrients

No plant mortality was recorded. Averages of water tissues content of *S. neei, A. graveolens* and *P. vaginatum* were 90.4 ± 0.1%, 80.5 ± 1.2% and 74.6 ± 1.0%, respectively. Micronutrient addition to the water (T2) had no effect on *S. neei* (Figure 1A-B) and *A. graveolens* (Figure 1C-D) vegetative growth and biomass in relation to T1 and T3 plants. *Apium graveolens* plants showed a poor growth and started bolting (i.e., growth of the inflorescence stem) between days 17^{th}-27^{th} of the experiment, but its plants in T3 did not flowering. Contrastingly, micronutrients addition in the water increased *P. vaginatum* shoot height ($F= 8.71$; $p < 0.001$), number of leaves ($F= 7.67$; $p < 0.001$) and shoot biomass ($F= 8.58$; $p < 0.001$) (Figure 1E-F). There was no difference ($p > 0.05$) for number of tillers and longest leaf of *P. vaginatum*.

S. neei plants under foliar spray treatment (T3) showed shorter shoot heights ($F= 7.43$; $p < 0.01$), reduced foliar index ($F= 4.11$; $p < 0.05$), branch number ($F= 3.98$; $p < 0.05$) and length of the longest branch ($F= 3.73$; $p < 0.05$), and lighter shoot biomass ($F= 5.29$; $p < 0.01$) than T1 and T2 (Figure 1A-B).

4. Discussion

4.1. Physical and chemical parameters

Clarified BFT system water applied to the decoupled aquaponic system had pH and salinity (i.e., electrical conductivity) meeting the acceptable limits for shrimp culture (Van Wyk and Scarpa, 1999). The one-year-old water was characterized by nitrifying condition and nitrate concentration with about 85-90 mg L^{-1}. Nitrate and other nitrogenous compounds were found in amounts within the tolerance values for *L. vannamei* (Van Wyk and Scarpa, 1999; Gaona et al., 2011; 2016). Total suspended solids were reduced after clarification to 71% of its original concentration in the breeding stock tanks (360.0 ± 19.6 mg L^{-1}) and phosphate water concentrations were intermediate between those found in intensive grow-out BFT system of *L. vannamei* (1.4-5.5 mg L^{-1}; Silva et al., 2013; Gaona et al., 2011, 2016; Pinheiro et al., 2017). Nitrogen sources and phosphate were on adequate concentrations for fulfill growth conditions of the three tested halophytes (see Chapter 2).

The irrigation of *P. vaginatum* with clarified BFT system water with increased iron, manganese and molybdenum concentrations (T2) improved the growth and biomass production of this species. In order to recommend micronutrient addition practice in saline aquaponics, we must consider the original sources and animal toxicity of the target elements. Manganese is an essential element for the production of *L. vannamei*, but its requirement was not established yet (NRC, 2011), being supplemented into the diet and its presence in the water can be mostly related to aged biofloc material (Kuhn et al., 2017), as for zinc and copper (Pereira, 2019). Iron and molybdenum are not supplied as essential micronutrients into shrimp diet, and their presence as trace elements might be related to the coastal surface waters used for production (rich in manganese and iron; Windom et al., 2006) and other management practices, such as feeding and addition of probiotic, limestone and/or molasses (Pereira, 2019). For instance, sugarcane molasses contains iron (Jain and Venkatasubramanian, 2017) and other microutrients like manganese, zinc and copper (Nogueira et al., 2009).

There is a lack of information on toxicity of micronutrients in the water for *L. vannamei* breeding stock, but micronutrients condition in the water recirculated in the aquaponic systems during our study certainly would not be considered proper for *L. vannamei* nursery and grow-out stages. Thus, caution would be required for a proper reuse of this water, after it leaves the aquaponic system in a closed cycle (i.e., back to the breeding stock) or in detour to another sector. Average zinc and copper concentrations were in accordance with the recommended limits for shrimp grow-out production (Van Wyk and Scarpa, 1999; zinc $\leq$ 100 µg L^{-1} and copper= 25 µg L^{-1}). However, zinc water concentration was above the maximum possible concentration [i.e., lethal concentration (LC_{50})/100)] for *L. vannamei* post-larvae (20.8 µg L^{-1}; Frías-Espericueta et al., 2003). Concentrations of manganese and boron were not an issue for *L. vannamei* post-larvae as showed for Frías-Espericueta et al. (2003) and Li et al. (2008). Iron supplementation to the BFT system water raised its concentration over the threshold of maximum possible concentration recommended for *L. vannamei* post-larvae (9.5 µg L^{-1}; Frías-Espericueta et al., 2003). Overall micronutrient concentrations supplied were below the threshold limits of water quality guidelines for glycophytic crop production with wastewater (Pescod, 1992). No similar guidelines are available for halophytes in saline aquaponics.

4.2. Growth parameters and shoot biomass

Each halophyte species showed a distinct response to micronutrients conditions. Micronutrient supplementation of the clarified BFT system water increased *P. vaginatum* growth. Among the micronutrients with concentration affected by the water supplementation, iron has been reported as a key factor for proper nutrition of *Paspalum* species. *Paspalum urvillei* can tolerate high iron concentration and increase its growth performance from 250 to 2,432 µg L^{-1} (Araújo et al., 2014), but under the highest iron concentration *P. urvillei* showed foliar modifications (i.e., wider and bronze colored leaves). Siqueira-Silva et al. (2018) also found leaf bronzing of *Paspalum densum* under very high iron exposure (390,915 µg L^{-1}), and they inform that it is a common symptom of iron toxicity. Concerning *P. vaginatum*, Pessarakli and Kopec (2004) found better shoot growth parameters of *P. vaginatum* cv. Sea Isle 2000 with supplementation with iron base fertilizer, but root growth inhibition when very high concentrations were applied. According to Tavares et al. (1999), *P. vaginatum* is very responsive to iron supply in alkaline condition. Although abundant in fresh waters and in soil, iron exists in insoluble form in aerobic environments at neutral or alkaline pH values, making it less available for plants (Weinberg, 1989). Thus, under alkaline condition, iron availability is an issue for plants in fresh water aquaponics, and Kasozi et al. (2018) pointed out that the optimal concentration of iron is still not established under this cultivation conditions, even for leafy and fruity vegetables. Marine alkaline condition reinforces low iron availability for halophytic plants (Singh et al., 2014). Neutral-alkaline pH condition is probably an unsatisfactory condition for acid tolerant species, like *P. vaginatum* (see Chapter 2), and a main factor for this species positive response to micronutrient supplementation in the saline water of BFT system. In relation to manganese, *Paspalum dilatatum* was consider very tolerant to high manganese concentration, and in sand culture it increased dry shoot biomass irrigated of with nutrient solutions containing 100 to 5,000 µg Mn L^{-1}, but growth inhibition was observed in concentrations above 40,000 µg Mn L^{-1} (Smith, 1979). Studies on molybdenum with grasses are rare and there are no established limits for turfgrass cultivation (Hull, 2003). Besides the highlighted micronutrient limitation, *P. vaginatum* growth and biomass production in aquaponics with clarified water of BFT system were higher or as good as this species in hydroponic cultivation with saline nutrient solutions (Beltrano et al., 1999; Ghiraldelli et al., 2019; Chapter 2).

Salicornia neei growing with extra supply of micronutrient in the clarified BFT system water showed no benefits, having similar shoot height, branch number, longest

branch, foliar index and shoot biomass of the control plants not supplemented. Plants of this species from both cited treatments had larger aerial structures and shoot biomass than *S. neei* grown for longer period of time (50-154 days) hydroponically in Hoagland solutions with different salinities (Souza et al., 2018) and in field plots with saline effluent from *L. vannamei* production in BFT system (Doncato and Costa, 2018a; Souza et al., 2018). In contrast to the lack of growth response of *S. neei* to high concentrations of iron, manganese and molybdenum added to the BFT system water, Singh et al. (2014) found chlorosis in *S. dolichostachya* plants grown hydroponically in alkaline artificial sea water solution, which was could be prevent with addition of Fe-EDDHA to the water. Furthermore, molybdenum addition (287.82 µg L^{-1}) significantly enhanced up to 30% shoot biomass of *S. europaea* in saline hydroponics (Ventura et al., 2010). BFT system water seems to attend micronutritional requirements of *S. neei*.

Apium graveolens growth was poor and similar among the micronutrient treatments. Its plants showed visual evidences of heat stress such as prostrated stems and growth impairment. During over half of days of the experiment, maximum daily temperatures about 30°C were recorded. Watts et al. (1984) working with *A. graveolens* tissue culture observed severe sensitivity to temperatures of 30 °C, not occurring biomass gain in a 15 days experiment. Other factor that inhibits vegetative growth *A. graveolens*, thus our evaluation of micronutrient nutrition, was the onset of reproduction during the experiment in the summer. *Apium graveolens* flowering and fruiting occur between November-May in the salt marshes of southern Brazil (Azevedo, 2000), and, according to Sachs and Rylski (1980), transference of well developed seedlings to the field on flowering period frequently stimulate bolting and inhibit vegetative growth. Thus, the experimental result was not conclusive about the micronutricional requirements of *A. graveolens* and further studies on optimal environmental conditions must be carry out.

