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CRESCIMENTO E SOBREVIVÊNCIA LARVAL DO MARISCO *Anomalocardia brasiliana* (GMELIN, 1791) ALIMENTADO COM DIFERENTES DIETAS ALGAIS

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PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

CRESCIMENTO E SOBREVIVÊNCIA LARVAL DO MARISCO *Anomalocardia brasiliana* (GMELIN, 1791) ALIMENTADO COM DIFERENTES DIETAS ALGAIS

Isabela Bacalhau de Oliveira

Tese apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco como exigência para obtenção do título de Doutor.

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Orientador

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.

Resumo

O presente estudo descreve a larvicultura do molusco bivalve *Anomalocardia brasiliana,* com avaliação de diferentes dietas e densidade de estocagem. As dietas avaliadas foram: microalgas *Isochrisys galbana* (Ig), *Phaeodactylum tricornutum* (Phaeo), *Chaetoceros calcitrans* (Cca), *Pavlova lutheri* (Pl) e as combinações (Ig + Cca), (Ig + Phaeo), (Cca + Phaeo) e (Cca + Pl), totalizando oito dietas fornecidas por um período de 15 dias de cultivo. A microalga *I. galbana* fornecida isoladamente, apresentou menor sobrevivência e crescimento em relação as demais dietas testadas. O uso de *C. calcitrans* e *P. tricornutum* isoladamente ou combinada as outras microalgas apresentou melhores valores de sobrevivência e crescimento. A dieta combinada Cca + Pl, obteve maior crescimento e sobrevivência nas larvas de *A. brasiliana* (261,67 ± 9,64 µm e 31,50 ± 0,87 %). Todas as dietas avaliadas obtiveram resultados satisfatórios quanto ao crescimento e sobrevivência de larvas de *A. brasilaina*, exceto a dieta *I. galbana* quando fornecida isoladamente. As densidades de estocagem avaliadas nas pós-larvas de *Anomalocardia brasiliana* foi de 40, 80 e 160 ind.cm^{-2} em um período experimental de 28 dias. A densidade de 40 ind.cm^{-2} apresentou a maior taxa de crescimento específico diária. As maiores sobrevivências das pós-larvas foram observadas nas menores densidades 53,24 ± 4,60 % (40 ind.cm^{-2}) e 52,95 ± 3,32 % (80 ind.cm^{-2}), diferindo significativamente da maior densidade de estocagem com 31,54 ± 0,70 %. Assim pôde-se concluir que podemos produzir larvas de *A. brasiliana* utilizando qualquer uma das dietas avaliadas, exceto *I. galbana* se fornecida isoladamente. No cultivo de pós-larvas desta mesma espécie devemos realizar o manejo na densidade de estocagem no decorrer do crescimento, a densidade de 160 ind.cm^{-2} pode ser utilizada até que as larvas alcancem 600µm de comprimento, larvas maiores que 600 µm devem ser cultivadas na densidade de 40 ind.cm^{-2} para manter a taxa de crescimento máximo diária.

Palavras-chave: microalgas, densidade de estocagem, larvas, pós-larvas, manejo.

Abstract

The present study describes the larval rearing of bivalve mollusc *Anomalocardia brasiliana*, with evaluation of different diets and stocking density. The diets were evaluated: microalgae *Isochrisys galbana* (Ig), *Phaeodactylum tricornutum* (Phaeo), *Chaetoceros calcitrans* (Cca), *Pavlova lutheri* (Pl) and combinations (Ig + Cca), (Ig + Phaeo) (Cca + Phaeo) and (Cca + Pl), totaling eight diets provided for a period of 15 days of cultivation. The microalgae *I. galbana* provided alone had lower survival and growth compared with other diets tested. The use of *C. calcitrans* and *P. tricornutum* alone or combined other microalgae showed highest values for survival and growth. The combined diet Cca + Pl, demonstrated the better growth and survival of larval *A. brasiliana* (261.67 ± 9.64 µm and 31.50 ± 0.87%). All diets evaluated with satisfactory results regarding the growth and survival of larvae of *A. brasilaina* except *I. galbana* diet when given alone. The stocking densities evaluated in post-larvae *Anomalocardia brasiliana* was 40, 80 and 160 ind.cm^{-2} in a trial period of 28 days. The density of 40 ind.cm^{-2} had the highest rate of daily specific growth. The highest survival of post-larvae were observed at lower densities 53.24 ± 4.60% (40 ind.cm^{-2}) and 52.95 ± 3.32% (80 ind.cm^{-2}), differing from greater stocking density with 31.54 ± 0.70%. So we concluded that we can produce larvae of *A. brasiliana* evaluated using any of the diets, except *I. galbana* was provided separately. In cultivation post-larvae of the same species must perform storage management in density during growth, density 160 ind.cm^{-2} can be used to achieve the larvae 600 µm in length, greater than 600 µm larvae must be grown at a density of 40 ind.cm^{-2} to keep the rate of maximum growth rate.

Key words: microalgae, stocking density, larvae, post-larvae, management.

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1 - Introdução

A produção aquícola mundial baseia-se no cultivo de seis grupos de organismos aquáticos: peixes de água doce, peixes diádromos, peixes marinhos, algas, crustáceos e moluscos. Em 2012 a produção mundial de moluscos foi de 15,2 milhões de toneladas, sendo mais que o dobro da produção de crustáceos com 6,4 milhões de toneladas (FAO, 2014).

No Brasil o cultivo de moluscos é representado por três espécies, a ostra japonesa (*Crassostrea gigas*) espécie exótica, a ostra nativa (*Crassostrea brasiliana*) e a vieira (*Nodipecten nodosus*). Entretanto, existem várias outras espécies nativas que apresentam potencial para o cultivo dentre elas pode-se citar o marisco *Anomalocardia brasiliana*, sendo cultivadas espécies da mesma família (Veneridae) em outras partes do mundo e que apresentam um mercado consolidado. O maior problema, para produção em laboratório, é conseguir uma produção regular de sementes, pois pouco se conhece sobre as exigências desta espécie para determinação de uma metodologia de produção.

As Microalgas marinhas são cultivadas para serem utilizadas como alimento para as várias fases de vida dos moluscos bivalves e, até o momento, têm constituído o principal alimento de larvas e sementes de bivalves cultivados (HELM et al., 2004). Outra função do cultivo de microalgas na aquicultura é proporcionar a melhoria da qualidade da água, através da absorção de produtos nitrogenados tóxicos (amônia e nitrito) e combate as bactérias patogênicas pela sua produção de substâncias antibióticas (LAVENS e SORGELLOS, 1996; REITAN et al. 1994). As microalgas mais utilizadas nas larviculturas de moluscos bivalves são as espécies dos seguintes gêneros: *Isochrysis, Pavlova, Chaetoceros, Phaeodactylum, Skeletonema, Thalassiosira, Dunaliella, Nannochloris, Tetraselmis* e *Rhodomonas* (COUTTEAU e SORGELOOS,

1992; MOUEZA et al., 1999; BROWN, 2002; HELM e BOURNE, 2004; CRAGG, 2006; LIU et al., 2009).

A larvicultura é uma das etapas mais importantes no cultivo de organismos aquáticos, e uma alimentação nutricionalmente balanceada, trará mais resistência aos animais quando forem cultivados fora do laboratório. No entanto, uma das principais causas de mortalidade na larvicultura é o desenvolvimento da comunidade bacteriana. Por exemplo larvas de vieira, *Pecten maximus*, são suscetíveis à contaminação bacteriana causando alta mortalidade nos cultivos intensos (COMELY, 1972; NICHOLAS et al., 1996). Além disso, fatores como alta temperatura, alta densidade de estocagem e excesso de nutrientes podem favorecer a proliferação de patógenos oportunistas (ANDERSEN et al., 2000).

O efeito da densidade de estocagem no crescimento e sobrevivência de larvas, juvenis e adultos de moluscos bivalves tem sido amplamente estudado (FRÉCHETTE, 2005), principalmente para aquelas espécies com importância comercial para aquicultura, como: *Crassostrea gigas*, *Ruditapes philippinarum*, *Perna perna*. A maioria dos estudos avaliaram o período de crescimento nas fases de D véliger até a metamorfose para pé de véliger (LIU et al., 2006; YAN et al., 2006; LIU et al., 2010), pois nesta fase as larvas são frágeis e altamente susceptíveis a doenças.

O conhecimento da melhor microalga, e/ou suas combinações na alimentação de *A. brasiliana* assim como o manejo adequado é essencial para aumentar o desenvolvimento e sobrevivência de larvas e pós-larvas de marisco.

2 - Objetivos

2.1 - Objetivo geral

A presente proposta visa contribuir com o aprimoramento de tecnologias para a larvicultura do marisco *A. brasiliana* na região Nordeste do Brasil.

2.2 - Objetivo(s) específico(s)

1. Avaliar o efeito de diferentes dietas microalgais no crescimento e sobrevivência de larvas do marisco *A. brasiliana*;
2. Analisar o efeito de dietas combinadas de microalgas no crescimento e sobrevivência de pós-larvas do marisco *A. brasiliana*
3. Avaliar o efeito da densidade de estocagem no crescimento e sobrevivência de pós-larvas do marisco *A. brasiliana*;
4. Analisar o efeito da densidade de estocagem no crescimento e sobrevivência de pós-larvas do marisco *A. brasiliana*, ao longo do tempo.

3 - Revisão de literatura

Anomalocardia brasiliana pertence ao Reino Animal, Filo Mollusca, Classe Bivalvia, Subclasse Heterodonta, Infraclasse Euheterodonta, Ordem Veneroida, Superfamília Veneroidea, Família Veneridae, Gênero *Anomalocardia* e espécie *brasiliana* (Gmelin, 1791). Reúnem aproximadamente 500 espécies viventes, pertencentes à aproximadamente cinquenta gêneros e doze subfamílias (CANAPA et al., 1996). Essa diversidade está relacionada à grande variedade de habitats que estão adaptados, tais como: praias arenosas, areno-lodosas, manguezais e fundos arenosos de ambientes coralíneos (CANTERA, 1991). No Brasil foram registradas 35 espécies de venerídeos, pertencentes a quatorze gêneros e sete subfamílias (RIOS, 1994).

A. brasiliana vive em profundidades de 0,3 – 5 m, superficialmente escavado na areia próximo ao manguezal, sensíveis a variações ecológicas, com alta mortalidade devido às chuvas, causando grandes flutuações no tamanho e na distribuição das populações (MOUEZA et al,. 1999; MONTI et al., 1991). Distribui-se desde a costa das Antilhas até o Uruguai (RIOS, 1994), e o tamanho máximo já encontrado foi de 38 mm segundo dados do Malacolog version 4.1.1. (A Database of Western Atlantic Marine Mollusca). Encontra-se em maior abundância na região entre marés nos bancos naturais formados da interação dos mesmos no sedimento com uma grande variedade de bancos naturais, *micro-habitats*. É conhecido popularmente na região sudeste e sul do Brasil como berbigão, papafumo, sarnambi ou vôngoli, e na região nordeste é chamado de marisco-pedra e maçunim (OLIVEIRA et al., 2014).

A *A. brasiliana* é uma espécie dióica, ou seja, apresenta sexos separados, não apresenta características morfológicas externas (diferença na coloração ou tamanho das conchas) ou internas (diferença na coloração das gônadas) que permitam a diferenciação macroscópica dos sexos, sendo necessária análise microscópica dos gametas ou estudos

histológicos (GROTTA & LUNETTA, 1980). A Reprodução é sexuada com lançamento de gametas na água e fecundação externa.

