**JULIANA RANGEL DE AGUIAR INTERAMINENSE**

# CONTROLE BACTERIANO NA ECLOSÃO E ENRIQUECIMENTO DE *Artemia* sp. PARA SUA APLICAÇÃO NA ALIMENTAÇÃO DE PÓS-LARVAS DE *Litopenaeus vannamei*

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**PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQÜICULTURA**

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**Juliana Rangel de Aguiar Interaminense**

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

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## Resumo

O presente trabalho teve por princípio avaliar os efeitos antibacterianos de diferentes suplementos na eclosão e no enriquecimento de *Artemia* sp. Os suplementos foram adicionados na água utilizada para eclosão de cistos de *Artemia* capsulados e descapsulados e à água de enriquecimento de metanáuplios de *Artemia*. O experimento de eclosão consistiu no acréscimo da diatomácea *Chaetoceros calcitrans*, de probiótico comercial (*Bacillus* spp.), do antimicrobiano Florfenicol e controle sem adição de agentes. O experimento de enriquecimento foi realizado pela aplicação de *C. calcitrans*, de probiótico comercial e de emulsão comercial rica em DHA/EPA à água de cultivo de metanáuplios e controle constituído por náuplios recém eclodidos. Os metanáuplios foram oferecidos para os estágios de $PL_7$ a $PL_{19}$ de *Litopenaeus vannamei*. A carga de *Vibrio* spp. da água de eclosão e náuplios recém eclodidos foram quantificadas no final do período de eclosão. A quantificação de *Vibrio* spp de pós larvas, metanáuplios, água de cultivo das pós larvas e enriquecimento de *Artemia* também foi realizada. As colônias presuntivas de *Vibrio* oriundas de náuplios recém eclodidos foram identificadas. Além disso, *Vibrio* spp. presente em náuplios submetidos ao congelamento e colônias de *Bacillus* spp. em amostras de *Artemia* enriquecida e pós-larvas do tratamento Probiótico foram quantificadas. Avaliando dos resultados gerais do estudo, o processo de descapsulação não demonstrou ser eficiente na redução da carga de *Vibrio* spp. nos náuplios e na água de todos os tratamentos. A adição de *C. calcitrans* na água de eclosão de *Artemia* provou ser uma alternativa eficaz para em alternativa a utilização de antibióticos. A utilização de probiótico deve ser também considerado para controlar a carga de *Vibrio* spp em náuplios de *Artemia*. No entanto, a utilização de suplementos para o processo de enriquecimento de *Artemia* pode favorecer o aumento da carga bacteriana e outros procedimentos para o seu controle deve ser avaliada.



**Palavras chave:** *Artemia, Vibrio,* eclosão, enriquecimento, *Litopenaeus vannamei*.

## Abstract

This study aimed to evaluate the antibacterial effects of different supplements on *Artemia* sp. hatching and enrichment. The supplements were added to the water used for *Artemia* hatching of capsulated and decapsulated cysts and for *Artemia* enrichment water. The experiment consisted in the addition of the diatom *Chaetoceros calcitrans*, a commercial probiotic (*Bacillus* spp.), antimicrobial Florfenicol to the hatching water and control without supplements. The enrichment experiment was performed by the application of *C. calcitrans*, probiotic and commercial emulsion DHA / EPA rich to enrichment water and control constituted by newly hatched nauplii. The enriched *Artemia* were offered for the $PL_7$ to $PL_{19}$ of *Litopenaeus vannamei* stages. The *Vibrio* spp. load of hatching water and newly hatched nauplii were quantified at the end of hatching. The *Vibrio* spp. quantification of postlarvae, enriched *Artemia* and water of enrichment and postlarvae rearing was also performed. The *Vibrio* presumptive colonies isolated from newly hatched nauplii were identified. Furthermore, *Vibrio* spp. present in nauplii subjected to freezing and *Bacillus* spp. colonies of *Artemia* and postlarvae of Probiotic treatment were quantified. Assessing the overall results of the study, the decapsulation process did not shown to be effective in reducing the *Vibrio* spp load of nauplii and water in all treatments. The *C. calcitrans* addition in *Artemia* hatching water has proven to be an effective alternative to antibiotic use. The probiotic use must also be considered to control *Vibrio* spp. load in *Artemia* nauplii. However, the supplements use to *Artemia* enrichment process may promote a bacterial increase and other procedures for its control must be evaluated.