Foliar spray did not demonstrate to be an appropriate technique for aquaponic cultivation of the tested halophytes, which did not show positive response to foliar fertilization. This result was different of improvement in *P. vaginatum* growth with iron foliar spray with 0.1% Tween® 20 recorded by Hegazi and Metwaly (2016), and better growth of Apiaceae species under nitrate foliar spray with 0.1% Tween® 20 (Elhindi et al., 2016). Additionally, *S. neei* biomass showed a 73% inhibition by foliar fertilization in relation to control treatment. This result contrast with findings of Ventura et al. (2010), which observed an up to 37% increment of *S. europaea* biomass by weekly foliar fertilization with molybdenum (287.82 µg Mo L^{-1}). No surfactant was applied and this

molybdenum concentration was 26 folds higher than used in our foliar fertilization. But our low concentration of molybdenum applied would not explain the inhibition of *S. neei* aerial structures. Additionally, Lv et al. (2015) evaluate the used of 0.1 % Tween® 20 surfactant in foliar spraying of *S. europaea* against a control treatment without this compound and did not find any negative effect on plants. According to Fageria (2009), foliar spray is a corrective technique applied when the root supply is ineffective, costly or prohibitive, not being considered a substitute. However, plant response to foliar fertilization can be species dependent. Usually micronutrients concentration applied as foliar spray is not high enough to lead to salt burn, a common feature of toxicity, although fast increase of micronutrients in cellular concentration is potentially toxic (Fernández et al., 2013). According to Yang (2008), low concentration of surfactants in spray solution can modify cell membrane properties and stimulate nutrient uptake through cuticle or stomata pores, whereas high concentration might lead to membrane integrity lost. Additionally, there is evidence that surfactants might cause inhibition of basipetal translocation, which is usually compensated by the increment of foliar uptake of elements (Coupland, 1989). Further studies are necessary to clarify if surfactant addition to the foliar fertilizer can negatively affect *S. neei* and to establish the best practices on the use of foliar spray in halophytes.

5. Conclusions

Saline aquaponics with clarified water from BFT system of *L. vannamei* breeding stock attends micronutritional requirements for vegetative growth and biomass production of *S. neei*. Micronutrient supplementation in water affected the concentrations of iron, manganese and molybdenum, and increased *P. vaginatum* growth, showing a high micronutritional requirement of this species, most probably for iron. Poor development of *A. graveolens* plants did not allow evaluation of their responses to micronutrient additions. Foliar spray with 0.1% Tween® 20 (surfactant) was not effective to improve halophytes growth.

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Fig. 1. Average values (± standard error) of different growth parameters and shoot biomass production of *Salicornia neei* (A-B), *Apium graveolens* (C-D) and *Paspalum vaginatum* (E-F) among micronutrients addition treatments T1 (control without supplementation), T2 (addition in the water) and T3 (addition by foliar spray) in saline aquaponics. For each growth parameters and shoot biomass, different lowercase letters represent significant differences between treatment averages ($p < 0.05$), according to the Tukey test.

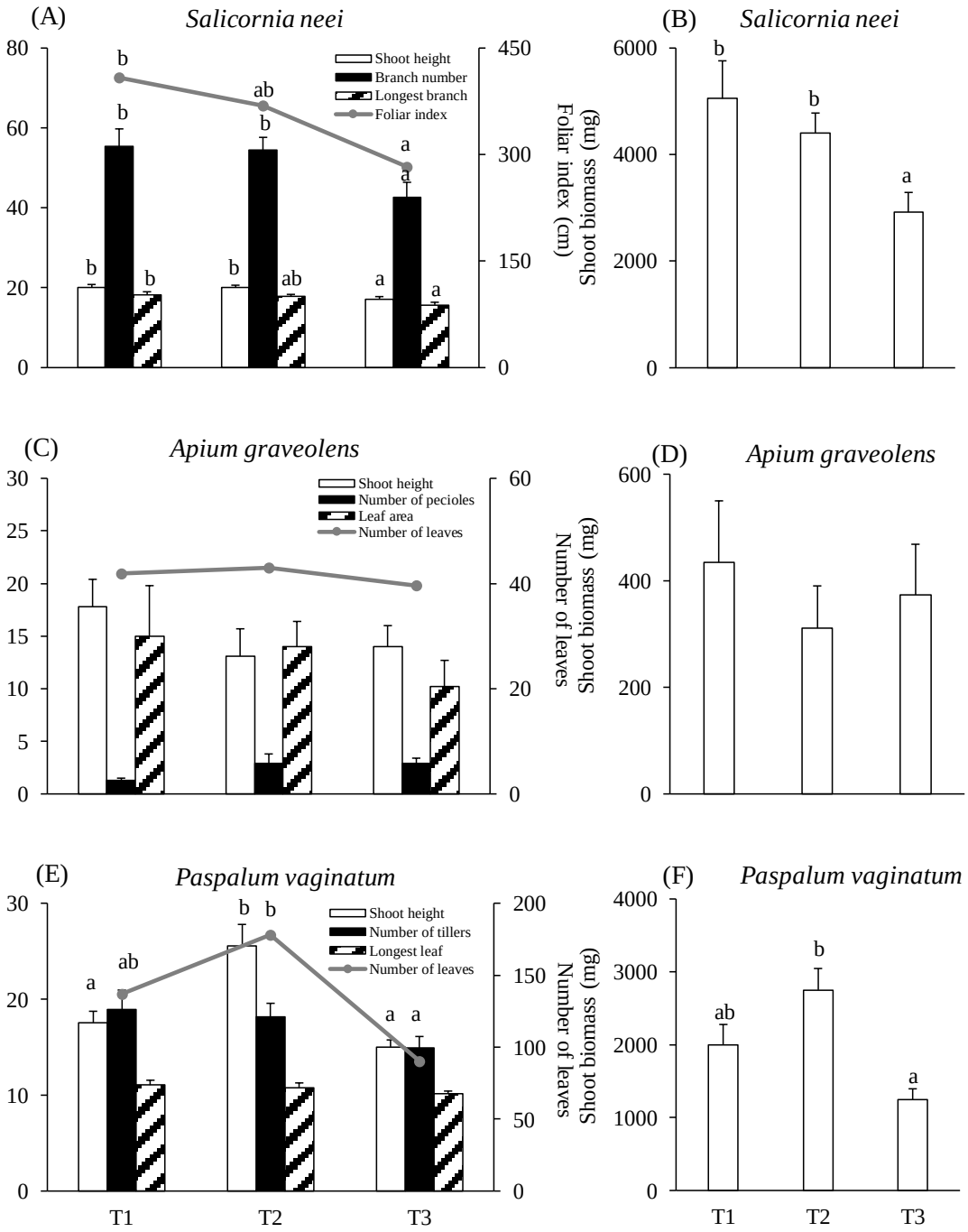
(A)
Salicornia neei
Shoot height
Branch number
Longest branch
Foliar index
Foliar index (cm)
(B)
Salicornia neei
Shoot biomass (mg)
(C)
Apium graveolens
Shoot height
Number of pecioles
Leaf area
Number of leaves
Number of leaves
(D)
Apium graveolens
Shoot biomass (mg)
(E)
Paspalum vaginatum
Shoot height
Number of tillers
Longest leaf
Number of leaves
Number of leaves
T1
T2
T3
(F)
Paspalum vaginatum
Shoot biomass (mg)
T1
T2
T3

Table 1

Average (± standard error) of macronutrients (Nitrogen Ammoniacal Total-NAT, Nitrite-NO_2, Nitrate-NO_3, Phosphate-PO_4, Potassium-K, Calcium-Ca, Magnesium-Mg and Sulfate-SO_4; mg L^{-1}) in the recirculating clarified water from BFT system of shrimp breeding stock used on aquaponic systems with halophytes. Results of one-way ANOVA among micronutrients addition treatments are presented; T1 (control without supplementation), T2 (addition in the water) and T3 (addition by foliar spray).

Treatment	NAT	NO_2	NO_3	PO_4	K	Ca	Mg	SO_4
T1	0.09	0.20	88.71	2.86	262.50	240.50	697.75	1404.00
	(0.03)	(0.02)	(19.40)	(0.35)	(37.77)	(44.56)	(123.52)	(177.29)
T2	0.06	0.16	84.86	2.72	250.50	226,00	657.25	1278.50
	(0.02)	(0.02)	(22.01)	(0.40)	(31.95)	(41.57)	(115.31)	(143.14)
T3	0.07	0.23	89.43	2.92	284.25	240.75	697,00	1339.75
	(0.01)	(0.05)	(20.40)	(0.32)	(29.12)	(40.61)	(115.33)	(243.34)
F treatment	0.56 ns	1.32 ns	0.01 ns	0.08 ns	0.27 ns	0.04 ns	0.04 ns	0.11 ns

ns: non-significant ($p > 0.05$).

Table 2

Average (± standard error) of micronutrients (Iron-Fe, Manganese-Mn, Zinc-Zn, Copper-Cu, Boron-B and Molybdenum-Mo; µg L^{-1}) in the recirculating clarified water from BFT system of shrimp breeding stock used on aquaponic systems with halophytes. Results of one-way ANOVA among micronutrients addition treatments are presented; T1 (control without supplementation), T2 (addition in the water) and T3 (addition by foliar spray).

Treatment	Fe		Mn		Zn	Cu	B	Mo	
T1	7.00	a	3,00	a	90,00	8.50	1716.25	3.75	a
	(1.00)		(0.41)		(19.13)	(1.50)	(285.31)	(0.48)	
T2	1072.75	b	56,00	b	125.25	9.75	1803.75	10.50	b
	(197.90)		(17.61)		(29.04)	(2.06)	(303.31)	(1.19)	
T3	11,00	a	3,00	a	70.25	8.00	1648.75	3.25	a
	(3.54)		(0.41)		(11.61)	(1.41)	(232.46)	(0.48)	
F treatment	162.38		20.09		1.73	0.23	0.06	22.59	
	***		***		ns	ns	ns	***	

Significance: *p < 0.05, **p < 0.01, ***p < 0.001, ns: non-significant. Different lowercase letters (within a column) represent significant differences between the averages (p < 0.05), according to Tukey test.