O desenvolvimento larval da *A. brasiliana* passa pelas fases trocófora, véliger (D-véliger, umbonada e pé-de-véliger) e pós-larva ou juvenil. MOÜEZA et al (1999) relataram o surgimento de larvas trocóforas após 9 horas do início da fertilização, e após 24 h de vida já apresentavam aproximadamente 95 µm de comprimento e estavam no estágio D-véliger. A partir do quinto dia passaram para véliger com comprimento médio de 150 µm. No sétimo dia inicia a metamorfose para fase pé de véliger, sendo esta fase considerada crítica, pois as larvas são frágeis e susceptíveis a doenças durante o manejo (OLIVEIRA et al., 2007). Aos 11 dias de vida, as larvas migram para o fundo, fase pé de véliger, 180 µm de comprimento, e com 15 dias apresentando todas as características de juvenil, sifão e o pé totalmente desenvolvidos, com aproximadamente 300 µm de comprimento (MOÜEZA et al.,1999).

No habitat natural a espécie pode reproduzir o ano todo, porém apresenta picos de reprodução em algumas épocas do ano. O período reprodutivo para uma mesma espécie pode variar substancialmente de região para região, principalmente em função de diferentes condições climáticas e ambientais (LAVANDER et al., 2011). Sendo o Brasil um país com grande extensão costeira, possívelmente o padrão maturacional para *A. brasiliana* é diferenciado entre as regiões do país.

Estudos realizados sobre a biologia reprodutiva da *A. brasiliana* no Brasil identificaram que em São Paulo, Santa Catarina e no Paraná a espécie apresenta ciclo reprodutivo contínuo, com dois períodos de liberação de gametas na primavera e outono, assim como um período de repouso parcial no inverno (NARCHI, 1976; BOEHS, 2000; ARAÚJO, 2001).

No estado da Paraíba, a produção de gametas ocorre durante todo o ano, devido às condições ambientais favoráveis, temperatura constante e baixo índice de precipitação (GROTTA & LUNETTA, 1980). No Ceará foi observado dois picos reprodutivos: julho a outubro (primavera e inverno) e fevereiro a abril (verão e outono) (BARREIRA & ARAÚJO, 2005). Em Pernambuco, da mesma forma, apresenta ciclo reprodutivo contínuo, com eliminação de gametas de outubro a junho (LAVANDER et al., 2011).

Concomitantemente aos estudos de biologia reprodutiva foram realizados estudos sobre a dinâmica populacional de *A. brasiliana* na costa brasileira (PEZZUTO e ECHTERNACHT, 1999; BOEHS, 2008; RODRIGUES, 2009; EL-DEIR, 2009; OLIVEIRA et al., 2011; RODRIGUES et al., 2013). Em Pernambuco, El-deir (2009) observou grandes variações na distribuição da espécie,apresentando uma densidade media de 1348 ind.m². Para o mesmo Estado, Oliveira et al. (2011) encontraram valores médios de densidade no período de verão e inverno de 298 ind.m² e 173 ind.m² respectivamente. Esses resultados são semelhantes aos encontrados por Rodrigues et al. (2013), que também estudaram a espécie no litoral de Pernambuco e verificou que em ambiente natural a espécie pode ser encontrada em até 268 ind.m².

Os dados de dinâmica populacional e biologia reprodutiva são necessários para o estabelecimento de medidas regulatórias da atividade pesqueira. Contudo, até o momento, as medidas legais tomadas não têm apresentado um resultado satisfatório na preservação dos estoques naturais da *A. brasiliana*.

A crescente demanda por frutos do mar e a facilidade com que os estoques costeiros podem ser capturados (DAME et al., 2002) resultou em uma super exploração e colapso de algumas espécies de pesca dos ecossistemas costeiros (MORA et al., 2009; FAO, 2010). Na costa brasileira, a pesca de *A. brasiliana* é amplamente difundida e intensa, com grande importância econômica para grupos de famílias de pescadores

artesanais (OLIVEIRA et al., 2014). A coleta de *A. brasiliana* está registrada desde 1970 em Pernambuco, quando a atividade era praticada apenas por mulheres, porém ao longo dos anos, o número de pescadores do sexo masculino tem aumentado significativamente (SILVA-CAVALCANTI, 2009).

Dentre as principais espécies de moluscos, no estado de Pernambuco, o marisco *A. brasiliana* é o que mais se destaca. A produção no ano de 2007 foi de 2.479,2 t responsável por 20% da produção total de moluscos (CEPENE, 2008). É o principal recurso pesqueiro entre os bivalves, e a principal fonte de renda de marisqueiras, e teve sua exploração quase que dobrada entre os anos de 2003 a 2005 (PEZZUTO & ECHTERNACHT, 1999). Infelizmente não há dados recentes sobre a produção pesqueira de *A. brasilaiana* no estado de Pernambuco, porém as comunidades tradicionais perceberam a diminuição deste recurso tanto em relação ao tamanho quanto ao volume (SILVA-CAVALCANTI, 2009). ARRUDA-SOARES et al. (1982) recomendam a captura de espécimes de *A. brasiliana* com comprimento acima de 20 mm,neste tamanho os indivíduos já teriam alcançado um grau de desenvolvimento reprodutivo que possibilitasse a reposição e manutenção dos estoques naturais.

O declínio dos bancos naturais de moluscos bivalves por todo mundo, tornou-se um insentivo ao crescimento das larviculturas para o fornecimento de sementes (PRADO et al., 2010). As atividades básicas de qualquer larvicultura de moluscos bivalves são condicionamento e desova do estoque reprodutor, cultivo e assentamento das larvas e sementes até um tamanho aceitável, assim como a produção de grandes quantidades de alimento (microalgas) para todas as fases do ciclo de produção (HELM et al., 2004).

A microalga *Isochrysis galbana* é considerada um ótimo alimento para as larvas dos moluscos bivalves *Mercenaria mercenaria* e *Tapes semidecussata*, porém para as

ostras *Crassostrea gigas* e *Crassostrea rhizophorae* e o marisco *Donax trunculus* não foi obtido um bom desenvolvimento larval (HELM e LAING, 1987; RUIZ-AZCONA et al., 1996). Liu et al. (2009) avaliaram o efeito de dietas de microalgas no crescimento e sobrevivência de larvas e pós-larvas de *Clinocardium nuttallii*. Os autores constataram que o uso de dietas espécie-específica de *I. galbana* (TISO) e *Chaetoceros muelleri* (CM) são suficientes para proporcionar um máximo crescimento de larvas e pós-larvas de *C. nuttallii*. Entretanto o uso destas microalgas utilizadas de forma combinada apresentou um melhor crescimento na fase larval ou no início da fase pós-larval, sendo recomendado o uso desta dieta bialgal para atender as necessidades nutricionais das sementes de *C. nuttallii*.

Martinéz-Fernandez e Southgate (2007) obtiveram uma maior taxa de crescimento na fase D-larva de *Pinctada margaritifera* para aquelas cultivadas com a combinação ternária de *Pavlova* sp. / *Pavlova salina* / TISO. No entanto, esta taxa de crescimento não foi significativamente maior do que a das larvas alimentadas com a combinação binária do *Pav. salina* / TISO que tem sido usado como uma "dieta padrão" para a larvicultura de *P. margaritifera* (SOUTHGATE e BEER, 1997). Martinéz-Fernandez e Southgate (2007) sugerem que a alimentação com uma única espécie de microalga para larvas de *P. margaritifera* pode ter uma finalidade prática, durante os primeiros 10 dias de larvicultura. No entanto, a adição de uma microalga diatomácea flagelada na dieta teve um incremento na taxa de crescimento e sobrevivência das larvas umbonadas de *P. margaritifera* quando comparada às combinações sem diatomáceas.

As haptophyta da classe Pavlovophycae são microalgas flageladas com tamanho de 3-5 µm, ou seja apropriado para ingestão de larvas de moluscos (PONIS et al., 2006), são ricas em ácidos graxos polinsaturados (PUFA) (KANAZAWA et al., 1979; VOLKMAN et al., 1991) . *Pavlova lutheri* é conhecida como um excelente alimento de

larvas de *Pecten maximus* e *Pecten fumatus*, contudo para larvas de *Crassostrea gigas* apresentou baixo valor alimentar, com menor sobrevivência das larvas (HEASMAN et al., 2000; PONIS et al., 2003a,b; LAING, 2004).

A utilização de *Pavlova pinguis* e *Radomonas salina* como alimento complementar a alimentação natural de juvenis da ostra do pacífico, *Crassostrea gigas*, em uma larvicultura comercial, produz aumento de custo-benefício na produção animal (BROWN et al., 1998). Estes mesmos autores observaram que a utilização de dietas mono-específica de *Tetraselmis* sp. CS-362 e *Nannochloropsis* sp. CS-246 foram mal ingeridas, e é provável que este fator tenha causado crescimento inferior das ostras.

Rivero-Rodríguez et al. (2007) constataram que a dieta mono-específica de *Chaetoceros calcitrans* para juvenis de *Crassostrea corteziensis* foi superior a outras dietas testadas (*Tetraselmis suecica, Isochrysis galbana* clone T-iso, *Phaedoctylum tricornutum, Chaetoceros muelleri, Chaetoceros calcitran*s e suas combinações bi-algaes). No geral, os juvenis de *C. corteziensis* alimentados com as dietas com qualquer combinação de *C. calcitrans* obtiveram melhor crescimento. O contrário foi observado para as microalgas *I. galbana, T. suecica, P. tricornutum* e suas combinações bi-algais.

Estudos afirmam que o uso da microalga *P. tricornutum*, em outros bivalves, afeta o crescimento, deixando-o mais lento (EPIFANIO et al., 1981; ALBENTOSA et al., 1996; RIVERO-RODRIGUEZ et al., 2007), devido à microalga ser de difícil digestão (RIVERO-RODRIGUEZ et al., 2007), possivelmente devido a falta de triptofano na dieta (EPIFANIO et al., 1981). Tang et al. (2006) obtiveram baixo crescimento de larvas de *Meretrix meretrix* quando alimentadas com *P. tricornutum* e *Pavlova viridis*.

O sistema Europeu de cultivo berçário de larvas de vieiras, inclui a troca completa da água várias vezes durante a semana, e alimentação uma ou duas vezes ao dia

(BUESTEL et al., 1982; COCHARD e DEVAUCHELLE, 1993; TAYLOR et al., 1994), que resulta em altos valores de matéria orgânica, e um ambiente microbiano instável, o qual é comumente controlado com uso profilático de agentes antibacteriano (ROBERT et al., 1996; TORKILDSEN et al., 2000). As larviculturas de moluscos bivalves são frequentemente afetadas por surtos de doenças, como infecções bacterianas do gênero *Vibrio* (PRADO et al, 2010). Andersen et al. (2000) observaram que o uso de antibióticos pode, hipoteticamente, alterar a atividade bacteriana ou a composição das espécies no sistema, ocorrendo menor degradação ou aumento na produção de amônia.

Prado et al. (2010) observaram em sua revisão que todos os tratamentos considerados (Filtração, Radiação Ultravioleta e Quimioterapicos) tem como objetivo a eliminação completa da microbiota presente na água, entretanto populações de bactérias ou parte delas tem efeito benéfico no desenvolvimento larval de bivalves. As bactérias parecem satisfazer as exigências metabólicas, fornecendo vitaminas ou outros fatores de crescimento (PRIEUR et al., 1990).

Foram realizados diversos estudos para estabelecer um protocolo de produção de larvas de bivalves, incluindo bivalves de areia (LIU et al, 2002; LIU et al, 2006; YAN et al, 2006; LIU et al, 2008; LIU et al, 2010), mexilhões (GALLEY et al, 2010; PETTERSEN et al, 2010), ostras (RICO-VILLA et al, 2006) e vieiras (RUPP e PARSONS, 2004; GOUDA et al, 2006), pois a identificação dos fatores que influenciam no crescimento e na sobrevivência das larvas de moluscos é essencial para o sucesso da larvicultura.

Fatores como temperatura, salinidade e alimentação podem influenciar na sobrevivência, crescimento e período de metamorfose larval para assentamento na espécie *A. brasiliana.* A sobrevivência e crescimento de moluscos são afetados por parâmetros físicos como temperatura e salinidade (KINNE, 1964), assim como manejo

e alimentação (DEVAKIE & ALI, 2000; OLIVEIRA et al, 2007). Para o molusco de areia mais cultivado no mundo, *Ruditapes philippinarum*, Delgado & Camacho (2007) descreveram que o aumento da temperatura influencia grande parte dos processos fisiológicos, afetando seu crescimento. Em *A. brasiliana* a temperatura também é fator primordial para seu crescimento (BARREIRA & ARAUJO, 2005).