**Keywords**: *Artemia*, *Vibrio*, hatching, enrichment, *Litopenaeus vannamei*.

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## 1. Introdução

O camarão branco *Litopenaeus vannamei* é vastamente cultivado em diversas partes do mundo. Seu crescimento rápido e capacidade de cultivo com altas densidades fazem dessa espécie uma boa escolha para estratégias semi-intensivas e intensivas de aquicultura (WILLIAMS et al., 1996; PONCE-PALAFOX et al., 1997).

A maioria das fazendas de camarão marinho utilizam pós-larvas produzidas em laboratórios especializados. Apesar da tecnologia em larga escala da produção de pós-larvas já se encontrar bem desenvolvida, a intensificação das atividades de carcinicultura têm aumentado a ocorrência de doenças, as quais são consideradas como a maior causa de mortalidade nas larviculturas de camarão prejudicando a produção e o desempenho das pós-larvas no sistema de engorda (MORIARTY, 1998; GOMEZ-GIL et al., 2000; COSTA, 2006).

Em geral as larviculturas utilizam diversos tipos de alimentos, vivos e inertes, na tentativa de suprir as necessidades nutricionais das diferentes fases larvais. Apesar dos avanços tecnológicos na produção de rações específicas para larvicultura, o uso de alimento vivo ainda se faz necessário. Entre os alimentos vivos que podem ser utilizados, os principais são as microalgas (ex. *Chaetoceros* sp., *Tetraselmis* sp.) e os microcrustáceos conhecidos popularmente como artêmia (*Artemia* sp.). Ambos possuem alto valor nutritivo, suprindo as exigências nutricionais das larvas de crustáceos (SORGELOOS e LÉGER, 1992).

As vantagens da utilização dos náuplios de artêmia são inúmeras, tais como seu tamanho adequado, mobilidade, aceitação pelo predador, alto teor protéico, atração organoléptica, carapaça quitinosa fina, facilidade de armazenamento dos cistos e praticidade na sua utilização (LÉGER et al., 1987). As artêmias ainda podem ser

oferecidas vivas (recém eclodidas ou enriquecidas), congeladas ou na forma de cistos descapsulados.

O cultivo intensivo de larvas sofre por grandes mortalidades, as quais podem ser atribuídas a bactérias introduzidas no sistema de cultivo com alimentos vivos (NICOLAS et al., 1989; KESKIN et al., 1994). A alta carga orgânica associada com a produção intensiva de culturas seletivas de alimentos vivos induz a um crescimento proporcional de bactérias oportunistas que podem ser patogênicas para as larvas (SKJERMO e VADSTEIN, 1999). A desinfecção, embora benéfica, pode não prevenir a re-colonização dos alimentos vivos em um curto período de tempo (MUNRO et al., 1999).

Os antibióticos têm desempenhado um importante papel no combate de doenças de animais aquáticos cultivados. Embora uma grande variedade e número de quimioterápicos tenham sido desenvolvidos e aplicados na aqüicultura, os utilizados para a carcinicultura são limitados, pois o uso em larga escala de antibióticos tende a selecionar cepas de bactérias resistentes aos mesmos. Dessa maneira a aplicação de probióticos no cultivo de organismos aquáticos está crescendo com a demanda por mais práticas de aqüicultura sustentáveis (BOYD e MASSAAUT, 1999; ESIOBU et al., 2002; CHYTHANYA et al., 1999; ROQUE e GOMES-GIL, 2003; HOLMSTRÖM et al., 2003).

Outro método de controle bacteriano pode ser relacionado ao uso de microalgas. Segundo Kellan e Walker (1989) e Olsen et al.(2000), algumas microalgas parecem ser naturalmente bacteriostáticas ou bactericidas.

Com base nessas informações faz-se necessário a investigação de métodos para manipular e controlar a contaminação bacteriológica em alimentos vivos,

especificamente na eclosão e enriquecimento de artêmia, no intuito de reduzir a carga de bactérias nos cultivos de larvas de interesse.