Capítulo 4:
O efeito dos regimes de corte no crescimento vegetativo e na qualidade da biomassa das halófitas *Paspalum vaginatum* Sw. e *Salicornia neei* Lag. em aquaponia salina

[The effect of cutting regimes on vegetative growth and biomass quality of the halophytes *Paspalum vaginatum* Sw. and *Salicornia neei* Lag. in saline aquaponics]

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RESUMO EXPANDIDO

INTRODUÇÃO

A integração das unidades de produção de hidroponia e aquicultura animal é conhecida como aquaponia, permitindo a utilização de águas e efluentes da produção animal como fonte de nutrientes para as plantas. O manejo de plantas que podem tolerar a salinidade (i.e., halófitas) é essencial para o desenvolvimento da aquaponia salina. Na última década, a aquaponia salina tem sido centrada em plantas de ciclo de vida curto (i.e., espécies anuais), como a comercial *Salicornia bigelovii*, que apresenta alta taxa de crescimento em curto período de tempo (Sagi e Ventura, 2013; Glenn et al., 2013), mas ainda há a necessidade de produzir novos propágulos para cada período de cultivo.

As espécies halófitas perenes também já foram cultivadas em aquaponia (Ventura et al., 2011; Sagi e Ventura, 2013; Glenn et al., 2013; Doncato & Costa, 2018a), devido à sua capacidade de produzir o ano todo por cortes e rebrote. Essa característica leva a uma produção contínua para o mercado, além de um ciclo de cultivo simples, redução da produção de mudas/propágulos que consomem muito tempo e insumos. Das halófitas perenes nativas do Brasil, *Paspalum vaginatum* Sw. (Pessarakli e Touchane, 2006; Lonard et al., 2015) e *Salicornia neei* Lag. (Costa, 2006; Costa et al., 2014) podem ser destacadas por sua capacidade de crescer rapidamente após o corte e o potencial econômico para usos múltiplos, por exemplo na alimentação de animais e humana (Lonard et al., 2015; Doncato e Costa, 2018b) e como nutracêuticos (Souza et al., 2018).

A otimização do regime de corte ainda não foi estabelecida para *P. vaginatum* e *S. neei*. Assim, o presente estudo visa avaliar os efeitos de diferentes regimes de corte em *P. vaginatum* e *S. neei* cultivadas em aquaponia salina com água clarificada do estoque de reprodutores de *Litopenaeus vannamei* B.. Os parâmetros de crescimento, produção de biomassa e teor de nitrogênio tecidual foram avaliados, bem como a captação total de nitrogênio da água em recirculação nas unidades hidropônicas submetidas a diferentes regimes de corte.

MÉTODOS

As plantas de *P. vaginatum* e *S. neei* foram dispostas em unidades hidropônicas NFT (*Nutrient Film Technique*) e a água clarificada de um tanque de estoque de reprodutores de *L. vannamei*, crescendo em sistema BFT, foi recirculada e substituída semanalmente. Os experimentos foram desenvolvidos ao longo de 28 dias. Três regimes de corte foram testados: nenhum corte ao longo do período de cultivo (controle); um corte

realizado no dia 0 (a cada 28 dias); e dois cortes, um realizado no dia 0 e outro no dia 14 - isto é, a cada 14 dias.

Durante os experimentos, a qualidade da água dos cultivos foi monitorada três vezes na semana, sendo medidos: pH (pHmetro FEP20 Mettler Toledo®), salinidade (refratômetro PAL-06S Atago®), temperatura da água (termômetro TDU-300 Unity®), sólidos em suspensão totais (Strickland e Parsons, 1972), total de nitrogênio amoniacal (UNESCO, 1983), nitrito (Bendschneider e Robinson, 1952), assim como os teores de nitrato e ortofosfato foram medidos de acordo com Aminot e Chaussepied (1983). Em relação aos parâmetros vegetativos, as biometrias foram realizadas no início e no final de cada experimento, e a biomassa fresca foi pesada e depois seca na estuda a 60 °C por 48 h, para então ser quantificada a biomassa seca. A diferença entre a biomassa fresca e seca estima o conteúdo de água das plantas. A partir da biomassa seca foi analisado o teor de nitrogênio dos tecidos de acordo com o protocolo da AOAC (1990).

Todos os experimentos foram analisados por Análises de Variância (ANOVA), precedidas pelos testes de normalidade (Shapiro-Wilk) e homoscedasticidade (Levene). Quando houveram diferenças significativas (5%) foi utilizado o teste de Tukey.

RESULTADOS E DISCUSSÃO

Os diferentes regimes de corte aplicados as duas halófitas não afetaram a qualidade da água recirculando nas unidades hidropônicas. O perfilhamento e o alongamento da altura de *P. vaginatum* apresentaram uma tendência ascendente com o aumento da frequência de corte (Figura 1; Tabela 2). Anteriormente, Duncan e Carrow (2000) mostraram que o corte de *P. vaginatum* estimula o perfilhamento, e assim a expansão lateral desta grama. Da mesma forma, *Paspalum dilatatum* (ecótipo André da Rocha) também aumentou o número de perfilhos após dois cortes consecutivos (Beck, 2012), como resultado da remoção de material morto naturalmente acumulado no centro da grama. Esta condição de melhoria da luz estimula a formação de novos perfilhos (Costa e Scheffer-Basso, 2003; Baréa et al., 2007; Beck, 2012). No entanto, o crescimento vigoroso das estruturas aéreas não gerou ganho semelhante na biomassa, pois o surgimento de novos perfilhos comprometeu a produção de folhas por perfilho (Figura 2; Tabela 2). O tratamento de um corte (colheita a cada 28 dias) foi considerado a prática mais adequada para *P. vaginatum*, devido ao perfilhamento intermediário e à produção de novas folhas para alimentação animal.

O corte de *S. neei* a cada 14 dias mostrou uma produção massiva de novas ramificações que tiveram um pequeno desenvolvimento em tamanho (Figura 3 e 4). Como resultado da dominância apical de *Salicornia* (Costa et al., 2006), pois o corte do caule principal pode estimular o desenvolvimento de ramificações laterais (Leyser, 2005). Essas ramificações jovens e minúsculas, embora possam ser utilizados para a produção de nutracêuticos (Costa et al., 2006; Souza et al., 2018), não são aceitáveis para o mercado como vegetais. Os cortes com intervalos de 28 dias produziram uma grande quantidade de ramificações de tamanho comercializável (≥ 10 cm) com menor lignificação do que as plantas não cortadas.

Paspalum vaginatum e *S. neei* captaram em seus tecidos, respectivamente, entre 2,27-2,45 mg N dia^{-1} e 4,59-6,43 mg N dia^{-1} da água clarificada do sistema BFT de estoque de reprodutores de *L. vannamei* (Figura 5). Os regimes de corte não afetaram o acúmulo de nitrogênio na biomassa de ambas as halófitas.

CONCLUSÕES

Ambas as halófitas foram capazes de regenerar após o corte em aquaponia salina com água clarificada de um sistema BFT de reprodutores de *L. vannamei*. O tratamento de corte a cada 28 dias permitiu estruturas foliares de *P. vaginatum* e caules de *S. neei* com melhor valor comercializável. Para *P. vaginatum*, um período de crescimento de 28 dias após o tratamento de corte evita o acúmulo de folhas mortas, permite perfilhamento abundante e uma grande produção de folhas novas, condições vantajosas para a produção de forragem. *Salicornia neei* cortada uma vez a cada 28 dias produziu uma grande quantidade de ramificações com tamanho aceitável pelo mercado, composto por biomassa jovem e não lignificada. A prática de corte não modificou a capacidade de absorção de nitrogênio da água do sistema aquapônico salino pelas halófitas.

ABSTRACT

Saline aquaponics (integration of hydroponics and marine aquaculture) have been centered in plants of short life cycle (i.e., annual species). Meanwhile the introduction of perennials can lead to continuous plant biomass production by consecutive harvests, simple growing cycles and reduction of costs with seedling production/maintenance. The present study evaluates the effects of different cutting regimes on the growth of perennial halophytes *Paspalum vaginatum* Sw. and *Salicornia neei* Lag. and their nitrogen uptake into tissues in saline aquaponics. Plants were established in NFT hydroponic units and clarified water from marine shrimp breeding stock tank was recirculated and replaced weekly. Trials were developed along 28 days for each species. The different cutting regimes applied to both halophytic species did not affect the quality of recirculating water in the hydroponic units. Tillering and stem elongation of *P. vaginatum* had an upward trend with increasing of cutting frequency. However vigorous regrowth of aerial structures did not generate similar gain in biomass, because of the sprout of new tillers compromises the production of leaves per tiller. The one cutting treatment (every 28 days harvest) was considered the most suitable practice for *P. vaginatum,* due to its intermediate tillering and new leaves production for animal feeding. *Salicornia neei* cut every 14 days showed a massive production of new branches that had a small development in size. Cuttings at 28-days intervals produced a large amount of marketable size branches (≥ 10 cm) with less lignified shoots than non-cut plants. Individual plants of *P. vaginatum* and *S. neei* uptook in their tissues between 2.27-2.45 mg N day^{-1} and 4.59-6.43 mg N day^{-1}, respectively. Cutting regimes did not affect markedly the accumulation of nitrogen into biomass of both halophytic species.

Keywords: clipping; *Litopenaeus vannamei*; harvest; integrated system; vegetable; prunning.

1. Introduction

The cultivation of vascular plants in aquaculture is becoming increasingly important for food production. Integration of hydroponics and animal aquaculture production units is known as aquaponics, allowing utilization of waters and effluents of aquatic animal production as total or partial source of nutrients for plants. The management of plants that can tolerate salinity (i.e., halophytes) is essential for the development of saline aquaponics. In the last decade, saline aquaponics have been centered in plants of short life cycle (i.e., annual species), like the commercial *Salicornia bigelovii*, which have high growth ratios in short periods (Sagi and Ventura, 2013; Glenn et al., 2013), but there is the necessity to produce new propagules for each cultivation period. Perennial species have also been cultivated in aquaponics (Ventura et al., 2011; Sagi and Ventura, 2013; Glenn et al., 2013; Doncato & Costa, 2018a), because of their ability to produce year-around by cuttings and regrowth. This characteristic leads to a continuous production for market, as well as simple growing cycle, reduction of time consuming seedlings/propagules production and of agricultural inputs. From the perennial halophytes natives from Brazil, *Paspalum vaginatum* Sw. (Pessarakli and Touchane, 2006; Lonard et al., 2015) and *Salicornia neei* Lag. (Costa, 2006; Costa et al., 2014) can be highlighted for their ability to readily growth after cutting and economic potential for multiple uses.