Outra importante variável a ser considerada, é a densidade de estocagem em larvas de moluscos bivalves. O efeito da densidade no crescimento e sobrevivência de larvas, juvenis e adultos tem sido amplamente estudado (FRÉCHETTE, 2005), principalmente nas espécies com importância comercial para aquicultura. A maioria dos estudos avaliaram o período de crescimento nas fases de D véliger até a metamorfose para pé de véliger (LIU et al., 2006; YAN et al., 2006; LIU et al., 2010), pois é considerada crítica, devido as larvas serem frágeis e susceptíveis a doenças durante o manejo.

Yan et al., (2006) avaliaram a dieta, densidade de estocagem, intensidade luminosa, filtração da água, troca de água e sedimento, tendo obtido que a densidade de 5-10 larvas.mL^{-1} mantém um crescimento normal em larvas de *Ruditapes philippinarum*. Para larvas de *Meretrix meretrix* a densidade de estocagem recomendada é de 10-20 larvas.mL^{-1} (LIU et al., 2006), enquanto que para larvas de *Clinocardium nuttallii* a densidade de estocagem deve ser igual ou inferior a 4 larval.mL^{-1} e ao se aproximar da metamorfose para a pé de véliger, reduz a densidade para 2,5 larvas.mL^{-1} (LIU et al., 2010).

Quando as larvas de moluscos bivalves realizam a metamorfose para a etapa pé de véliger, estas tornam-se mais resistentes ao manejo e passam a ser cultivadas em densidades mais elevadas, por um período maior de tempo (semanas) até alcançarem o tamanho de semente (1mm) quando poderão ser cultivadas a campo (NICOLAS e

ROBERT, 2001; EPELBAUM et al., 2011; LIU et al., 2011). Portanto avaliar a densidade de estocagem durante a fase pós-larval, tem como finalidade promover um ambiente de cultivo adequado para que estas alcancem a fase de semente em um menor tempo possível.

Para larvas de *Clinocardium nuttallii* é recomendado a densidade de estocagem de 160 ind.cm^{-2} até alcançarem 1 mm de comprimento, passando para 40 ind.cm^{-2} até 2 mm, depois 20 ind.cm^{-2} até 3 mm, finalizando com 10 ind.cm^{-2} até 4 mm (LIU et al., 2011). Resultados similares foram encontrados por Jones et al. (1993), sendo 150-200 pé de veliger.cm^{-2} de Manila clam *Tapes philippinarum* em down-wellers.

Uma larvicultura comercial tem como meta melhorar o cultivo, aumentando a produção por unidade em um volume limitado de água com o menor custo possível. Nas larviculturas de moluscos é comum utilizar os sistemas de fluxo contínuo de água, diferenciados pelo sentido da corrente, sentido de cima para baixo (Down-welling) e de baixo para cima (Up-welling). Contudo quando se utiliza unidades de pequeno volume, o sistema de cultivo fechado com circulação da água também é bastante utilizado.

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5. - Artigo científico

5. 1. - Artigo científico I

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Growth and survival of *Anomalocardia brasiliana* larvae (Bivalvia: Veneridae) fed microalgal diets

ABSTRACT. Laboratory production of bivalve molluscs depends of the use of microalgae. For this reason, the aim of this study was to evaluate the effect of using different microalgal diets on growth and survival of *Anomalocardia brasiliana* larvae for 15 days of cultivation, between the trochophore and pediveliger stages. The diets were evaluated in two separate experiments. The first experiment tested the microalgae *Isochrisys galbana* (Ig), *Phaeodactylum tricornutum* (Phaeo), *Chaetoceros calcitrans* (Cca) and the combinations (Ig + Cca), (Ig + Phaeo) and (Cca + Phaeo). The second tested the microalgae *C. calcitrans* (Cca), *Pavlova lutheri* (Pl) and the combination (Cca + Pl). The microalgae *I. galbana* when given alone had lower survival and growth when compared to other diets tested. The use of diatoms *P. tricornutum* and *C. calcitrans* in this bivalve diet alone or combined other microalgae achieved better values for survival and growth. The diet (Cca + Pl) showed higher data concerning the growth and survival of *A. brasiliana* larvae (261.67 ± 9.64 μm and 31.50 ± 0.87 %). The use of microalgae *C. calcitrans* combined with *P. lutheri* is recommended to provide a good development in *A. brasiliana* larvae. Alone, microalgae *P. lutheri* and *C. calcitrans* no showed significant difference, however, there is a synergistic effect when they are used in combination. The use of a specific diet of *P. lutheri* combined with *C. calcitrans* is recommended to provide satisfactory performance in *A. brasiliana* larvae, cultured from the trochophore stage to pediveliger.

Keywords: *Anomalocardia brasiliana* microalgae, larvae, growth, clam, diet, pediveliger.

Efecto de las dietas de microalgas en crecimiento larvario y la supervivencia de *Anomalocardia brasiliana* (Bivalvia: Veneridae)

RESUMEN. La producción de moluscos bivalvos en laboratorio depende del uso de microalgas. En este sentido, el objetivo de este estudio fue evaluar el efecto del uso de diferentes dietas de microalgas sobre crecimiento y supervivencia de larvas de *Anomalocardia brasiliana* durante los 15 días de cultivo entre las fases trocófora y pediveliger. Las dietas fueron evaluadas en dos experimentos separados. En el primer experimento, se evaluaron las microalgas *Isochrisys galbana* (Ig), *Phaeodactylum tricornutum* (Phaeo), *Chaetoceros calcitrans* (Cca) y las combinaciones (Ig + Cca), (Ig + Phaeo) y (Cca + Phaeo). En el segundo experimento, las microalgas, *C. calcitrans* (Cca), *Pavlova lutheri* (Pl) y su combinación (Cca + Pl). En el primer experimento, las dietas que contienen *C. calcitrans* mostraron la mayor longitud de la concha al final del cultivo, con una diferencia significativa entre Cca e Ig, donde la longitud promedio final fue de 251,33 ± 5,66 µm y 230,00 ± 7,42 µm, respectivamente. Para el segundo experimento, con la dieta (Cca + Pl) se obtuvieron los mejores resultados para crecimiento y sobrevivencia del cultivo de larvas de *A. brasiliana* (261,67 ± 9,64 µm y 31,50 ± 0,87 %). Cuando las microalgas se usan de manera aislada *P. lutheri y C. calcitrans* no presentan significativas diferencias. Sin embargo hay un efecto sinérgico cuando se utilizan en combinación. El uso de la dieta específica de la especie de *P. lutheri* combinada con *C. calcitrans*, se recomienda para proporcionar un rendimiento satisfactorio en las larvas de *A. brasiliana*, cultivadas a partir de la etapa trocófora paseo pediveliger.

Palabras clave: *Anomalocardia brasiliana*, microalgas, larvas, crecimiento, molusco, dieta, pediveliger

INTRODUCTION

In 2010, global production of marine molluscs reached 13.9 million tons, and 75.5% of all aquatic organisms produced by mariculture in the world were molluscs, mostly bivalves, particularly oysters, mussels, clams and cockles (FAO, 2012). In Brazil, mollusc culture is still limited to a few species including Mytilidae *Perna perna*, Ostreidae *Crassostrea gigas, Crassostrea rhizophorae* and *Crassostrea brasiliana* and in the Pectinidae family *Nodipecten nodosus*. Considering the potential of various species for cultivation, opportunity exists for the diversification of aquaculture.

Brazilian northeast region has favorable climatic conditions for the development of mollusc culture, but hatcheries have been established for few native species and are limited mainly to oysters, *C. rhizophorae* and *C. brasiliana.* There is a need to develop new technologies for aquaculture of other native species, such as the bivalve *Anomalocardia brasiliana* (Gmelin,1791).

Anomalocardia brasiliana is a resource of great importance for Brazilian coastal communities (Silva-Cavalcanti & Costa, 2009; Oliveira *et al.*, 2011). Laboratory production of its seed could be a management tool for restocking the heavily expoited populations along the Brazilian coastline, as well as for diversification of mariculture.

One of the obstacles to establish successful larval cultures is the availability of an appropriate diet (Ponis *et al.*, 2006; Liu *et al.*, 2009; Pettersen *et al.*, 2010). Microalgae have been the main food source for larvae and seeds in bivalve hatchery (Helm & Bourne, 2004). In addition, microalgae can provide improved water quality by absorbing toxic nitrogen products (ammonia and nitrite) and combating pathogenic bacteria for produce antibiotic substances (Lavens & Sorgeloos, 1996).

It is important that the microalgae are of the appropriate size to allow ingestion, and that they meet the nutritional requirements of the cultivated species. The cost of the microalgae must also be competitive with other species and be easy to produce on a commercial scale (Heasman *et al.*, 2000; Ponis *et al.*, 2008). Most microalgae used in bivalve hatcheries are species from the genera *Isochrysis, Pavlova, Chaetoceros, Phaeodactylum, Skeletonema, Thalassiosira, Dunaliella, Nannochloris, Tetraselmis* and *Rhodomonas* (Coutteau & Sorgeloos, 1992; Moueza *et al.*, 1999; Brown, 2002; Helm & Bourne, 2004; Cragg, 2006; Ponis *et al.*, 2008; Liu *et al.*, 2009).

Precise knowledge of microalgae as well as proper management is essential to provide greater shellfish larvae survival and growth. There isn’t studies on the use of

microalgae in hatcheries of this species, and the purpose of this study was to evaluate survival and growth of larvae of *A. brasiliana* when fed different microalgal diets.

MATERIALS AND METHODS

A total of 500 adults longer than 20 mm were collected on the beach of Mangue Seco (07°49' 44,19"S, 035°50' 03,06"W), Igarassu municipality, 30 km from Recife, Pernambuco state, Brazil, transported to the Laboratory of Sustainable Mariculture (LAMARSU). and acclimated during 24 h in 500 liter tanks at 25 ºC, 30 g L^{-1} salinity and 6 mg L^{-1} mean dissolved oxygen. .

After this period, they were fed twice daily a mixture of *Isochrysis galbana* and *Chaetoceros calcitrans* at a cell ratio of 1:1, with a total ration of $20x10^4$ cell. mL^{-1} .day^{-1}. In the first experiment, after 10 days, spawning occurred spontaneously, and the fertlized eggs were filtered 50 µm mesh opening and kept in a 500 L tank. Yet to the second experiment, spawns occurred on the same day that the breeding arrived at the laboratory, through the induction, release of gametes and gradual temperature increase (1 ºC.h^{-1})

After 24 hours D-veliger larvae (n = 30) had an average length of 69.94 ± 1.54 mm and were stocked with an initial density of five larvae.mL^{-1}, in triplicate for each treatment, in plastic containers with two liters of seawater (three µm filtered and UV-treated).

Temperature and salinity were measured daily, oxygen twice a day and the water of larval cultures was renewed completely every three days. Feeding was once a day, and microalgae supplied to the larvae were from three days-old cultures, in exponential growth.

The microalgae used in the experiment were obtained from LAMARSU stock strains. The seawater with salinity of 32 ± 2 g L^{-1} was filtered through paper with 1 µm pores, autoclaved, and enriched with a Conway sterilized medium (Walne, 1966), supplemented with sodium metasilicate (40 mg L^{-1}) to provide a silica source for the diatom *C. calcitrans* and *P. tricornutum*.

The effect of microalgal diets on larval growth of *A. brasiliana* was evaluated in two completely randomized experiments. The first experiment tested the microalgae *I. galbana* (Ig), *Phaeodactylum tricornutum* (Phaeo) and *Chaetoceros calcitrans* (Cca) and the combinations (Ig + Cca), (Ig + Phaeo) and (Cca + Phaeo). The larval rearing

period was 15 days, starting with the D-veliger larval stage and ending with pediveliger larvae. The total algal cell density provided was $30x10^3$ cell mL^{-1} and for bialgal diets a 1:1 ratio was used.