## 2. Revisão de literatura

O camarão *Litopenaeus vannamei* é amplamente cultivado em países ocidentais onde 90% da produção total de camarões são desta espécie (WURMANN et al., 2004). Entre as atividades de aqüicultura que mais vêm se destacando encontra-se a carcinicultura.

Atualmente, o *L. Vannamei* também é a espécie o mais cultivada no Brasil, respondendo por mais de 95% da produção nacional. A escolha desta espécie para o cultivo foi devido principalmente a sua fácil adaptação às condições climáticas, tecnologias bem desenvolvidas, boa produtividade, grande aceitação no mercado internacional, como também maior adaptação em cativeiro (SIQUEIRA et al, 2009; ABCC, 2004; RODRIGUES, 2005).

Em 2008, a contribuição do cultivo de peneídeos para a produção mundial de crustáceos atingiu 73,3%. Além disso, o camarão continua a ser a maior commodity, única em termos de valor, contabilizando15% do valor total dos produtos da pesca comercializados a nível internacional em 2008 (FAO, 2010).

Aumentos nos cultivos de camarão têm desencadeado uma crescente demanda na produção de pós-larvas (NAEGEL e RODRÍGUEZ-ASTUDILLO, 2004). Em larviculturas comerciais, a artêmia está entre os alimentos naturais mais completos as exigências nutricionais de peixes e camarões, sendo adotado como alimento padrão (SORGELOOS et al., 1998; SORGELOOS et al., 2001).

O consumo de cistos de *Artemia* sp. no Brasil é centrado em sua quase totalidade (>95%) nos laboratórios de produção de larvas de camarão marinho. Em 2001, o Brasil produziu 7.915 bilhões de pós-larvas de *L. vannamei* e foram necessárias quatro

toneladas de cistos de *Artemia* sp. para cada bilhão de pós-larvas produzidas (YFLAAR e OLIVERA, 2003).

Por mais de 30 anos o uso de náuplios de artêmia como alimento vivo durante os estágios de desenvolvimento de Misis e pós-larval, tem sido uma prática comum em larviculturas de camarão, por causa de suas vantagens nutricionais e operacionais, além de serem amplamente reconhecidos como os melhores alimentos vivos armazenáveis avaliados (COOK e MURPHY, 1966; KUNGVANKIJ et al., 1986; LAVENS e SORGELOOS, 1986).

As pós-larvas são morfologicamente iguais a um camarão adulto e apresentam hábito bentônico. Nesta fase, em seu habitat natural apresentam hábito alimentar omnívoro, e em condições de cativeiro alimentam-se de ração e biomassa de *Artemia* adulta (TREECE e FOX, 1993). O passo inicial para o cultivo de larvas de organismos aquáticos ocorreu com a descoberta, por Seale (1933), de que náuplios de *Artemia* (Leach 1819, Crustacea, Branchiopoda, Anostraca, Artemiidae), constituem uma excelente fonte de alimento para larvas (SORGELOOS, 1980).

De acordo com Léger et al., (1986) e Sorgeloos et al., (1988), o náuplio de artêmia tem se apresentado como um dos melhores alimentos para a maioria de organismos de cultivo, pois é considerado como vetor para promover o crescimento, carregar drogas para aplicações terapêuticas (AGUILAR-ÁGUILA et al., 1994; OZKIZILCIK e CHU, 1994a; DIXON et al., 1995; TOURAKI et al., 1996; BURBOA-ZAZUETA, 1997), bem como para realizar um controle biológico em sistemas de aqüicultura (OESTMAN et al., 1995). Além disso, pode ser utilizado de diferentes formas durante uma larvicultura de camarões. Estas podem ser oferecidas como cistos descapsulados ou como náuplios congelados (SMITH et al., 1992).

O escudo exterior duro do cisto de artêmia, a camada alveolar, pode ser completamente removida através de um processo químico denominado descapsulação (SORGELOOS et al., 1983). Os cistos descapsulados podem então serem utilizados imediatamente ou desidratados em uma solução de salmoura para armazenamento, ou podem ainda serem submetidos a um processo de secagem para armazenamento a longo prazo. As vantagens da descapsulação incluem a desinfecção dos cistos, melhoria da eclosão dos cistos para obtenção de náuplios, maior conteúdo energético dos náuplios, e a isenção de risco da larva sofrer obstrução intestinal durante a ingestão do cisto (BENGTSON et al., 1991).