Paspalum vaginatum Sw. is a cosmopolitan perennial grass widely spread in the Brazilian coastal salt marshes and coastal dunes (Costa and Davy, 1992). *Paspalum vaginatum* biomass is composed of 1.3% fatty acids, 10.4% crude protein and 77.1% carbohydrates (DesRochers et al., 2009), and high concentration of free phenolic compounds (Souza et al., 2018). Due to its vigorous growth, even when irrigated with brackish water, *P. vaginatum* is used for covering sports fields as golf (Lonard et al., 2015), landscaping (Ntoulas and Nektarios, 2015), goat and cattle fodder and phytoremediation of saline soils (Lonard et al., 2015). Weekly cuttings of rooted plants were satisfactorily performed under hydroponic cultivation by Pessarakli and Touchane (2006), but there are no studies evaluating cutting effect on *P. vaginatum* growth in saline aquaponics. Cuts from this species are likely to be used for cut-and-carry forage system, as for *Paspalum atratum* (Hare et al., 2001).

Salicornia neei Lag. [syn. *Salicornia gaudichaudiana* Moq. and *Sarcocornia ambigua* (Michx.) M.A. Alonso & M.B. Crespo] is a perennial shrub distributed in the intertidal marshlands along most of the Atlantic and Pacific coast of South America

(Costa et al., 2019). *Salicornia neei* biomass has high nutritional quality, rich in macronutrients and micronutrients (Doncato and Costa, 2018a; 2018b), as well as high concentrations of phenolic acids, flavonoids and vitamin C (Costa et al., 2006; Souza et al., 2018). Vegetative biomass can be used for human and animal consumption (Doncato and Costa, 2018b) and source of nutraceuticals (Souza et al., 2018). Like other *Salicornia* species (Ventura et al., 2011; Ventura and Sagi, 2013), *S. neei* has a great potential as leafy vegetable product. Regrowth of *S. neei* shoots was successful after cutting in soil cultivated plants (Costa, 2006; Costa et al., 2014) and other perennial *Salicornia* species, irrigated with saline water, could withstand 7 harvest rounds spread over one year of continuous cultivation, producing twice more biomass than annual *Salicornia* in the same period (Ventura and Sagi, 2013). However, cutting effects on morphological features of *Salicornia* with marketable value are little known (Ventura and Sagi, 2013).

Cutting of halophytes might stimulate vegetable product quality and quantity by generation of young shoots with more nutritional value and the accumulation of biomass between cuttings (Leite et al., 2001; Ventura et al., 2011; Ventura and Sagi, 2013). For instance, Costa et al. (2006) showed that cutting of *S. neei* plants generate sprouting shoots with higher phenolic compounds content than of intact plants. However, the optimization of cutting regime has not been established for *P. vaginatum* and *S. neei*. The present study evaluates the effects of different cutting regimes on *P. vaginatum* and *S. neei* plants grown in saline aquaponics with clarified Biofloc Technology (BFT) system water from a shrimp breeding stock tank. Growth parameters, biomass production and tissue nitrogen content were evaluated, as well as total nitrogen uptake from the recirculating water in the aquaponic system subjected to different cutting regimes.

2. Methods

2.1. Plant acquisition

All the plants were obtained from the active germplasm bank of the Laboratório de Biotecnologia de Halófitas, Instituto de Oceanografia-IO, Universidade Federal do Rio Grande-FURG. *Paspalum vaginatum* plants were originally obtained from a salt flat of the estuary of Caravelas (17º45'03"S; 39º13'04"W). *Salicornia neei* plants were from lineage BTH2 selected from a wild population of Patos Lagoon estuary (32°01'S, 52°06'W; Doncato and Costa, 2018a). Both species were grown from shoot fragments, disposed individually into 50 cm^3 plugs filled with fine sand beach and placed within polyethylene trays with 10% of Hoagland solution (Hoagland and Arnon, 1950), which

were kept in an unheated greenhouse for 6 and 12 weeks, respectively. Nutrient solution was weekly replaced and, previously to the experiment, all plants were uprooted and roots cleaned with water to remove the substrate.

2.2. Recirculating water

Decoupled aquaponic systems used saline water from a tank of *Litopenaeus vannamei* B. breeding stock located at Estação Marinha de Aquacultura (EMA, IO, FURG). Shrimps were reared in a 40 m³ tank with BFT system and a density of 555 shrimps per tank (approximately 50 g). Feeding was provided twice a day of extruded feed (Poti Evolution 35 Guabi®; 35% of crude protein). Residence time of the tank water was one year and due to the management practice there was a partial replacement of the water along the experiment.

Before the water being conduct by gravity to a reservoir (3,000 L) of the aquaponic system, solids in suspension were removed by clarification in a conical cylinder clarifier tank of 500 L (similar principle of Gaona et al., 2011) and by filtration with BP-420-50 Pentair® filter bags of 50 microns. Storage water was then pumped to individual reservoirs of three hydroponic units. Water was renewed every week after the cleaning with sodium hypochlorite of the clarifier tank, filter bags, pipes and aquaponic system reservoirs.

2.3. Experimental design

Two experiments were carried out; one for each halophytic species. Both experiments used the same set up and were developed along 28 days, being the trial of *P. vaginatum* in the summer-autumn 2019 and the *S. neei* trial conducted in the autumn 2019. Plants of *P. vaginatum* (shoot height: 10.95 ± 0.43 cm; average ± standard error) and *S. neei* (shoot height: 5.63 ± 0.40 cm) were transferred to net pots (made of plastic cup) fulfilled with small gravels and randomly disposed in the Nutrient Film Technique (NFT) benches. In each hydroponic unit were placed 22 plants and 13.1 L of clarified shrimp water min $^{-1}$ was recirculate every 15 minutes (393 L^{-1} h^{-1}) between the bench tubes and water reservoir of 250 L. Three cutting regimes were tested and one decoupled aquaponic system per treatment was used: no cutting along the cultivation period (control); one cutting performed in day 0 (every 28 days); and two cuttings, one performed in day 0 and other in day 14 (every 14 days). Plants were cut about 4 cm above the gravel level (height between the gravel surface and the top border of the net pot), in order to preserve the branchs' apical meristems of *S. neei* and ensure a viable photosynthetic area for the basal regrowth of *P. vaginatum* tillers. Few plants were damaged during net cup transfer and/or biometry procedure and were removed from data

analyses. The final numbers of plants account for each experimental trial were: *P. vaginatum*, 19, 18 and 18 for the first, second and third treatments, respectively; and *S. neei*, 22, 18 and 18 for the first, second and third treatment, respectively.

Local experimental conditions (mean ± standard error) during *P. vaginatum* trial was temperature of 22.01 ± 0.52 °C and daily solar radiation of 16.96 ± 0.82 MJ m^{-2} day^{-1}. During *S. neei* trial average temperature was 19.87 ± 0.42 °C and daily solar radiation averages 11.20 ± 0.88 MJ m^{-2} day^{-1}. Both data were obtained from the automatic station of Instituto Nacional de Meteorologia-INMET (32°04'43" S; 52°10'03" W).

2.4. Physical and chemical water parameters

Water physical-chemical parameters were monitored three times per week in both experiments. Water temperature measured with a TDU-300 Unity® thermometer, pH using a FEP20 Mettler Toledo® pHmeter and salinity by PAL-06S Atago® refractometer. Total suspended solids (TSS) were estimate according to Strickland and Parsons (1972). Total ammonia nitrogen (TAN) quantification followed the described at UNESCO (1983). Nitrite (NO_2) was analysed by the methodology of Bendschneider and Robinson (1952), while nitrate (NO_3) and orthophosphate (PO_4) were measured according to Aminot and Chaussepied (1983). All the sampling bottles were cleaned with 10% of chloridric acid and rinsed with distilled water three times.

2.5. Vegetative parameters, biomass and tissue nitrogen content

Biometry procedures were carried out in the beginning of each experimental trial and at each cutting date. For *S. neei*, we measured shoot height, length of all branches, branch number and shoot biomass, and for *P. vaginatum* were measured shoot height, number of tillers, number of leaves and shoot biomass. Plants were individualized by their position in the benches, and biotic data is presented as absolute growth rates between harvests (i.e., discounting initial values) and results of two cuttings treatment are sums of two regrowth periods. Foliar index of *S. neei* plants was estimated by the sum branch lengths measured (cm). In the beginning of the experiment there were no roots out of the net pots, and final root biomass of both species were measured at the final harvest by cutting all roots external to the net pots.

Shoot and root biomass components were weighted on a precision scale to determine fresh biomass and then oven dried at 60 °C for 48 h to quantify dry biomass. All the biomass data was presented as dry matter. The difference between fresh and dry matter estimates the water content in shoot tissue. Nitrogen content in shoot and root tissues was measured by Kjeldahl digestion following the protocol of AOAC (1990). The

amount of nitrogen accumulated into plant biomass under different cutting regimes was estimated by the multiplication of averages plant biomass produced along the experiment and their tissue nitrogen content.