The second experiment evaluated the algae *C. calcitrans* (Cca), *P. lutheri* (Pl) and the combination (Cca + Pl). *C. calcitrans* was assessed again because it had the highest relative growth in the first experiment and was characterized as an excellent diet for weight gain in molluscs (Rivero-Rodriguez *et al*., 2007). The microalgae *P. lutheri* has been described in some studies (Ponis *et al*., 2006, 2008) as an excellent diet for bivalve molluscs. The methodology and algal density provided in the second experimet were the same adopted for the first experiment.

To assess larval survival at the end of experiment the entire volume of each experimental unit was filtered. The larvae were concentrated in a 50 mL container, from which one milliliter (1 mL) samples were drawn. The larval counting was done with a Sedgewick-Rafter counting chamber and optical microscope, three samples of each experimental unit were analyzed.

For the evaluation of larval growth, one milliliter samples of each experimental units were removed on the first and last day of the experiment and images of 30 larvae chosen at random were obtained using an optical microscope coupled to a camera lens, and their length (maximum anterior-posterior dimension) and width (maximum dorsal-ventral dimension) were measured using version 2.0 software ImageTool (Texas University, Health Science Center, San Antonio, USA)..

Relative growth (K) was calculated using the equation $K = (\ln L2 - \ln L1) / t$ (Walen, 1963), where *L1*, *L2* represent the lengths at the beginning and end of the experiment in µm, respectively, and *t* is the duration of the experiment in days.

Survival data, length, width and relative growth between diets, generated in both experiments, were previously checked for normality using the Kolmogorov-Smirnov test and for homogeneity of variance with Cochran's C test. Analysis of Variance (ANOVA) was used to determine the effect of diets on the growth and survival of larvae over time of cultivation. Duncan's test was performed to detect the mean levels which differed significantly between treatments. The level of significance was $P < 0.05$.

RESULTS

The variables of temperature (ºC), salinity (g L^{-1}) and dissolved oxygen (mg L^{-1}) of the water were maintained within acceptable limits for shellfish growing. The temperature ranged from 25.05 °C to 25.60 °C, salinity from 29.88 g L^{-1} to 30.18 g L^{-1} and dissolved oxygen had a minimum value of 5.89 mg L^{-1} and a maximum of 6.66 mg L^{-1}.

First Experiment

There was a significant difference in larval survival of *A. brasiliana* as assessed at the end of cultivation, between diets (ANOVA). The Phaeo diet had the highest survival of approximately 25%, which was not significantly different of Cca, Ig + Phaeo and Ig + Cca diets. The lowest survival values were found in diets of Ig and Cca + Phaeo which averaged 6.83% and 5.27%, respectively, significantly lower than the Phaeo diet (Table 1).

No significant differences in shell width was found in larvae fed with the different algal diets tested (Table 1). Significant differences were observed between the relative growth rate in the diets tested (Table 1). The Cca and Ig + Phaeo diets had higher relative growth, than Ig diet.

Larval survival obtained in the first experiment reached a maximum value of 24.83 ± 3.03 % in the Phaeo diet. The survival of larvae fed with *C. calcitrans* (12.33%) was not significantly different from other diets tested (5.2- 4.83%). Larvae fed with microalgae *I. galbana* and a mixture of *C. calcitrans* with *P. tricornutum* had the lowest survival.

By analyzing the larval length as a growth factor, it was possible to determine significant differences between the diets tested ($P < 0.05$). The Cca diet had the greatest shell length at the end of cultivation (251.33 ± 5.66 µm), differing significantly from the Ig diet, which had the lowest growth in length (230.00 ± 7.42 µm). The other tested diets showed no significant difference regarding the growth in length (Fig. 1).

The microalgae *I. galbana* when given alone had lower survival and growth when compared to other diets tested. The use of diatoms *P. tricornutum* and *C. calcitrans* in this bivalve diet alone or combined other microalgae achieved better values for survival and growth.

Insert Table 1.

Insert Figure 1

Second experiment

Larval survival of *A. brasiliana* assessed at the end of cultivation was significantly different among the three diets tested (Table 2). The Cca + Pl diet had the highest survival with a mean of 31.50%.

When analyzing the width of the shell as a growth parameter, no significant differences were found between the larvae fed with Cca + Pl and Cca diets (Table 2). The combined use of microalgae *C. calcitrans* and *P. lutheri* led to the highest growth in width after 15 days of culture with 242.00 ± 10.02 µm.

There were significant differences in the relative growth rate in larva with different diets (Table 2). The Cca + Pl diet showed higher relative growth significantly different than the Cca diet ($P < 0.05$), demonstrating its positive effect on the growth of *A. brasiliana* larvae when microalgae *C.calcitrans* and *P. lutheri* are used in combination. Confirming the positive effect observed in larval survival, with a Cca + Pl diet.

By analyzing the length of the larvae, significant differences were found between the diets tested ($P < 0.05$). The diet containing both algae *C. calcitrans* and *P. lutheri* had the highest growth in length of larvae at the end of the experiment, with a significant difference from the Cca diet (Fig. 2).

Insert Table 2.

Insert Figure 2.

DISCUSSION

The water parameters, oxygen, temperature and salinity remained within the limits recommended for bivalve culture during all the experimental period. The absorption efficiency of some microalgae species, due to the maintenance of water quality standards can explain good growth in bivalves (Grizzle *et al.*, 2001; Resgalla Jr. *et al.*, 2007).

The use of a closed recirculation system, with daily water exchange can lead to better survival of *A. brasiliana* larvae. Liu *et al.* (2009) were able to increase survival to 68.0% by using a continuous water renewal system (50% daily and 100% every three days) when *Clinocardium nutallii* larvae were fed some diet for nine days of cultivation. Thus, the low survival rate (25%) observed in *A. brasiliana* larvae may be related to the use of closed cultivation without daily renewal, but with 100% renewal every three days.

In addition to maintaining water quality, a second factor of great importance for successful development of shellfish larvae is the nutritional value of the diet. Lourenço (2006) revised the chemical composition of some marine microalgae species, *C. calcitrans* (34% protein, 16% fat and 6% carbohydrate), *P. tricornutum* (30% protein, 14% fat and 8.4% carbohydrate), *I. galbana* (29% protein, 23% fat and 12.9% carbohydrate) and *P. lutheri* (29% protein, 12% lipids and 9% carbohydrate), indicating similar chemical compositions. However, slight differences in the concentration of fatty acids, vitamins and amino acids supplied in the diet can compromise survival and larval development.

The relative growth of larvae fed with Cca and Ig + Phaeo diets were higher only than that of larvae fed an Ig diet, with no significant differences with the other diets tested. The microalga *C. calcitrans* is considered the most suitable for feeding bivalve larvae (Brown & Robet, 2002), not only because of its biochemical composition, but also because of its cell size, digestibility and absence of toxins (Pettersen *et al.*, 2010).

Studies have found that the use of the microalgae *P. tricornutum* for feeding other bivalves, causes slow growth (Epifanio *et al.*, 1981; Albentosa *et al.*, 1996; Rivero-Rodriguez *et al.*, 2007), because it is difficult to digest (Rivero-Rodriguez *et al.*, 2007), probably due to its lack of tryptophan (Epifanio *et al.*, 1981). Tang *et al.* (2006) achieved a relatively low growth in *Meretrix meretrix* larvae when fed *Phaeodactylum tricornutum* (0.0388) and *Pavlova viridis* (0.0361).

A diet composed of the microalgae *C. calcitrans* showed the longest shell at the end of the cultivation (251.33 ± 5.66 µm). Rivero-Rodriguez *et al.* (2007), evaluating the growth of *Crassostrea corteziensis* seeds found significant growth when using *C. calcitrans* alone or in combination with other diets, which was up to twice the growth when this microalga is present in the diet. This growth was affirmed to be related to the fact that *C. calcitrans* contains high levels of arachidonic acid (ARA). These authors

confirmed that the mixed use of *C.calcitrans* and *C. muelleri* is the second best diet in terms of weight gain for *C. corteziensis*. Similar results were found by Liu *et al*. (2009)

In the second experiment in this study, there was an increase in survival of *A. brasiliana* larvae when *C. calcitrans* and *P. lutheri* microalgae were used in combination, reaching average survival above 31%. Ponis *et al.* (2008), evaluating the effect of *P. lutheri* in *Crassostrea gigas* larvae obtained survival above 78% when *C. calcitrans* was added to the diet. This corroborates our findings that after 15 days of culture, larvae fed a bialgal Cc + Pl diet had better survival that significantly differed from those fed only monoalgal diets (Cca and Pl). Other studies have also achieved good results when adding other species of diatom microalgae (Epifanio, 1979; Romberger & Epifanio, 1981; Laing & Millican, 1986; O'Connor & Heasman, 1997), which has been attributed to better essential nutrient balance (Webb & Chu, 1983).

The relative growth for the Cca + Pl diet (0.0867) was higher than the other diets tested in both experiments. There was no significant difference in growth when the microalgae *P. lutheri* and *C. calcitrans*, were used singly, however there is a synergistic effect when they are used together. The final average length of *A. brasiliana* larvae fed a combination of *P. lutheri* and *C.calcitrans* was significantly higher than larvae fed only *C. calcitrans*.

Bialgal diets are often used in feeding bivalve larvae, and it is common to combine species, using a flagellate with a diatom, to maximize growth and larval development (Galley *et al.*, 2010; Liu *et al.*, 2009; Martinéz-Fernandez & Southgate, 2007). The flagellated species commonly used are *Isochrysis galbana* and *Pavlova lutheri* and the diatoms include *Chaetoceros calcitrans*. Spolaore *et al.* (2006) affirm that the combination of different algae species offers a better nutritional balance and improves animal growth compared to a monoalgal diet, which agrees with the result of this experiment, which found that the bialgal diet (Cca + Pl) led to better growth at the end of cultivation.

In hatchery of the mollusc *Pinctada margaritifera*, Martinéz-Fernandez & Southgate (2007) had a higher growth rate in the D-larvae phase when cultured with the ternary combination of *Pavlova* sp./*Pavlova salina*/*I. galbana*. However, the growth rate was not significantly higher than those of larvae fed with the binary combination of *P. salina*/*I. galbana* which has been used as a "standard diet" for *P. margaritifera* larvae (Southgate & Beer, 1997). Martinéz-Fernandez & Southgate (2007) suggest that feeding a single species of microalgae to *P. margaritifera* larvae may be more practical during

the first 10 days in a hatchery. However, the addition of diatom microalgae to the diet increased growth rate and survival in umbonate larvae of *P. margaritifera* when compared to treatments without diatoms.

Protein and vitamin content are important factors for determining the nutritional value of microalgae. Furthermore, high amounts of polyunsaturated fatty acids (eg. eicosapentaenoic [EPA], arachidonic acid [AA] and docosahexaenoic acid [DHA]), (Hemaiswarya *et al.*, 2011), can lead to better growth and survival of larvae fed with the microalgae *P. lutheri*, which is rich in DHA/EPA, and *C. calcitrans*, which is used to increase vitamin levels (Hemaiswarya *et al*., 2011).

Pavlova lutheri is used frequently in the diet of the reproductive stock of bivalves, such as oysters, clams, mussels and scallops (Hemaiswarya *et al.*, 2011). *C. calcitrans* is rich in fatty acids such as arachidonic acid (ARA) and vitamins, and is widely used in bivalve hatchery (Hemaiswarya *et al.*, 2011; Rivero-Rodriguez *et al.*, 2007).

This study found that monoalgal and bialgal diets present satisfactory results in terms of survival. Prymnesiophyceae *P. lutheri* is commonly used in aquaculture as live food for marine invertebrates (molluscs, crustaceans, zooplankton) and particularly for bivalves (larvae, juveniles and breeding stock) (Webb & Chu, 1983; Borowitzka, 1997; Wikfors & Onho, 2001; Brow, 2002; Rico-Villa *et al.*, 2006), but its use alone may present low growth when compared to use with other diatoms (Rico-Villa *et al.*, 2006; Ponis *et al.*, 2008). The microalga *C. calcitrans* is used in some studies as a reference diet that offers good results for growth and survival (Ponis *et al.*, 2006; Ponis *et al.*, 2008).