A artêmia congelada geralmente é utilizada em caráter de urgência, porém seu uso contínuo não só causa a deterioração da qualidade da água, mas também o costume da larva se alimentar de artêmia morta e a mesma pode não voltar a capturar presas móveis (SMITH et al., 1992). Contudo, Soares et al. (2006) em estudo realizado com *Farfantepenaeus paulensis*, indicaram que a artêmia congelada é recomendada apenas para pós-larvas com um a dez dias de idade (PL1-PL10).

Atualmente, a maioria dos cistos de *Artemia* encontrada no mercado é produzida no lago Great Salt Lake, cidade de Salt Lake, Estado de Utah, Estados Unidos (GSL), esses microcrustáceos possuem como habitat natural ecossistemas aquáticos continentais, sendo, portanto, supostamente inadequados para a alimentação de organismos marinhos (PONTES e ANDREATTA, 2003). Para suprir a deficiência de ácidos graxos altamente insaturados, característica dos cistos tipo água doce, desenvolveu-se a técnica de bioencapsulação (WATANABE et al., 1980), também conhecida como enriquecimento.

O valor nutricional dos náuplios de artêmia pode ser aumentado pela técnica de enriquecimento. A técnica explora o fato de que a artêmia é um organismo filtrador não

seletivo no segundo estágio (Instar II ou metanáuplio) de desenvolvimento, que ocorre após oito horas depois da eclosão (SORGELOOS et al., 2001). Isto permite a introdução de substâncias desejáveis nos metanáuplios, como ácidos graxos, probióticos, etc.

A maioria das bactérias associadas aos cistos de artêmia podem ser eliminadas por tratamento químico (SORGELOOS et al., 1977; GÓMEZ GIL, 1993; MERCHIE et al., 1997), no entanto larviculturas de camarão são relacionadas a mortalidades larvais com a presença de bactérias, especialmente no estágio de Zoea III, onde normalmente elas começam a serem alimentadas com artêmia (LÓPEZ-TORRES e LIZÁRRAGA-PARTIDA, 2001).

Duan et al., (1995) indicaram que bactérias produzem substâncias orgânicas que se desenvolvem em filmes em superfícies expostas à água do mar. Estes filmes são compostos por polissacarídeos, principalmente glicose e galactose (RODRÍGUEZ e BHOSLE, 1991), que podem proteger a bactéria contra lavagens e cloração das paredes dos tanques. Essas afirmações, em conjunto com a limpeza deficiente dos tanques de cultivo, podem explicar como espécies de *Vibrio* são encontradas e mantidas durante as operações de cultivo.

Espécies de *Vibrio* começam a ser dominantes depois de 24 horas, provavelmente por que durante a eclosão, os cistos de artêmia são rompidos e uma substância de reserva orgânica, glicerol, é excretada para a água de cultivo (SORGELOOS et al., 1986). Glicerol é um substrato orgânico que é utilizado eficientemente por espécies de *Vibrio* (BIANCHI, 1976).

A preocupação com os efeitos do uso de antibiótico contra enfermidades ocorrentes no ambiente aquático tem sido crescente nos últimos anos. Os antibióticos podem ser aplicados diretamente na água ou incorporados em alimentos vivos (HIRSCH et al., 1999;. SHAO, 2001). Em ambos os casos, estas substâncias ou seus

metabólitos, eventualmente, podem atingir o meio ambiente e causar efeitos adversos sobre os organismos selvagens (FERREIRA et al., 2007).

Estudos realizados apontaram que dentre os antibióticos e antimicrobianos mais comuns de uso na aquicultura, encontram-se compostos da classe das tetraciclinas, fluoroquinolonas, sulfonamidas e anfenicóis como o tianfenicol e o florfenicol (LALUMERA et al.,2004; DIETZE et al., 2005; CHRISTENSEN et al., 2006; LYLE-FRITCH et al., 2006).