2.6. Statistics

For each halophyte, one-way Analyses of Variances (ANOVA) were carried out to compare physical-chemical parameters of the recirculated water, vegetative attributes, biomass and nitrogen tissue content among cutting regimes (no cutting, one cutting and two cuttings). In order to fulfill the requirements of normality (Shapiro-Wilk test) and homoscedasticity (Levene test) described in Zar (2010), some variables were transformed. Shoot height, number of tillers and number of leaves of *P. vaginatum*, as well as shoot height, foliar index and number of branches of *S. neei* were transformed by square root (x). Log_{10} (x) was used for shoot biomass of *P. vaginatum* and shoot biomass, shoot and total nitrogen content of *S. neei*. Log_{10} (x) was applied for TAN of *P. vaginatum* experiment and water temperature, TAN, NO_2, NO_3, PO_4. When significant (α= 0.05) results were obtained, Tukey post-hoc was performed.

Paired t-tests were performed to contrast plants' responses of the first and second cutting in the two cuttings treatment. For these analyses, number of tillers and leaves of *P. vaginatum* and foliar index and branch number of *S. neei* were transformed by square root (x), while shoot biomass of *P. vaginatum* and *S. neei* were transformed by log_{10} (x). Histograms of *S. neei* branch lengths at the beginning and each harvest date of all treatments illustrate the impact of the different cutting regimes on this marketable value attribute.

3. Results

3.1. Physical and chemical water parameters

No statistically significant difference was found for water quality parameters among the cutting regimes in both *P. vaginatum* and *S. neei* trials (data not presented). The global average conditions (average ± standard error) of the hydroponics units for *P. vaginatum* experiment were TSS of 165.15 ± 3.99 mg L^{-1}, water temperature of 23.43 ± 0.36 °C, pH of 7.55 ± 0.02, salinity of 20.96 ± 0.44, TAN of 0.14 ± 0.03 mg L^{-1}, NO_2 of 0.14 ± 0.01 mg L^{-1}, NO_3 of 57.08 ± 2.51 mg L^{-1} and PO_4 of 2.45 ± 0.07 mg L^{-1}. The global average conditions of the hydroponics units during *S. neei* experiment were TSS of 193.10 ± 7.65 mg L^{-1}, water temperature of 20.66 ± 0.29 °C, pH of 7.60 ± 0.03, salinity of 24.58

± 1.49, TAN of 1.40 ± 0.29 mg L^{-1}, NO_2 of 7.06 ± 1.40 mg L^{-1}, NO_3 of 23.71 ± 2.59 mg L^{-1} and PO_4 of 1.27 ± 0.15 mg L^{-1}.

3.2. Vegetative parameters, biomass and tissue nitrogen content

Increasing cutting regimes affected significantly some vegetative characteristics of *P. vaginatum* (Figure 1a-d; Table 1-2). Growth rates of shoot height ($F= 90.80$; $p < 0.001$) and number of tillers ($F= 10.64$; $p < 0.001$) of *P. vaginatum* ($F= 215.73$; $p < 0.001$) increased with the increment of the cutting frequency, which did not result in biomass gain. Water content and nitrogen content in two cuttings treatment were higher than no cutting treatment (Table 1). For the two cuttings treatment, averages of all vegetative parameters, shoot biomass and shoot tissue water content of *P. vaginatum* plants showed statistically higher values in the second harvest than in the first harvest (Figure 2a; Table 2). Shoot biomass response of the second harvest (175% higher than the first one) was mainly determine by the production of new tillers (increased 124%) and leaves (increased 120%) (Table 2).

On the other hand, few parameters of *S. neei* were affected by cutting treatments (Figure 3a-d; Table 1-2). Shoots of two cuttings treatment showed slightly lower ($p < 0.05$) water content than one cutting treatment plants (Table 1). Only *S. neei* branch formation was stimulated with the increasing cutting frequency ($F= 45.06$; $p < 0.001$) (Figure 3b). This branching process did not result in biomass gain (Figure 2b) since there was a very small elongation for the great number of branches sprouted (Figure 4). Initially, *S. neei* plants of all treatments had less than 1% of their branches with 10 cm length (international marketable size; Ventura et al., 2011) and modal values of branch length lower than 5 cm. At the final harvest, 39.5% and 26.5% of the branches of no cutting and one cutting treatments reached marketable size, respectively. By contrast, plants of the two cuttings treatment had only 0.7% and 0.1% of their sprouted branches after the first and second harvests with marketable sizes, respectively.

3.3. Nitrogen uptake by plant biomass

Paspalum vaginatum and *S. neei* did not show marked differences in the total uptake of nitrogen into shoot and root biomass between cutting regimes. Considering individual average values of biomass production and nitrogen tissues content, the total nitrogen uptake by *P. vaginatum* and *S. neei* ranged between 2.27-2.45 mg N day^{-1} and 4.59-6.43 mg N day^{-1}, respectively (Figure 5). Due to their differential growth patterns, *P. vaginatum* accumulated 62.8% of total nitrogen uptake into its root biomass, whereas *S. neei* concentrated 53.2% of the total nitrogen absorption into shoot biomass.

4. Discussion

4.1. Physical and chemical water parameters

The different cutting regimes applied to both halophytic species did not affect the quality of recirculating water in the hydroponic units. Nitrate and phosphate concentrations in the water during *S. neei* experiment were distinctly lower than the water used in the *P. vaginatum* experiment, because of management practice of partial replacement of the water volume in the shrimp tank. These changes and augments of NAT and nitrite in *S. neei* experiment can be explained by a new startup of biofloc formation with predominance of heterotrophic bacteria and ammonia-oxidizing bacteria (AOB) (Timmons and Ebeling, 2010; Silva et al., 2013). However, nitrogen compounds and phosphate concentrations were not limiting for the growth of *P. vaginatum* and *S. neei* (see Chapter 2).

4.2. Vegetative parameters, biomass and tissue nitrogen content

Tillering and stem elongation of *P. vaginatum* had an upward trend with increasing of cutting frequency. Previously, Duncan and Carrow (2000) showed that the cutting of *P. vaginatum* shoots stimulates tillering, thus lateral expansion of its turfs. *Paspalum dilatatum* ecotype André da Rocha also increased the number of tillers after two consecutive cuttings (Beck, 2012), as result of the removal of dead material naturally accumulated in the center of turfs. Light improving condition stimulates the basal buds to form new tillers (Costa and Scheffer-Basso, 2003; Baréa et al., 2007; Beck, 2012). By contrast, *P. atratum* (Hare et al., 2001) and *P. dilatatum* (biotype Virasoro: Scheffer-Basso et al., 2007; ecotype Bagual: Beck, 2012) showed no cutting effect on tiller production, and *P. guenoarum* ecotype Azulão decreased the number of tillers after two consecutive cuts (Lopes at al., 2016). The species may also differ in their capacity to regrowth and produce new leaves. Repetitive cuttings are a common management practice on *Paspalum* species, and, likewise *P. vaginatum* in our aquaponic experiment, some species can improve shoot height (vertical) regrowth with increasing cutting intervals (*P. atratum*, Hare et al., 2001; *P. dilatatum*, Scheffer-Basso et al., 2007). *Paspalum vaginatum* demonstrates powerful recuperation even in a short 14 days period, a better responses than *P. atratum* cv. Pojuca that had its growth inhibit by cutting interval equal or shorter than 21 days (Leite et al., 2001).

No effect of cutting regimes was observed in *P. vaginatum* biomass production. Similar lack of biomass response was observed in *P. dilatatum* (Holt and McDaniel, 1963; Bungenstab et al., 2003). Otherwise, *Paspalum notatum* declines its aboveground yield

and root growth with cutting (Kaminski et al., 1998), whereas *P. guenoarum* improved its biomass when subject to two cuttings than one cutting (Lopes at al., 2016). Vigorous regrowth of aerial structures not always generates similar gain in biomass, due to the different energetic and structural prices of plant modules that need to be formed. In our experiment, the production of new tillers seems to compromise the production of leaves; plants of treatments no cutting, one cutting and two cuttings showed averages of 9.4, 6.2 and 4.6 leaves per tillers. Thus, although *P. vaginatum* shoot height and number of tillers were improved by frequent cuttings, no expected accumulation of biomass occurs due to negative effect on leaf formation per tiller.

Considering the point of view of forage production and the utilization of *P. vaginatum* biomass generate in aquaponics for cut-and-carry forage system (Hare et al., 2001), the one cutting treatment (every 28 days harvest) can be considered the most suitable practice, due to its intermediate tillering and new leaves production for animal feeding. *Paspalum vaginatum* tillering can become an issue on its aquaponics practice, since intensive tillering fill up rapidly net cups and periodic thin out are necessary. From the biomass quality point of view, highest nitrogen content and water content in shoots of *P. vaginatum* subject to cuttings every 14 days characterize a more nutritious young biomass, which can be used as supplement of animal diets or for extraction of bioactive compounds (Souza et al., 2018). Leite et al. (2001) and Baréa et al. (2007) reported similar nitrogen enrichment of *P. atratum* and *P. dilatatum* new tiller biomass after repetitive cutting. Furthermore, shoot nitrogen content of *P. vaginatum* was higher than observed in *P. atratum* (1.48-1.94% N; 14-28 days of cultivation; Leite et al., 2001), *P. dilatatum* (1.81% N; Baréa et al., 2007) and *P. guenoarum* (2.25% N; Costa and Saibro, 1995).

Salicornia neei was less responsive to cuttings than *P. vaginatum*. The only growth parameter affected was the number of branches. Due to apical dominance of *Salicornia* (Costa et al., 2006), cutting of the main stem can stimulate the development of lateral branches, which assume the role as primary apical meristem (Leyser, 2005). When cut every 14 days (i.e., two cuttings), this species showed a massive production of new branches that had a small development in size. This young tiny branches although could be used for production of nutraceuticals (Costa et al., 2006; Souza et al., 2018), being not acceptable for the market as vegetables. Otherwise plants subject to no cutting or only one cutting every 28 days had approximately 26-40% of their branches reaching commercial size (≥ 10 cm; Ventura et al., 2011). Previously Ventura and Sagi (2013) highlighted that frequent harvests led to shorter shoot size of *Salicornia* species cultivated

in Israel. The choice of time intervals between subsequent harvests must consider plant regrowth pattern, and in the present study cuttings at 28-days intervals produced a large amounts of marketable size branches with less undesired lignified ("wooden") shoot parts than non-cut plants.