Our results confirm the potential of the microalgae *C. calcitrans* for offering good growth of mollusc larvae. In hatching the mussel *Mytilus galloprovincialis*, was observed an increase in larval mortality when a diet of *C. calcitrans* was replaced with *C. muelleri*, with a consequent decrease in DHA content in the larvae (Pettersen *et al.*, 2010).

A comparison of the results of the first and second experiment in the current study indicated that the Cca + Pl diet had the best results in both survival (31.50 ± 0.87%) and growth (261.67 ± 9.64 µm). The combination of the microalgae *C. calcitrans* and *P. lutheri* proved to be an excellent diet for the larval culture of *A. brasiliana*. Used alone, the microalgae C. calcitrans and P. lutheri showed no significant difference, however, there is a synergistic effect when they are used in combination.

The use of microalgaes *C. calcitrans* combined with *P. lutheri* can provide good growth in *Anomalocardia brasiliana* larvae. The study found that the use of bialgal diets leads to better growth and survival, and is more recommended due to better nutritional balance. There is a need for new studies to improve dietary practices and increase survival between the larval stages, trochophore and pediveliger.

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Table 1. Mean (± SE) survival, width and relative growth (K) of larvae of *A. brasiliana* fed different diets over 15 days of culture. Cca: *Chaetoceros calcitrans*, Ig: *Isochrysis galbana*; Phaeo: *Phaeodactylum tricornutum*, mixed diets Cca+Ig: *C. calcitrans* and *I. galbana*; Cca + Phaeo: *C. calcitrans* and *P. tricornutum*, and Ig + Phaeo: *I. galbana* and *P. tricornutum*. Different letters in the same column have a significant difference ($P < 0.05$).

Diets	Survival (%)	Width (µm)	K (µm.day^{-1})
Ig	6.83 ± 1.92^{b}	212.33 ± 6.79	0.0779 ± 0.0022^{b}
Cca	12.33 ± 5.84^{ab}	229.00 ± 5.19	0.0848 ± 0.0015^{a}
Phaeo	24.83 ± 3.03^{a}	222.33 ± 5.27	0.0830 ± 0.0020^{ab}
Ig + Cca	13.50 ± 4.91^{ab}	226.33 ± 5.04	0.0821 ± 0.0020^{ab}
Ig + Phaeo	18.27 ± 2.42^{ab}	226.00 ± 7.81	0.0839 ± 0.0019^{a}
Cca + Phaeo	5.27 ± 1.36^{b}	220.00 ± 6.49	0.0807 ± 0.0020^{ab}

Table 2. Mean (± SE) survival, width and relative growth (K) of larvae of *A. brasiliana* fed different diets over 15 days of culture. Cca: *Chaetoceros calcitrans*, Pl: *Pavlova lutheri*, mixed diet Cca+Pl: *C. calcitrans* and *P. lutheri*. Different letters in the same column have a significant difference ($P < 0.05$).

Diets	Survival (%)	Width (µm)	K (µm.day^{-1})
Cca	4.42 ± 1.58^{c}	211.67 ± 8.60^{b}	0.0781 ± 0.0028^{b}
Pl	16.80 ± 5.63^{b}	219.33 ± 6,69^{ab}	0.0805 ± 0.0021^{ab}
Cca+Pl	31.50 ± 0.87^{a}	242.00 ± 10.02^{a}	0.0867 ± 0.0024^{a}

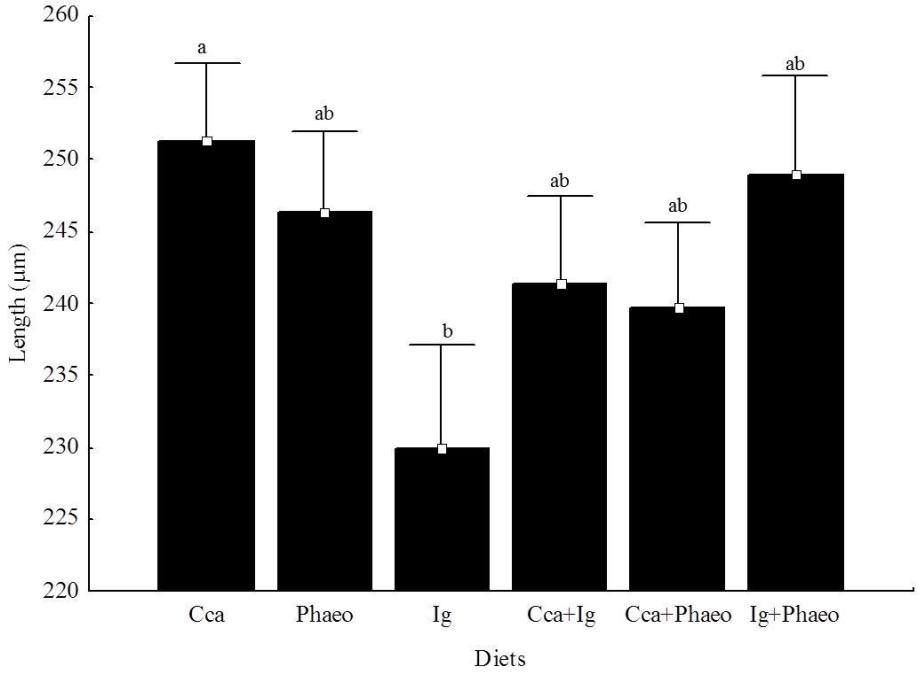

Figure 1. Mean length of larvae *A. brasiliana* fed different diets of single or mixed algae over a period of 15 days of growth. Cca: *Chaetoceros calcitrans*, Ig: *Isochrysis galbana*; Phaeo: *Phaeodactylum tricornutum*, mixed diets of Cca + Ig: *C. calcitrans* and *I. galbana*; Cca + Phaeo: *C. calcitrans* and *P. tricornutum*, and Ig+Phaeo: *I. galbana* and *P. tricornutum*. Means with different letters differ significantly ($P < 0.05$).

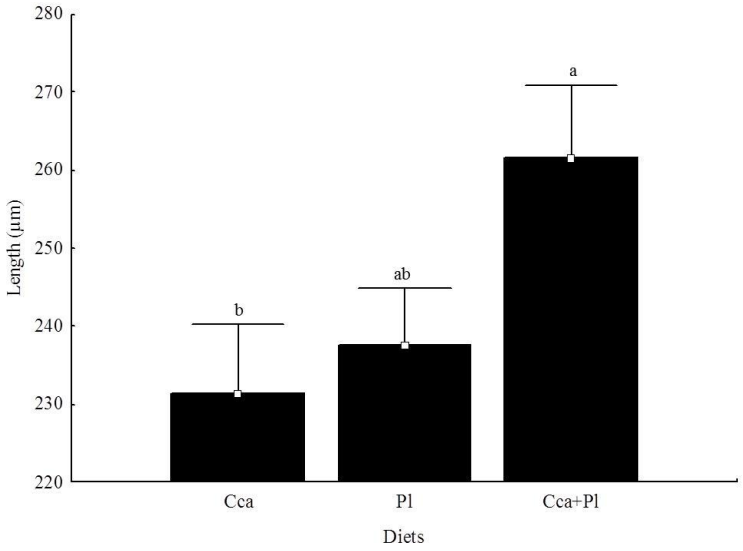

Figure 2. Mean length of larvae of *A. brasiliana* fed microalgal diets for a period of 15 days. Ig: *Isochrysis galbana*; Cca: *Chaetoceros calcitrans*; Pl: *P. lutheri*. Means with different letters have a significant difference ($P < 0.05$).

5.2. - Artigo científico II

Artigo científico a ser encaminhado a Revista Aquaculture.

Todas as normas de redação e citação, deste capítulo, atendem as estabelecidas pela referida revista (em anexo).

Efeito da densidade de estocagem no crescimento e sobrevivência de pós-larvas de *Anomalocardia brasiliana* (Bivalvia: Veneridae)

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Resumo

As pós-larvas de *Anomalocardia brasiliana* foram cultivadas por 28 dias para avaliação do efeito da densidade de estocagem no crescimento e sobrevivência, sendo alimentados com a mistura das microalgas *Pavlova lutheri* e *Chaetoceros calcitrans*. Três densidades de estocagem foram testadas 40, 80 e 160 ind.cm^{-2}, em unidades experimentais de pequeno volume (2 litros) com sistema de cultivo estático e renovação total da água feita a cada 48h. O delineamento experimental utilizado foi inteiramente casualizado com três tratamentos (densidades) e três repetições cada. Os animais cultivadas na densidade de estocagem de 40 ind.cm^{-2} alcançaram 1mm de comprimento aos 24 dias de cultivo. Ao final dos 28 dias de cultivo apenas 18% dos animais cultivadas na densidade de 80 ind.cm^{-2} apresentavam comprimento de 1mm, enquanto que o tratamento com 160 ind.cm^{-2} 100% não atingiram 1mm de comprimento. Ao avaliarmos todo o período experimental (0-28 dias) a densidade de 40 ind.cm^{-2} apresentou a maior taxa de crescimento específico diária, 4,98±0,08 %.dia^{-1}. A taxa de sobrevivência das pós-larvas para as menores densidades (40 e 80 ind.cm^{-2}) apresentaram maiores médias (53,24 ± 4,60 % e 52,95 ± 3,32 %) respectivamente, diferindo significativamente da maior densidade de estocagem com 31,54 ± 0,70 %. Na larvicultura das pós-larvas de *A. brasiliana* deve-se realizar o manejo da densidade de estocagem no decorrer do crescimento deste molusco. A densidade de 160 ind.cm^{-2} pode ser utilizada até que as pós-larvas alcancem 600µm de comprimento, pós-larvas maiores que 600 µm devem ser cultivadas na densidade de 40 larvas.cm^{-2} para manter a taxa de crescimento máximo diária.

Palavras-chave: crescimento, sobrevivência, pós-larvas, larvicultura.

1. Introdução

O marisco, *Anomalocardia brasiliana*, é um recurso pesqueiro de grande importância para os estados brasileiros, principalmente, nas comunidades costeiras da região nordeste (Nishida et al., 2004; Silva-Cavalcanti & Costa, 2009; Oliveira et al., 2011; Lavander et al., 2011; Oliveira et al., 2014). Pertence à família Veneridae, vive em profundidades de 0,5 a 1,5 m, superficialmente escavado na areia próximo ao manguezal, é sensível a variações ambientais, com alta mortalidade devido às chuvas, que causam grandes flutuações no tamanho e na distribuição das populações (Monti et al., 1991; Mouëza et al., 1999; Oliveira et al., 2014).

A região nordeste do Brasil apresenta condições climáticas favoráveis para o desenvolvimento da malacocultura, no entando para as espécies nativas ainda é pouco desenvolvida a larvicultura. A espécie *A. brasiliana* apresenta potencial para ser cultivada, contudo o estabelecimento de um protocolo de produção larval para uma nova espécie exige a avaliação de diversos fatores tais como: densidade de estocagem, qualidade e quantidade da dieta a ser fornecida, e parâmetros de qualidade da água de cultivo (temperatura e salinidade).

Foram realizados diversos estudos para estabelecimento de um protocolo de produção de larvas de moluscos, tais como: bivalves sedentários (Liu et al, 2002; Liu et al, 2006; Yan et al, 2006; Liu et al, 2008; Liu et al, 2010), mexilhões (Galley et al, 2010; Pettersen et al, 2010), ostras (Rico-Villa et al, 2006) e vieiras (Rupp e Parsons, 2004; Gouda et al, 2006). Observou-se que os fatores que influenciam o crescimento e a sobrevivência das larvas é essencial para o sucesso de uma larvicultura.