Análises preliminares de uma variedade de produtos do mar têm revelado traços de cloranfenicol e nitrofuranos, que são antibióticos de amplo espectro de utilização e que apresentam alta taxa de risco de toxicidade para seres humanos. O cloranfenicol pode causar doenças potencialmente fatais como anemia e leucemia, enquanto que os nitrofuranos são carcinogênicos. O uso destes antibióticos na produção de alimentos para animais é proibido há mais de uma década na maioria dos países (GLOBAL AQUACULTURE ALLIANCE, 2002). Recentemente, a disseminação do Florfenicol tem crescido rapidamente por sua eficácia e pelo fato de que, mesmo tendo estruturas semelhantes ao cloranfenicol, não existem estudos que associem seu uso ao aparecimento de anemia (PEZZA, 2006). Segundo Roiha et al (2010), o microcrustáceo artêmia pode ser utilizado como vetor de partículas de florfenicol para estágios larvais de organismos aquáticos.

Além das vantagens da bioencapsulação, as artêmias quando ainda encistadas podem passar por um protocolo de desinfecção que é frequentemente realizado para reduzir a carga bacteriana antes da sua adição em tanques de cultivo. O protocolo de descontaminação dos náuplios geralmente envolve um ou mais agentes antimicrobianos, como desinfetantes, formaldeído, hipoclorito de sódio, peróxido de hidrogênio ou ozônio (GILMOUR et al., 1975; BENAVENTE e GATESOUPE, 1988; GOMEZ-GIL

et al., 1994; HAMEED e BALASUBRAMANIAN, 2000; GATESOUPE, 2002; TOLOMEI et al., 2004; GIMENEZ et al., 2006; SMITH e RITAR, 2006).

Um estudo realizado por Hoj et al., (2009), demonstrou que depois do tratamento de náuplios de *Artemia* com formalina, Virkon S (desinfetante), e uma mistura de antibióticos, a abundância total de bactérias cultiváveis foi reduzida para todos os náuplios de artêmia enriquecidos. Métodos moleculares mostraram, contudo, que o DNA de *Vibrio* spp. foi enriquecido depois do estágio de desinfecção. Isso demonstra que o DNA do *Vibrio* foi melhor protegido do que o DNA de outros gêneros de bactérias, sugerindo que as células do gênero *Vibrio* foram mais resistentes ao tratamento do que outras populações bacterianas. Uma alta incidência de genes multiresistentes a antibióticos na população de *Vibrio* seria uma possível explicação para esta observação. Vários autores têm relatado a existência de *Vibrios* multiresistentes a antibióticos, o que pode ser atribuído a genes presentes em plasmídios localizados no cromossomo bacteriano (MOLINA-AJA et al., 2002; AKINBOWALE et al., 2006; ROWE-MAGNUS et al., 2006; NEELA et al., 2007; LE ROUX et al., 2009).

Uma vez que os cistos de artêmia podem ser eclodidos em larviculturas sem condições estéreis, o enriquecimento alimentar de náuplios de artêmia permite a manipulação de sua composição bioquímica. Inoculando o trato digestivo dos organismos alvo a serem cultivados com bactérias probióticas, através do fornecimento de artêmia enriquecida, pode ter um efeito positivo pela melhora das propriedades da microflora indígena das larvas cultivadas. Esse efeito positivo dos probióticos pode ser atribuído a sua habilidade de competir com outras bactérias ou produzir micronutrientes importantes para o desenvolvimento das larvas (SUGITA et al., 1991; HAVENNAR et al., 1992; RINGØ et al., 1992; GATESOUPE, 1994; AUSTIN et al., 1995).

As propriedades quantitativas e qualitativas da microflora bacteriana dos alimentos vivos podem ser ajustadas para evitar efeitos negativos de uma sobrecarga de bactérias (BENAVENTE E GATESOUPE, 1988; NICOLAS et al., 1989; SKJERMO e VADSTEIN, 1993; KESKIN et al., 1994), e ao mesmo tempo realizar uma colonização bem sucedida do intestino de larvas de interesse (MUNRO et al., 1999). A incubação em curto prazo dos organismos do alimento vivo em uma suspensão bacteriana constituída por uma ou várias cepas probióticas é um possível caminho para substituir bactérias oportunistas por outras bactérias menos agressivas (REITAN et al., 1993).