As for other perennial *Salicornia* species cultivated with saline irrigation (Ventura et al., 2011; Ventura and Sagi, 2013), *S. neei* grows vigorously and withstands periodic cutting and it was able to sustain continuous production of biomass in saline aquaponics. Previously, Costa (2006) and Costa et al. (2014) showed that *S. neei* was able to sustain periodic cuttings growing in field plots irrigated with saline waters/effluent from shrimp farming. No cutting effect was observed on shoot biomass production of *S. neei* in the present study. Ventura et al. (2011) tested cutting intervals of 14, 21 and 28 days over 5 months of cultivation and also reported no effect of cutting regime on the accumulated biomass of *Salicornia fructicosa* (ecotype EL). Contrastingly, annual *Salicornia persica* (ecotype RN) accumulated more biomass than the former and showed the highest total production with cutting every 21 days. Concerning biomass quality, nitrogen content of *S. neei* shoots produced in saline aquaponics with BFT system water was higher than values found for the same lineage cultivated in field plots (2.05% N, *S. neei* lineage BTH2; Doncato and Costa, 2018a), *Salicornia europaea* (2.40% N; Tikhomirova et al., 2008) and the commercial *Salicornia bigelovii* (0.72 to 1.36% N; Coronado, 1991), but lower than *S. neei* lineage BTH1 (2.92% N; Doncato and Costa, 2018a). Thus, saline aquaponics with BFT system water generates a high quality *S. neei* biomass.

4.3. Nitrogen accumulated into biomass

Cutting regimes did not affect markedly the accumulation of nitrogen into biomass of both halophytic species. This result occurs because of very little effect of cuttings on growth and tissues nitrogen content of plants. Although *P. vaginatum* had high shoot nitrogen contents in plants subjected to two cuttings, the values of shoot and root biomass, and root nitrogen content were similar, resulting in very alike total nitrogen uptake into biomass of all treatments. Both Brazilian halophytes accumulate less nitrogen per day than *Aster tripolium* (shoot= 4.41 mg N day^{-1} and roots= 8.75 mg N day^{-1}), *Bolboschoenus maritimus* (shoot= 3.49 mg N day^{-1} and roots= 11.56 mg N day^{-1}) and *Spartina anglica* (shoot= 3.02 mg N day^{-1} and roots= 6.86 mg N day^{-1}), evaluated for remediation of eutrophicated coastal waters (Lange and Paulissen, 2016). These results were obtained with plants grown for 63 days and reaching large total dry individual biomass of 78000, 122500 and 138000 mg, respectively. Additionally, *P. dilatatum* showed an uptake of

3.62 mg N day^{-1} cultivated for 270 days, but plants reached 54125 mg of shoot dry biomass (Baréa et al., 2007), what explains its much larger nitrogen accumulation in tissues than *P. vaginatum* plants growing in saline aquaponics (shoots reached 700-800 mg). On the other hand, *S. neei* cultivated with clarified BFT system water accumulated more nitrogen than *S. neei* growing in field plots (total uptake nitrogen, lineage BTH1= 3.25 mg N day^{-1}; lineage BTH2= 5.80 mg N day^{-1}; Doncato and Costa, 2018a), but less than *S. europaea* in hydroponics (shoot uptake nitrogen, 5.83 mg N day^{-1}; Tikhomirova et al., 2008). These results were either due to best growth and percentage nitrogen content of *S. neei*, showing high quality conditions for growth of this species in aquaponics with BFT system waters.

5. Conclusions

Both studied halophytes were able to regrowth after cutting under saline aquaponics condition with clarified water of a BFT system from a shrimp breeding stock tank. The one cutting treatment every 28 days allowed foliar structures of *P. vaginatum* and *S. neei* plants with best marketable value. For *P. vaginatum*, a growth period of 28 days after cutting treatment prevents accumulation of dead leaves in the center of turfs, plentiful tillering and a great production of leaves, advantageous conditions for fodder production. *Salicornia neei* cut once every 28 days produced a large amount of branches with sizes equal or larger than acceptable by the market, composed by young and not lignified biomass. Cutting practice did not modify the capacity of halophytes to uptake nitrogen into their biomass from water of the aquaponic systems.

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Fig. 1. Average (± standard error) of cumulative shoot height (A), numbers of tillers (B) and of leaves (C), and final root biomass (D) of *Paspalum vaginatum* plants subject to three cutting regimes along 28 days on saline aquaponics. The d14 and d28 codes indicate data from consecutive harvests at days 0 and 14 of the two cuttings treatment, respectively.

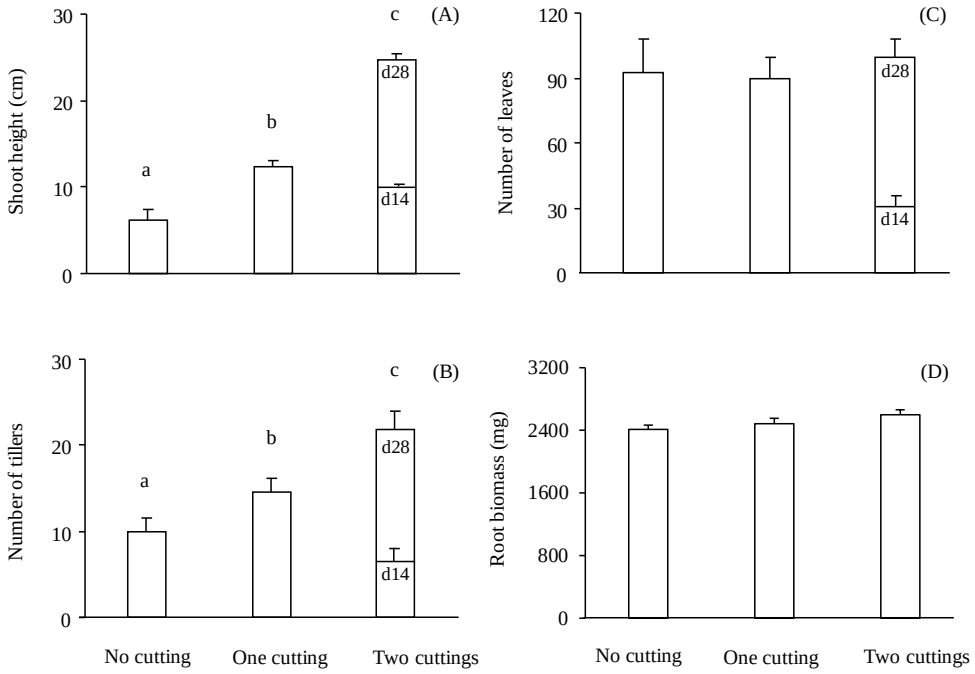
Shoot height (cm)
30
20
10
0
a
b
c
d28
d14
(A)
Number of leaves
120
90
60
30
0
d28
d14
(C)
Number of tillers
30
20
10
0
a
b
c
d28
d14
(B)
Root biomass (mg)
3200
2400
1600
800
0
(D)
No cutting
One cutting
Two cuttings
No cutting
One cutting
Two cuttings

Fig. 2. Average (± standard error) of total shoot biomass of *Paspalum vaginatum* (A) and *Salicornia neei* (B) plants subject to three cutting regimes along 28 days on saline aquaponics. The d0, d14 and d28 codes indicate data from consecutive harvests at days 0, 14 and 28, respectively.

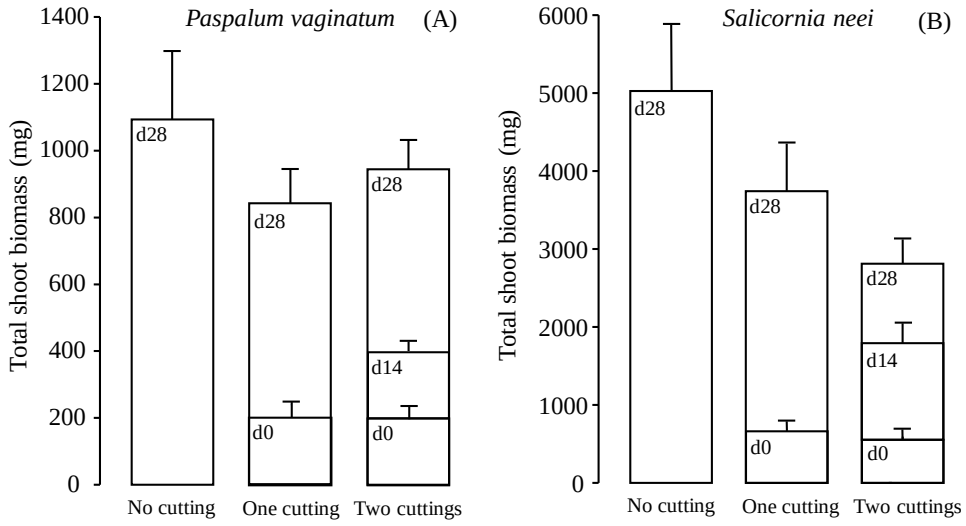
Paspalum vaginatum
(A)
Total shoot biomass (mg)
1400
1200
1000
800
600
400
200
0
d28
d28
d0
d28
d14
d0
No cutting
One cutting
Two cuttings
Salicornia neei
(B)
Total shoot biomass (mg)
6000
5000
4000
3000
2000
1000
0
d28
d28
d0
d28
d14
d0
No cutting
One cutting
Two cuttings

Fig. 3. Average (± standard error) of cumulative shoot height (A), branch number (B) and foliar index (C), and final root biomass (D) of *Salicornia neei* plants subject to three cutting regimes along 28 days on saline aquaponics. The d14 and d28 codes indicate data from consecutive harvests at days 0 and 14 of the two cuttings treatment, respectively.