Uma importante variável a ser considerada é a densidade de estocagem, pois afeta no crescimento e sobrevivência de larvas, juvenis e adultos bivalves tem sido amplamente estudado (Fréchette, 2005), principalmente para aquelas espécies com

importância comercial para aquicultura. A maioria dos estudos avaliaram o período de crescimento nas fases de D véliger até a metamorfose para pé de véliger (Liu et al., 2006; Yan et al., 2006; Liu et al., 2010), pois são fases críticas, onde as larvas são frágeis e susceptíveis a doenças durante o manejo.

Quando as larvas realizam metamorfose para a etapa pé de véliger, ficam mais resistentes ao manejo e passam a ser cultivadas em densidades mais elevadas, por um período maior de tempo (semanas) até alcançarem o tamanho de semente (1mm) quando poderão ser transferidas para o campo (Nicolas e Robert, 2001; Epelbaum et al., 2011; Liu et al., 2011). A Avaliação da densidade de estocagem durante a fase pós-larval tem como objetivo promover um ambiente de cultivo adequado para que estas alcancem a fase de semente em um menor tempo possível.

Uma larvicultura comercial tem por meta melhorar os dados de cultivo, aumentando a produção em um volume limitado de água com o menor custo possível. Nas larviculturas de moluscos é comum utilizar os sistemas de fluxo contínuo de água, diferenciados pelo sentido da corrente, de cima para baixo (Down-welling) e de baixo para cima (Up-welling). Contudo, quando se utiliza unidades de pequeno volume, o sistema de cultivo fechado com circulação da água também é bastante utilizado.

Neste sentido, este estudo tem como objetivo avaliar o efeito da densidade de estocagem sobre o crescimento e a sobrevivência de pós-larvas de *Anomalocardia brasiliana* em um sistema de cultivo fechado.

2. Material e Métodos

Para obtenção de larvas de *A. brasiliana* foi necessário à coleta de indivíduos adultos no ambiente natural. Os reprodutores foram capturados na praia de Mangue Seco (07° 49' 44,19"S e 035° 50' 03,06"O), litoral norte do estado de Pernambuco –

Brasil. Foram coletados 500 indivíduos adultos, com comprimento médio de 24,50±2,00 mm e transportados ao Laboratório de Maricultura Sustentável (LAMARSU). No laboratório os animais foram aclimatados em tanques de 500 litros com controle das variáveis de qualidade da água, temperatura (25 ºC), salinidade (30 g.L-1) e oxigênio dissolvido médio de (6 mg.L-1). Após a identificação dos mariscos que estavam com as gônadas maduras foram realizados estímulos para desova tais como: aumento da temperatura, oferta de alimento e lançamento de gametas na água. Após estes estímulos foram obtidas as desovas. Os ovos fecundados foram filtrados através de uma malha com abertura de 50 µm e mantidos em incubadoras com 30L de volume útil por 15 dias, com troca total da água a cada 48h. Durante esse tempo as larvas foram alimentadas com uma mistura das microalgas *Pavlova lutheri* e *Chaetoceros calcitrans*, na proporção celular de 1:1 e a concentração algal foi diferenciada para cada estágio larval e está descrito na Tabela 1.

Inserir Tabela 1

Larvas pé de véliger com 15 dias de vida foram mensuradas (n=30), obtendo-se um tamanho médio de 307,89±50,92 µm de comprimento (medida entre as extremidades ântero-posterior da concha). Em seguida, foram transferidas para as unidades experimentais que eram constituídas de recipientes plásticos transparentes com dois litros de volume útil, internamente suspenso um cilindro de PVC (diâmetro 10 cm; área 78,5 cm^2), sendo na base do cilindro colocado uma malha de 250 µm ficando a uma distância de fundo de 5 cm e adicionado um airlift para circulação da água (Figura 1).

Inserir Figura 1

As pós-larvas foram cultivadas por um período de 28 dias, com avaliação de três diferentes densidades de estocagem, 40 ind.cm^{-2}, 80 ind.cm^{-2} e 160 ind.cm^{-2}, em umsistema fechado de circulação da água com troca total a cada 48 horas. A água utilizada na larvicultura foi previamente esterelizada por radiação ultravioleta, para posterior utilização nas unidades experimentais. Diariamente foram aferidas as variáveis temperatura, salinidade e oxigênio dissolvido. A qualidade da água nas unidades experimentais foi mantida dentro dos limites aceitáveis para a larvicultura de bivalves, com temperatura média de 25,89±0,50 °C, salinidade média de 28,5±0,88 g.L^{-1} e com média de oxigênio dissolvido em 5,66 ±0,55 mg.L^{-1}).

A alimentação foi realizada uma vez ao dia, com as microalgas *P. lutheri* e *C. calcitrans*, fornecidas às pós-larvas a partir do terceiro dia de cultivo algal, na fase exponencial da curva de crescimento, a uma concentração de 50x10^{3} cel.mL^{-1}. As microalgas foram obtidas do banco de cepas do LAMARSU, e mantidas em tubos de ensaio com água marinha de salinidade 32±2 g.L^{-1} e enriquecida com meio Conway esterelizado, sendo adicionado metassilicato de sódio (40 mg.L^{-1}) como fonte de sílica no cultivo da diatomácea *C. calcitrans.*

Para a avaliação do crescimento larval, foram retiradas amostras de um mililitro das unidades experimentais e com auxílio da câmara de Sedgewick-Rafter, imagens foram tomadas utilizando uma câmara fotográfica objetiva acoplada a um microscópio óptico. Foram realizadas fotografias aleatórias em 30 pós-larvas de cada amostra, a cada semana. As medidas de comprimento (máxima dimensão antero-posterior) e largura (máxima dimensão dorso-ventral) das larvas foram obtidas utilizando o software ImageTool versão 2.0 (Texas University, Health Science Center, San Antonio, USA).

Durante o experimento a taxa de crescimento das pós-larvas foram estimadas usando a seguinte fórmula:

$$TCE = 100 (\ln C2 - \ln C1) / t,$$

Onde TCE é a taxa de crescimento específico (% dia^{-1}); *C1*, *C2* representam os comprimentos no início e no fim do experimento em µm, respectivamente, e *t* é o tempo de duração do experimento (dias).

Para a avaliação da sobrevivência larval no final do cultivo, foi realizada uma filtragem de todo o volume de cada unidade experimental. As pós-larvas foram concentradas em recipientes de 50 mL, e retiradas amostras de um mililitro (mL). A contagem dos indivíduos foi realizada com auxílio da câmara Sedgewick-Rafter e microscópio óptico, e analisado três amostras de cada unidade experimental.

Os dados de sobrevivência, comprimento e crescimento das densidades de estocagem avaliadas, foram previamente checados quanto à normalidade dos dados usando o teste de Kolmogorov-Smirnov e para homogeneidade de variância com o teste de Cochran C. A Análise de Variância (ANOVA) foi usada para determinar o efeito das densidades no crescimento e sobrevivência das pós-larvas ao longo do tempo de cultivo. O teste de Tukey foi realizado para detectar qual média entre os tratamentos diferem significativamente, a nível de significância de 5% ($p < 0,05$). Os dados estão apresentados em média ± erro padrão.

3. Resultados

O comprimento médio das pós-larvas de *A. brasiliana* estocadas em diferentes densidades de estocagem, estão apresentados na Figura 2.

Ao término da primeira semana do experimento (7 dias) não foram encontradas diferenças ($p>0,05$) no comprimento dos animais entre as densidades testadas. A partir

da segunda semana o comprimento das pós-larvas foram menores a medida que se aumentou a densidade de estocagem. Aos 21 dias de cultivo não foi identificado diferença ($p>0,05$) no comprimento das pós-larvas cultivadas com as densidades 40 ind.cm^{-2} e 80 ind.cm^{-2}. As pós-larvas de *A. brasiliana* cultivadas na maior densidade de estocagem (160 ind.cm^{-2}) apresentaram menor média de comprimento ($p<0,05$), tornando-se mais evidente ao final dos 28 dias de cultivo.

As pós-larvas cultivadas na densidade de 40 ind.cm^{-2} alcançaram nos 24 dias de cultivo 1mm de comprimento, e ao final dos 28 dias do experimento apenas 18% das larvas cultivadas na densidade de 80 ind.cm^{-2} apresentavam comprimento de 1mm, enquanto que no tratamento com 160 ind.cm^{-2} não atingiram este comprimento.

Inserir Figura 2

A tabela 2 apresenta a TCE (% dia^{-1}) das três diferentes densidades de estocagem para cada período do cultivo. O rápido crescimento ocorreu para os primeiros sete dias do experimento. A menor densidade (40 ind.cm^{-2}) continuou apresentando o maior crescimento até 14 dias de cultivo, diferindo significativamente das outras densidades avaliadas ($p<0,05$). Aos 21 dias de cultivo as pós-larvas de todos os tratamentos apresentaram uma queda acentuada na taxa de crescimento (Tabela 2).

As pós-larvas cultivadas na densidade de 40 ind.cm^{-2} apresentram a maior taxa de crescimento, diferindo significativamente das outras densidades testadas. Ao avaliarmos todo o período experimental (0-28 dias) a densidade de 40 ind.cm^{-2} permaneceu com a maior taxa de crescimento específico diária.

Inserir Tabela 2

A sobrevivência larval das menores densidades (40 e 80 ind.cm^{-2}) apresentaram média de 53,24 ± 4,60 % e 52,95 ± 3,32 % respectivamente, diferindo significativamente da maior densidade de estocagem com 31,54 ± 0,70 % (Figura 3). Portanto, observa-se o efeito negativo na sobrevivência quando ocorre aumento na densidade de estocagem de pós-larvas de *A. brasiliana*.

Inserir Figura 3

4. Discussão

O alimento é um fator limitador do crescimento, de larvas de moluscos bivalves quando são cultivadas em altas densidades. Estudos evidenciam que (Loosanoff e Davis, 1963; Sprung, 1984a,b), o excesso de alimento pode causar um gasto energético com a produção de pseudofazes e outros efeitos inibitórios relacionados à fisiologia de digestão, resultando em uma diminuição do crescimento larval, além de ocasionar contaminação bacteriana e toxidez alimentar pela acumulação de ectometabolitos (Loosanoff e Davis, 1963; Sprung, 1984a,b).

Ao estudar o efeito da densidade de estocagem de uma espécie-alvo deve se garantir que o alimento não ultrapasse a concentração máxima desejável (Liu et al., 2010). No presente estudo, o residual algal foi verificado diariamente, sendo ajustado quando necessário e mantido na mesma concentração da dieta em cada tratamento. Desta forma, supõe-se que o alimento não tenha sido um fator limitante.

Altas densidades de estocagem no cultivo de larvas de bivalves podem ocasionar redução no crescimento, isto pode ser atribuído além da escassez do alimentação, também ao reduzido espaço de confinamento que causará deterioração na qualidade da

água (Reghavan e Gopinathan, 2008; Velasco e Barros, 2008). Larvas pelágicas utilizam o velum para nadar e se alimentar, logo um aumento na densidade de cultivo aumenta a possibilidade de colisão entre os indivíduos, que pode resultar numa inibição da atividade alimentar (Liu et al., 2006).

Na metamorfose de larvas pelágicas para pé de véliger, quando estas passam a ter hábitos bentônicos, há uma queda na taxa de crescimento (Liu et al., 2006). Além do fator biológico, estudos evidenciam que a área de fundo pode ser um fator limitante de densidade, para larvas com hábitos bentônicos. Em larvas pé de véliger há uma redução no crescimento quando os indivíduos ocupam 100% ou mais da área de cobertura de fundo (Liu et al., 2011). Heasman et al. (2002) observou que ocupar 70% da área de cobertura de fundo na larvicultura de juvenis de vieiras *Pecten fumatus* é um fator limitador da densidade para garantir a manutenção da máxima taxa de crescimento.