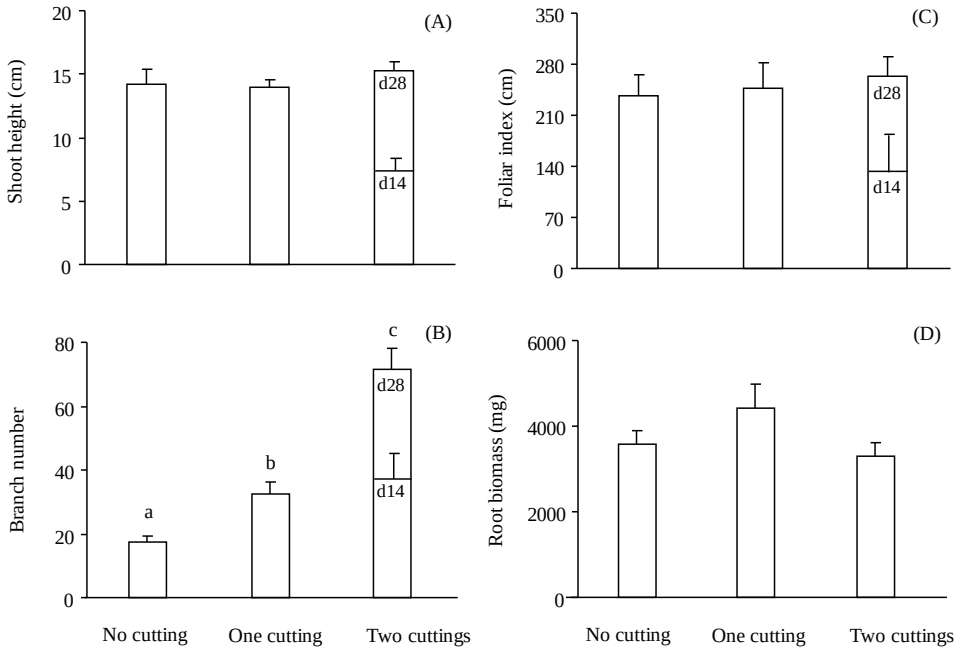
(A)
Shoot height (cm)
20
15
10
5
0
d28
d14
(C)
Foliar index (cm)
350
280
210
140
70
0
d28
d14
(B)
Branch number
80
60
40
20
0
a
b
c
d28
d14
(D)
Root biomass (mg)
6000
4000
2000
0
No cutting
One cutting
Two cuttings
No cutting
One cutting
Two cuttings

Fig. 4. Histograms of *Salicornia neei* branch lengths at the beginning (A-C) and each harvest date of all cutting regime treatments (D-G) along 28 days on saline aquaponics. The d0, d14 and d28 codes indicate data from consecutive harvests at days 0, 14 and 28, respectively. Treatment codes: no cutting (NC), one cutting (1C) and two cuttings (2C).

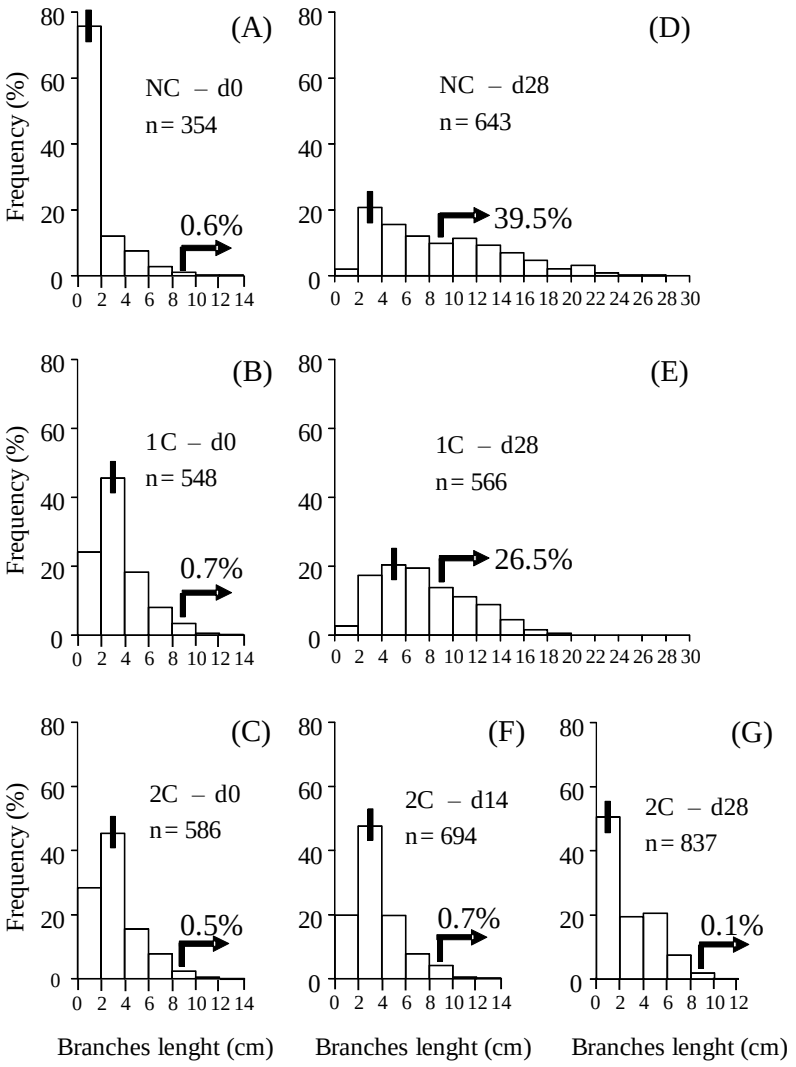
(A)
NC – d0
n = 354
0.6%
(D)
NC – d28
n = 643
39.5%
(B)
1C – d0
n = 548
0.7%
(E)
1C – d28
n = 566
26.5%
(C)
2C – d0
n = 586
0.5%
(F)
2C – d14
n = 694
0.7%
(G)
2C – d28
n = 837
0.1%
Frequency (%)
Branches lenght (cm)

Fig. 5. Estimated daily amount of nitrogen accumulated (uptake) into *Paspalum vaginatum* (A) and *Salicornia neei* (B) biomass under three cutting regimes along 28 days on saline aquaponics. Values calculated by multiplication of averages plant biomass produced along the experiment and their tissue nitrogen content (shoots+roots).

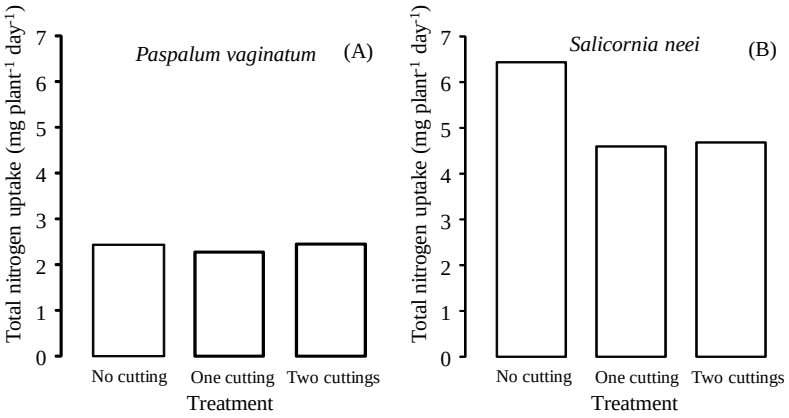
Total nitrogen uptake (mg plant-1 day-1)
Paspalum vaginatum
(A)
0
1
2
3
4
5
6
7
No cutting
One cutting
Two cuttings
Treatment
Total nitrogen uptake (mg plant-1 day-1)
Salicornia neei
(B)
0
1
2
3
4
5
6
7
No cutting
One cutting
Two cuttings
Treatment

Table 1

Average (± standard error) of shoot tissue water content and nitrogen content in shoots and roots of *Paspalum vaginatum* and *Salicornia neei* subject to three cutting regimes along 28 days on saline aquaponics. Results of one-way ANOVA among cutting regimes are presented (significance: *p < 0.05, **p < 0.01, ***p < 0.001, ns: non-significant). Different lowercase letters (within a row) represent significant differences between the averages (p < 0.05), according to Tukey test.

Paspalum vaginatum							
	No cutting		One cutting		Two cuttings		F
Water content (%)	75.68 (0.63)	a	77.73 (0.77)	ab	78.99 (0.30)	b	7.90 ***
Shoot N (%)	3.19 (0.01)	a	3.21 (0.08)	a	3.41 (0.06)	b	4.51 *
Root N (%)	1.56 (0.05)		1.73 (0.02)		1.67 (0.05)		4.03 ns
Salicornia neei							
Water content (%)	90.33 (0.42)	ab	90.86 (0.16)	b	89.65 (0.25)	a	3.40 *
Shoot N (%)	2.37 (0.09)		2.55 (0.08)		2.41 (0.05)		1.43 ns
Root N (%)	1.96 (0.09)		1.76 (0.11)		1.64 (0.08)		3.23 ns

Table 2

Average (± standard error) of vegetative parameters, shoot biomass, shoot tissue water content and nitrogen content of *Paspalum vaginatum* and *Salicornia neei* subject to two cuttings treatment along 28 days on saline aquaponics (1st cutting = regrowth between days 0-14; 2nd cutting = regrowth between days 14-28). Results of paired "t" tests between harvests are presented (significance: *p < 0.05, **p < 0.01, ***p < 0.001, ns: non-significant).