Usando a área de fundo (100%) como fator limitador da densidade para larvas pé de véliger de *Clinocardium nuttallii*, foi possível determinar que 160 ind.cm^{-2} seria o recomendado para indivíduos com até 1mm de comprimento (Liu et al., 2011). O fator área de cobertura de fundo não foi limitador de crescimento para *A. brasiliana*, pois utilizando a metodologia de Liu et al. (2011) obtivemos ao final do cultivo 26,74 %, 29,88 % e 25,29 % de cobertura de fundo para as densidades 40, 80 e 160 ind.cm^{-2}, respectivamente. Para pós-larvas de *A. brasiliana* a densidade de 160 ind.cm^{-2} seria recomendado até alcançar 600 µm de comprimento e 40 ind.cm^{-2} até alcançar 1mm, mantendo o limite máximo de crescimento.

A taxa de crescimento específico em larvas de *Meretrix meretrix* foi inferior a 2% quando estavam no período de metamorfose e assentamento, independente do densidade de estocagem (Liu et al., 2006). No cultivo de pós-larvas de *A. brasiliana*, aos 21 dias, observou-se queda na TCE (<2%). Aos 24 dias de cultivo as pós-larvas do tratamento

com menor densidade (40 ind.cm^{-2}) alcançaram 1mm de comprimento, aumentando a taxa de crescimento. O aumento na taxa de crescimento não ocorreu nas outras densidades, onde as larvas ainda não haviam alcançado 1mm de comprimento.

As pós-larvas cultivadas em altas densidades apresentaram o menor crescimento. Essa baixa taxa de crescimento diário afetou o comprimento final, pois aos 28 dias de cultivo as pós-larvas cultivadas na maior densidade apresentaram o menor comprimento.

A sobrevivência final foi menor na densidade 160 ind.cm^{-2}. A densidade de estocagem afetou diretamente na sobrevivência de pós-larvas de *A. brasiliana*. A taxa de sobrevivência de larvas de bivalves diminui continuamente ao longo do tempo, durante o desenvolvimento das fases D-véliger para pé de véliger, mesmo quando as condições de criação se mantiveram favoráveis (Gouda et al., 2006; Magnesen et al., 2006; Saucedo et al.,2007; Velasco e Barros, 2008). No cultivo de larvas de *Argopecten nucleus* há uma queda na sobrevivência quando as larvas são cultivadas em altas densidades (Velasco e Barros, 2008). Variação na taxa de sobrevivência entre 50 % e 90 % é considerada satisfatória para o cultivo de larvas de moluscos (Utting e Spencer, 1991), o que corrobora com a sobrevivência obtida para as densidades de 40 ind.cm^{-2} e 80 ind.cm^{-2}.

5. Conclusão

Na larvicultura de larvas assentadas de *A. brasiliana* deve-se realizar o manejo na densidade de estocagem no decorrer do crescimento. Para larvas pé de véliger de *A. brasiliana* a densidade de 160 larvas.cm^{-2} seria recomendado até alcançar 600 µm de comprimento e 40 larvas.cm^{-2} até alcançar 1mm, mantendo o limite máximo de crescimento.

A densidade de estocagem afeta diretamente o crescimento e a sobrevivência de larvas assentadas de *A. brasiliana*. Em um sistema de cultivo fechado a densidade de 40 larvas.cm^{-2} pode ser utilizada mantendo a taxa de crescimento específico em torno de 5% ao dia.

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Tabela 1. Concentração algal fornecida às larvas de *A. brasiliana* até atingir o estágio juvenil.

Etapas	**Dias**	**Fases**	**Concentração Algal**
1	1 - 7	D véliger / Umbonada / véliger	30.000 cél.mL^{-1}
2	8 - 12	Pé de véliger	40.000 cél.mL^{-1}
3	13 - 45	Pé de véliger / Juvenil	50.000 cél.mL^{-1}

Tabela 2. Taxa de crescimento específico de larvas pós-assentamento de *A. brasiliana* em três diferentes densidades de estocagem para cada período cultivado (dias).

	TCE (%.dia^{-1})				
Densidade de estocagem (Larvas.cm^{-2})	Período do cultivo (dias)				
	0-7	7-14	14-21	21-28	0-28
40	8,21±0,33^{a}	6,00±0,32^{a}	0,37±0,32^{b}	5,02±0,32^{a}	4,98±0,08^{a}
80	8,44±0,27^{a}	3,91±0,30^{b}	1,47±0,26^{a}	2,27±0,30^{b}	4,08±0,07^{b}
160	8,52±0,23^{a}	2,73±0,23^{c}	1,19±0,34^{ab}	0,82±0,29^{c}	3,37±0,07^{c}

Médias com caracteres diferentes diferem significativamente ($p<0,05$).

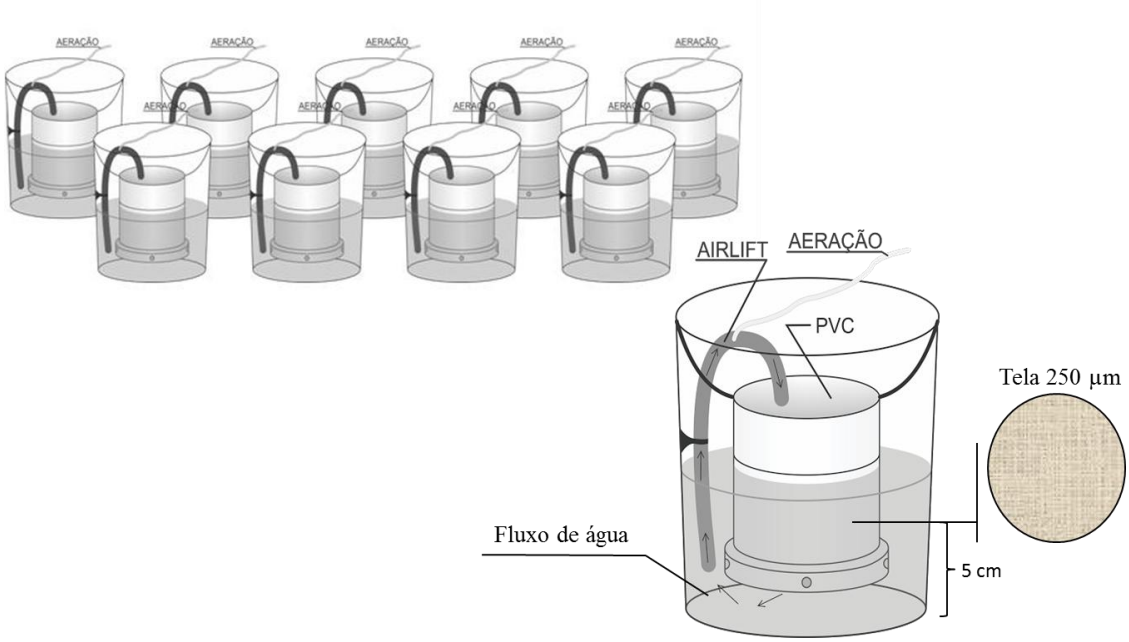

Figura 1. Desenho esquemático das unidades experimentais utilizadas para avaliação do efeito da densidade de estocagem no crescimento e sobrevivência de larvas pé de véliger de *A. brasiliana*.

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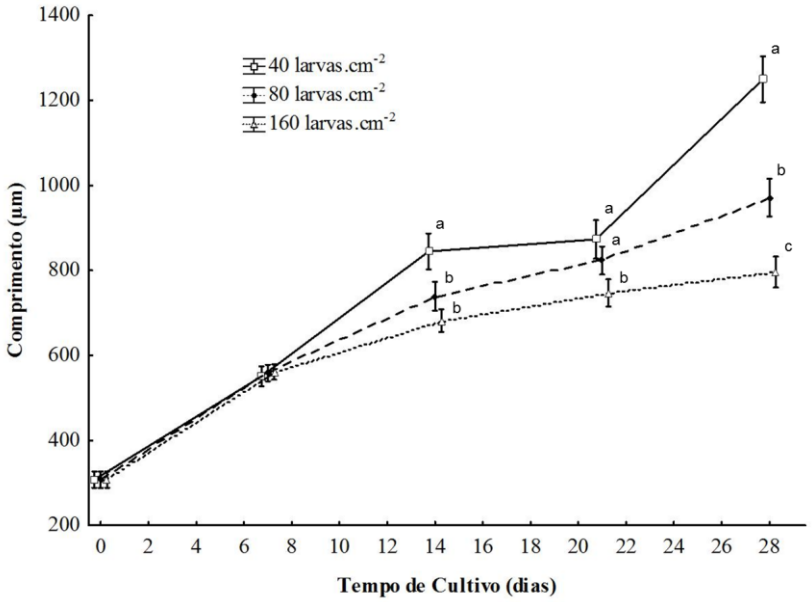

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411 Figura 2. Comprimento médio (± EP) de larvas pé de véliger de *A. brasiliana* para cada
412 período do cultivo (7, 14, 21 e 28 dias) em três diferentes densidades de estocagem.
413 Médias com letras sobrescritas diferentes diferem significativamente ($p<0,05$).

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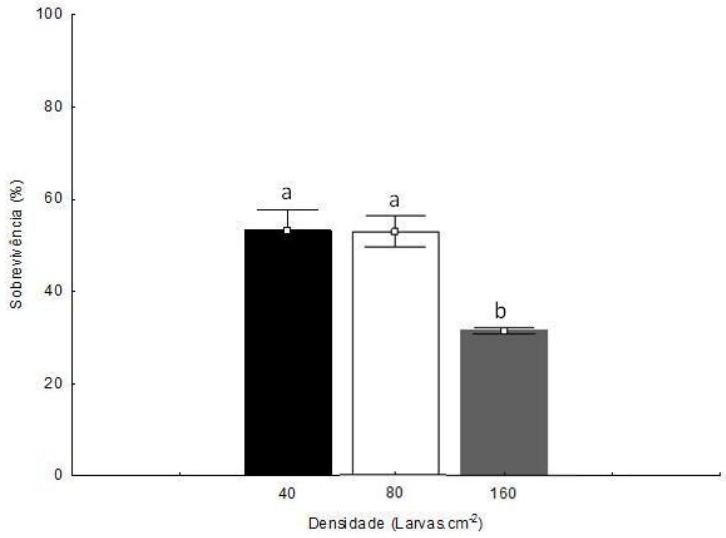

Figura 3. Sobrevivência larval de *Anomolocardia brasiliana* cultivada por 28 dias em diferentes densidades de estocagem. Médias com caracteres diferentes diferem significativamente ($p<0,05$).

6. CONSIDERAÇÕES FINAIS

A larvicultura do molusco bivalve *Anomalocardia brasiliana* pode ser realizada utilizando diversos tipos de dietas microalagais, porém a microalga *I. galbana* quando fornecida isoladamente não apresentou crescimento e sobrevivência larval satisfatório, devendo ser utilizada sempre combinada a uma outra espécie, principalmente, diatomáceas.

No cultivo de pós-larvas do molusco bivalve *A. brasiliana* deve-se considerar o manejo na densidade de estocagem no decorrer do crescimento das pós-larvas. No início da metamorfose para pé-de-véliger pode-se utilizar a densidade de 160 ind.cm² até que estes alcancem 600µm de comprimento (eixo dorso ventral), posteriormente reduzir a densidade de estocagem para 40 ind.cm² até 1mm de comprimento.

A fim de melhorar os resultados obtidos neste estudo, novos trabalhos deverão ser realizados com objetivo de determinar o melhor sistema de cultivo, aberto ou fechado com recirculação, diferentes condições de temperatura e salinidade, além do uso de probióticos na larvicultura de *A. brasiliana*.

ANEXOS

Normas da Revista Latin American Journal of Aquatic Research

Instructions for Authors

General Publishing Instructions for the Authors

Latin American Journal of Aquatic Research - LAJAR continues the work of the journal *Investigaciones Marinas* published since 1970 by the Escuela de Ciencias del Mar, Facultad de Recursos Naturales of the Pontificia Universidad Católica de Valparaíso. This journal publishes original research articles, short communications and reviews, in English or Spanish, on aquatic science and technology derived from research done in Latin America's continental and marine waters.