Paspalum vaginatum					
	1st cutting		2nd cutting		t
Shoot height (cm)	10.14 (0.40)	a	14.49 (0.61)	b	7.62 ***
Number of tillers	6.72 (0.90)	a	15.06 (1.70)	b	6.57 ***
Number of leaves	31.06 (3.81)	a	68.22 (6.51)	b	5.20 ***
Shoot biomass (mg)	198.41 (23.96)	a	545.54 (62.90)	b	7.71 ***
Water content (%)	78.03 (0.50)	a	79.95 (0.26)	b	3.66 *
Shoot N (%)	3.37 (0.05)		3.41 (0.06)		0.82 ns
Salicornia neei					
Shoot height (cm)	7.14 (0.75)		8.09 (0.32)		1.11 ns
Foliar index (cm)	135.39 (29.74)		127.57 (8.73)		1.22 ns
Branch number	37.94 (6.82)		33.44 (2.54)		0.11 ns
Shoot biomass (mg)	1,239.48 (254.89)		1,017.73 (75.26)		0.47 ns
Water content (%)	89.54 (0.42)		89.76 (0.24)		0.38 ns
Shoot N (%)	2.75 (0.11)		2.41 (0.05)		2.22 ns

Discussão geral

A aquaponia é um sistema de produção integrado que tem sido praticado há muito tempo, porém a comunidade científica vem estudando esta área há apenas algumas décadas. No caso do uso de águas salinas para a aquaponia, se faz necessário o uso de plantas tolerantes a salinidade (*i.e.* halófitas). Tal cultivo de plantas halófitas vem expandindo nos últimos anos, não apenas pelo seu uso bioremediador de águas e solos salinos, mas devido a potencialidade para comercialização, visto que espécies como a *S. neei*, *A. graveolens* e *P. vaginatum* podem ser consumidas por seres humanos e/ou animais. Entretanto, em vista do número limitado de informações referentes ao cultivo de halófitas em sistemas aquapônicos salinos, a presente tese buscou otimizar a propagação, requerimentos nutricionais e regime de poda das espécies halófitas nativas do Brasil em sistema aquapônico salino.

A primeira hipótese da tese (H1) foi confirmada. As sementes selvagens do aipo (*A. graveolens*) só germinaram quando a incubação foi em temperaturas alternadas durante o dia (20/30 ºC) do que sob temperatura constante. Apesar desta espécie ser cosmopolita, ficou caracterizada uma marcada adaptação da variedade testada ao clima regional onde foi coletada. Quanto ao controle da infestação das sementes por fungos, ao contrário do que foi hipotetizado, concentrações muito altas de desinfetantes, particularmente ácido acético, inibiram a germinação. Devido a sensibilidade das sementes, concentrações intermediárias de desinfetantes (hipoclorito de sódio 5-10%) produzem uma maior germinação de sementes e controle da infestação fúngica. Ressalta-se que o estabelecimento de um protocolo de germinação da variedade selvagem de *A. graveolens* (Capítulo 1) foi um estudo primordial para o uso da variedade brasileira desta espécie, a qual tem uma notória dificuldade de propagação.

A hipótese de que as halófitas teriam diferentes preferências quanto à forma de nitrogênio (nitrato ou nitrogênio amoniacal) se comprovou para *Salicornia neei* e *A. graveolens* (H2, Capítulo 2). Ambas tiveram melhor desenvolvimento vegetativo e de formação de biomassa com nitrato como fonte nitrogenada única ou combinada, sendo esta forma nitrogenada tipicamente acumulada nas águas da produção de animais aquáticos como por exemplo o sistema BFT. Todas as espécies tiveram um melhor crescimento quando submetidas aos altos teores dissolvidos de nitrogênio e fósforo na água de cultivo, condição característica do sistema BFT. Apesar de não previsto no início do trabalho, a nutrição com alta concentração de nitrogênio amoniacal (10 mg L^{-1})

mostrou causar acidificação da rizosfera e inibição do crescimento de *A. graveolens*. Essa condição de alta concentração de amônio é incomum no sistema BFT, no qual ao ser atingido uma concentração de aproximadamente 1 mg L^{-1} de nitrogênio amoniacal total, se realiza a adição de uma fonte de carbono orgânico para estimular o desenvolvimento das bactérias heterotróficas, e consequente há redução da concentração de nitrogênio amoniacal total. A manipulação do pH (alcalinização) e a presença de nitrato junto em solução com o alto teor de nitrogênio amoniacal (típicas no processo de nitrificação) mostraram impedir o efeito tóxico do nitrogênio amoniacal. Este estudo demonstrou a capacidade de todas as halófitas de se desenvolverem plenamente em cultivo aquapônico salino, com águas apresentando variações das concentrações de macronutrientes encontradas nas diferentes fases de desenvolvimento dos micro-organismos quimioautotróficos.

A hipótese de que águas de sistemas intensivos de camarão marinho, advindos de sistema de bioflocos clarificado, não poderiam suprir as necessidades micronutricionais do cultivo aquapônico das halófitas (H3, Capítulo 3) foi confirmada para *P. vaginatum*. Visto que *P. vaginatum* apresenta ser uma espécie de alto requerimento micronutricional, particularmente de ferro, e o incremento do seu crescimento vegetativo e produção de biomassa poderão ser alcançados com suplementação na água. Ressalta-se que ainda não há estudos que corroborem com o reuso das águas suplementadas com micronutrientes na água na produção de camarão, especificamente para o estoque de reprodutores. A suplementação de micronutrientes através da fertilização foliar não mostrou ser eficiente para as halófitas testadas. O desempenho pequeno de *A. graveolens* impossibilitou a avaliação da resposta à adição de micronutrientes. Com isto, foi possível observar que a produção de aquapônica salina de halófitas pode vir a ser limitada por micronutrientes, exceto para *S. neei*.

A produção de biomassa e a assimilação de nitrogênio não foram maiores em manejo com uma alta frequência (a cada 14 dias) de podas consecutivas (H4, Capítulo 4). Os dois processos citados foram afetados pelo regime de poda, sendo podas a cada 28 dias o melhor tratamento encontrado para *P. vaginatum* e *S. neei,* visando seus usos para alimentação animal e humana, respectivamente. O experimento do Capítulo 4 revelou um marcado aumento no perfilhamento de *P. vaginatum* e na formação de ramos nos caules de *S. neei* com o aumento do regime de poda que, no entanto, são acompanhados de reduções nos tamanhos das estruturas fotossintéticas associadas (*e.g.* folhas e comprimento das ramificações). Estas mudanças morfológicas têm impacto negativo no

valor comercial para o consumo direto das plantas produzidas em aquaponia salina. No entanto, as plantas geradas com este tipo de manejo de poda podem ter um apelo comercial para indústria de ração/suplementação nutricional e/ou farmacêutica.

A qualidade nutricional para o consumo da biomassa das três halófitas (*i.e.* teor de nitrogênio) foi maior em cultivos com maiores concentrações de nitrogênio na água (Capitulo 2), confirmando a hipótese inicialmente formulada (H5). No entanto, quanto ao efeito da poda sobre a qualidade nutricional das halófitas perenes, apenas as plantas de *P. vaginatum* submetidas a podas múltiplas tiveram seus teores teciduais de nitrogênio aumentado (Capitulo 4). Assim, esta prática de podas consecutivas pode garantir uma alta qualidade da biomassa dessa grama para dieta animal.

Em síntese, melhores técnicas de propagação, o atendimento as preferências nutricionais e o uso de regime de poda apropriadas na aquaponia salina com as halófitas *S. neei, A. graveolens* e *P. vaginatum* contribuem para o aumento da produção vegetativa destas espécies comercializáveis. Entretanto, outros estudos são necessários para compreender as necessidades micronutricionais de *A. graveolens* em condições ambientais ótimas para a espécie. A fertilização foliar de halófitas também deve ser estudada mais profundamente, tanto em relação aos teores de nutrientes que causem respostas significativas no crescimento das espécies, como também verificar possíveis efeitos celulares e anatômicos de surfactantes empregados na facilitação da fertilização foliar. Estudos de longo prazo (por vários meses) de podas consecutivas permitirão uma melhor avaliação dessa prática na produção aquapônica de halófitas.

Conclusões gerais

- A incubação de sementes da variedade selvagem de *A. graveolens* em temperaturas alternadas é imprescindível para o sucesso da germinação, sendo também necessário realizar o pré-tratamento de desinfecção das sementes com hipoclorito de sódio (5-10%) para germinação;

- O nitrato é a forma preferencial de nitrogênio para *S. neei* e *A. graveolens*, havendo uma melhora do crescimento e da produção de biomassa com o aumento da sua disponibilidade como fonte única e combinada de nitrogênio, desde que sejam supridas concentrações $\geq$ 10 mg L^{-1}. A alta concentração de amônio (10 mg L^{-1}) como fonte única de nitrogênio pode causar toxidez, porém o gerenciamento do pH pode aliviar o efeito negativo em *A. graveolens*. *Paspalum vaginatum* é uma espécie sem preferência por forma nitrogenada e sem sensibilidade a alta concentração de amônio. As formas de nitrogênio combinadas promoveram um aumento no crescimento das três halófitas testadas, concomitante com o incremento da concentração de nitrato nos tratamentos. O nível de fósforo só foi limitante para *S. neei* e *A. graveolens* em condição altamente nitrificante (*e.g.* 50 mg NO_3-N L^{-1}), porém não é uma situação comum no cenário atual da aquicultura;

- A condição micronutricional do sistema aquapônico salino supriu as necessidades para o desenvolvimento vegetativo de *S. neei*. Diferentemente de *P. vaginatum*, que aumentou seu crescimento e produção de biomassa com suplementação de micronutrientes na água. O pequeno desempenho de *A. graveolens* não possibilitou a avaliação das respostas a adição de micronutrientes. Adicionalmente, a fertilização foliar não foi efetiva para melhorar o crescimento das halófitas; e

- *Paspalum vaginatum* e *S. neei* foram capazes de rebrotar após de podas consecutivas, sendo que uma poda a cada 28 dias possibilitou a obtenção de biomassa vegetativa com melhores características para a comercialização e para consumo animal e humano, respectivamente.