Decisions regarding publication are based on the opinion of anonymous reviewers. Manuscripts are also subjected to an editorial evaluation process; *LAJAR* reserves the right to edit accepted papers in order to maintain editorial standards.

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- SUBMISSION OF PAPERS
- MANUSCRIPT PREPARATION
- FIGURES AND TABLES
- REVIEW ARTICLES
- SHORT COMMUNICATIONS
- PROOFS AND REPRINTS
- PUBLICATION FEE

SUBMISSION OF PAPERS:

Papers should be submitted electronically (http://www.rlajar.equipu.cl/index.php/rlajar) as Microsoft Word files typed at space and a half; all tables and figures should be included in the same Microsoft Word file. The final version of accepted papers must be sent in Word, using Word or Excel for the tables and Corel Draw or Surfer for the figures. Figure and table captions should be sent in a separate file.

Authors should suggest at least three potential reviewers who are recognized for quality work in the field (provide names, addresses, and e-mails). Manuscripts should be written in English or Spanish, typed in Times New Roman 12 pt. The papers should be organized as follows:

Title:
Brief and descriptive, written in English or Spanish. A running head of no more than 50 characters should also be provided.

Authors:
Indicate name, last name (paternal only, when applicable), affiliation, address, and e-mail.

Abstract:
In English and Spanish, 250 words maximum, indicating the main results or findings presented in the text.

Keywords:
Maximum of six, arranged in order of importance.

- Introduction
- Material and Methods
- Results
- Discussion
- Conclusions (optional)
- Acknowledgements

References:
Indicate only the works mentioned in the text, organized alphabetically by the first author's last name. The authors' initials and last names should be written using both upper and lowercase letters. If a reference has more than one author, the second and following authors' initials should precede their last names; use a comma to separate the authors' names.

a) Entries in the reference list should follow this format: Author(s). Year of publication. Article title. Abbreviated journal name (See: Journal Title Abbreviations), Journal volume (and number in parentheses): First and last page numbers.

- Coelho,V., R.A. Cooper & S. Rodrigues. 2000. Burrow morphology and behaviour of the mud shrimp Upogebia omissa (Decapoda, Thalassinidea, Upogebiidae). Mar. Ecol. Progr. Ser., 200: 229-240.

b) Book references should indicate: Author(s). Year of publication. Book title. Editorial, City, Pages.

- Thurman, H. & A. Trujillo. 2002. Essentials of oceanography. Prentice Hall, New Jersey, 524 pp.

c) Articles published in books should indicate: Author(s). Year of publication. Article title. Editor(s). Book title. Editorial, City, Pages.

- Brummet, R.E. & B.A. Costa-Pierce. 2002. Village-based aquaculture ecosystems as a model for sustainable aquaculture development in Sub-Saharan Africa. In: B. Costa-Pierce (ed.). Ecological aquaculture: evolution of the blue revolution. Blackwell Science, Oxford, pp. 145-160.

d) Articles published on Internet should indicate: Author(s). Year of publication. Article title. Web site. Date reviewed.

- Walker, J.R.1997. MLA-Style citations of Internet sources. [http://www.cas.usf.edu/english/walker/janice.html]. Reviewed: 24 January 2008.

e) References to articles or books published in CD-Rom should indicate: author(s), year of publication, (CD-ROM), article or book titles, editorial, city.

- Retamal, M.A. 2000. (CD-ROM). Decápodos de Chile. ETI-Universidad de Concepción. Springer-Verlag, Berlin.

RESEARCH ARTICLES - MANUSCRIPT PREPARATION

1. Manuscripts, when possible, should not exceed 30 pages including tables and figures; the position of these should be indicated in the margin of the text.

2. Units should be expressed according to the Systeme International (SI). Should it be necessary to use another system, this must be explained at its first use in the paper.

3. Citations in the text should be ordered chronologically, whether for a single author, two or more authors, or several works by a given author within one year. The author's last name and year of publication should be cited (Muñoz et al., 2002; Alvarez, 2004; Johnson & Smith, 2004; Palmer, 2006a, 2006b).

4. All citations should indicate works that are published or in press. In the latter case, the work should be listed in the references, giving the author(s) name(s), article title, and journal, followed by the words (in press). Personal communications should be cited as (author, pers. comm.) and included in the text only. Example: (J. Smith, pers. comm.).

FIGURES AND TABLES

1. Graphs, maps, schemes, drawings, or photographs are referred to as figures (abbreviated in the text as Fig.). Figures (Corel Draw, Surfer) should be numbered consecutively using Arabic numerals; captions should be self-explanatory and typewritten on a separate page in English. The figures, including text (Arial Narrow) and symbols within these should not require more than three reductions in order to fit the final size; symbols should be no smaller than 1.5 mm high. Figures can be a maximum of 15 cm wide by 21 cm long (including the caption). Figures should be 1:1 and high resolution.

2. Photographs should be sent in digital format (JPG, TIFF or PNG) at 300 dpi considering an adequate range of tones and contrasts.

3. The tables (Excel or Word) are numbered consecutively with Arabic numerals. Legends should be self-explanatory and written in English. The heading of each column must clearly express its content and measurement units.

REVIEW ARTICLES

A review article is a scientific paper which provides a synthesis of current state information on specific research topic. The review consists of an abstract (maximum 200 words) and keywords written in English and Spanish, plus a continuous free-style section. The manuscript should not exceed 20 pages including the figures and tables.

SHORT COMMUNICATIONS

Short works on a specific topic that describe methods or preliminary results are published as Short Communication. These notes consist of an abstract (**maximum 200 words**) and keywords written in English and Spanish, plus a continuous single section encompassing the Introduction, Methods, Results, and Discussion; no subheadings should be used. The manuscript should not exceed nine pages including the figures and tables.

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Normas da Revista Aquaculture

Introduction

Types of paper

Original Research Papers should report the results of original research. The material should not have been previously published elsewhere. Articles are expected to contribute new information (e.g. novel methods of analysis with added new insights and impacts) to the knowledge base in the field, not just to confirm previously published work.

Review Articles can cover either narrow disciplinary subjects or broad issues requiring interdisciplinary discussion. They should provide objective critical evaluation of a defined subject. Reviews should not consist solely of a summary of published data. Evaluation of the quality of existing data, the status of knowledge, and the research required to advance knowledge of the subject are essential.

Short Communications are used to communicate results which represent a major breakthrough or startling new discovery and which should therefore be published quickly. They should not be used for preliminary results. Papers must contain sufficient data to establish that the research has achieved reliable and significant results.

Technical Papers should present new methods and procedures for either research methodology or culture-related techniques.

The Letters to the Editor section is intended to provide a forum for discussion of aquacultural science emanating from material published in the journal.

Contact details for submission

Papers for consideration should be submitted via the electronic submission system mentioned below to the appropriate Section Editor:

Nutrition:

D.M. Gatlin

The Nutrition Section welcomes high quality research papers presenting novel data as well as original reviews on various aspects of aquatic animal nutrition relevant to aquaculture. Manuscripts addressing the following areas of investigation are encouraged:

1) determination of dietary and metabolic requirements for various nutrients by representative aquatic species. Studies may include environmental/stress effects on animal's physiological responses and requirements at different developmental stages;

2) evaluation of novel or established feedstuffs as well as feed processing and manufacturing procedures with digestibility and growth trials. Such studies should provide comprehensive specifications of the process or evaluated ingredients including nutrients, potential anti-nutrients, and contaminants;

3) comparison of nutrient bioavailability from various ingredients or product forms as well as metabolic kinetics of nutrients, food borne anti-nutrients or toxins;

4) identification of key components in natural diets that influence attractability, palatability, metabolism, growth reproduction and/or immunity of cultured organisms;

5) optimization of diet formulations and feeding practices;

6) characterization of the actions of hormones, cytokines and/or components in intracellular signaling pathway(s) that influence nutrient and/or energy utilization;

7) evaluation of diet supplementation strategies to influence animal performance, metabolism, health and/or flesh quality.

Manuscripts concerning other areas of nutrition using novel or advanced methods are also welcome. Please note that in regard to various diet additives such as probiotics, prebiotics, herbal extracts, etc., a very large number of papers have already been published. Therefore, Aquaculture will not continue to accept manuscripts that present initial and preliminary investigations of such additives. Manuscripts addressing these and other feed additives will be accepted for review only if they are of the highest scientific quality and they represent a significant advance in our knowledge of the mechanisms involved in their metabolism. Manuscripts may also be considered if they present clinical efficacy data generated in large-scale trials and economic cost-benefit analysis of these applications.

Aquaculture Production Science:

B.Costa-Pierce
AQUACULTURE PRODUCTION SCIENCE (PS) is one of 5 sections of the international journal AQUACULTURE dedicated to research on improvements and innovations in aquatic food production.

This section supports worldwide dissemination of the results of innovative, globally important, scientific research on production methods for aquatic foods from fish, crustaceans, mollusks, amphibians, and all types of aquatic plants. Contributions are encouraged in the following areas: 1) Improvement of production systems that results in greater efficiencies of resource usage and sustainability of aquaculture; 2) Effective applications of technologies and methods of aquaculture production for improved stocking regimes; 3) The use of new species and species assemblages; and, 4) Investigations to minimize aquaculture wastes and improve water quality, including technologies for nutrient recycling in aquaculture ecosystems, and potential synergy of aquaculture and other food production systems using methods such as polyculture and integrated aquaculture. Aspects of seafood processing and technology will not be considered in this section although aquaculture techniques that may influence the

nutritional value of aquatic food products may be considered in the Nutrition Section.

Physiology:

Fish: A. P. (Tony) Farrell
Invertebrates: J. Benzie
The Physiology Section welcomes high quality papers that present either novel research data or original reviews. The content must be relevant to solving aquaculture problems on all aspects of the physiology of cultured aquatic animals and plants.

Submitted manuscripts must have a valid hypothesis or objective, clearly state the relevance to aquaculture, have proper experimental design with appropriate controls and utilize appropriate statistical analysis. Mention of trade names is limited to the main text.

Relevant physiological topics include, but are not limited to:

- Reproductive and endocrine physiology, including control of development and sex differentiation, induced ovulation and spermiation, gamete quality, storage and cryopreservation, physiology of gynogenetic, and triploid and transgenic organisms
- Cardiorespiratory, muscle and exercise physiology
- Osmoregulatory physiology
- Digestive physiology, including endocrine and environmental regulation of growth
- Larval physiology and ontogeny, including metamorphosis, smolting and molting
- Performance under variable culture conditions, including temperature, water quality, rearing density, and stress and disease physiology
- Physiology of harvest and handling techniques

Genetics:

G. Hulata
The Genetics Section welcomes high-quality research papers presenting novel data, as well as critical reviews, on various aspects of selective breeding, genetics and genomics. Submitted manuscripts must have a valid hypothesis or objective, clearly state the relevance to aquaculture, have proper experimental design with appropriate sample size and controls and utilize appropriate statistical analysis.

Relevant genetics topics include, but are not limited to:

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- Applications of crossbreeding and interspecific hybridization
- Evaluation of commercially important phenotypes among cultured strains, populations or stocks
- Applications of biotechnology and genetic manipulation methods
- Development of linkage maps, identification of QTL or association of commercially important traits with specific gene(s). Where appropriate, linkage maps should include co-dominant markers, such as microsatellite DNA and SNP

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Sustainability and Society:

D.C. Little
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Disease
B. Austin

The Disease sections welcomes critical reviews and high quality articles containing novel data on all aspects concerning diseases of farmed aquatic species. The aims of the section are: description of new and emerging diseases including characterization of the causal agent(s), development in the understanding of fish pathogens for example including new methods of growth where this has been a problem for fastidious organisms, pathogenicity and epizootiology, developments in the diagnosis of disease going beyond the use of standard well used methods, and methods of disease control, notably new developments in vaccines, immunostimulants, dietary supplements, medicinal plant products, probiotics, prebiotics and genetically-disease resistant stock. Relevance to aquaculture must be demonstrated. Articles, which adapt well known methods without further refinement of those methods, are unlikely to be accepted.

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