Bactérias com várias características têm sido incorporadas em metanáuplios de artêmia por desafios orais (CHAIR et al., 1994; GRISEZ et al., 1996). Esta rota tem sido utilizada para vacinar alevinos de peixes e juvenis de carpas (CAMPBELL et al., 1993; JOOSTEN et al., 1995). Alguns estudos sugerem que as infecções bacterianas são iniciadas através da rota oral em larvas de peneídeos e suas pós-larvas (LAVILLA-PITOGO, 1990), portanto a adição de probióticos via alimentação pode ser um eficiente método para introduzir cepas probióticas.

O estudo da interação bacteriana com crustáceos como artêmia e camarões peneídeos é importante, pois presume-se que as bactérias proporcionam direta ou indiretamente elementos nutricionais como vitaminas, aminoácidos essenciais, ácidos graxos, poliaminas e enzimas (AUSTIN, 1988; BERGH, 1995; GRIFFITH, 1995; GOROSPE et al., 1996; VERSCHUERE et al., 2000). Douillet (1987) demonstrou que alimentos secos como farelo de arroz desengordurado, farelo de soja, *Spirulina*, e levedura Fleischmann resultaram em uma baixa ou nenhuma sobrevivência de larvas de artêmia, em condições axênicas. No entanto os mesmos alimentos resultaram em mais de 60% de sobrevivência larval, quando as culturas axênicas foram inoculadas com uma microflora selecionada.

As microalgas também compõem um papel importante ou até mesmo vital no cultivo de animais aquáticos como moluscos, camarões e peixes e têm um interesse estratégico na aqüicultura (MULLER-FEUGA, 1977). Elas são necessárias na alimentação na segunda fase de desenvolvimento larval de camarões peneídeos (Zoea), e em combinação com o zooplâncton para a terceira fase (Misis). A alimentação de larvas de interesse consiste em uma combinação de microalgas e estágios iniciais de desenvolvimento de *Artemia sp*., bem como rações comerciais específicas para cada fase de desenvolvimento. Os principais gêneros de microalgas utilizados são *Skeletonema, Chaetoceros, Tetraselmis, Chlorella, e Isochrysis* (MULLER-FEUGA, 2000). Dentre essas linhagens de microalgas, a espécie *C. calcitrans* é uma das mais adequadas para alimentar artêmia por causa de seu tamanho apropriado, digestibilidade, ausência de toxinas, e valor nutricional (KHOI et al., 2009).

Os benefícios nutricionais das algas e seu potencial como agentes biocontroladores na aquicultura têm sido reconhecidos. Kogure et al. (1979), por exemplo, demonstraram que espécies de *Pseudomonas* e *Vibrio* em cultura mista foram inibidos pela Bacillariophyta marinha *Skeletonema costatum.* Do mesmo modo, Austin et al. (1992) observaram que vários patógenos foram inibidos pelo sobrenadante e extratos de células de *Tetraselmis suecica.*

Os efeitos biológicos já descritos, para as microalgas abrangem atividades imunomoduloras, antivirais, antitumorais, antibacterianas e anticoagulantes, entre outras. Essas atividades benéficas são atribuídas às macromoléculas de polissacarídeos encontrados nas microalgas como material de reserva (BOHN e BeMILLER, 1995; SPOLAORE, 2006). Além disso, as microalgas quando participam do processo de enriquecimento de artêmia podem melhorar o valor nutritivo desse alimento vivo

através do fornecimento de ácidos graxos altamente insaturados (HUFAs) (LÉGER et al.,1986).

Como os náuplios de artêmia oriundos de fontes continentais não possuem ácidos graxos poliinsaturados de cadeia longa, a sua suplementação com emulsões lipídicas, ou microalgas com alto teor de ácidos graxos poliinsaturados através do processo de enriquecimento é recomendada (SORGELOOS et al., 2001).

Do ponto de vista energético, os lipídeos constituem a mais rica classe de nutrientes, por serem importantes fornecedores de energia metabólica, e possuírem valores superiores de energia bruta. Além de atuarem como fonte de ácidos graxos e outras classes de lipídeos essenciais, como os fosfolipídeos, também são importantes na absorção das vitaminas lipossolúveis A, D, E e K, constituem parte da estrutura da membrana celular, além de serem precursores de hormônios esteróides (TACON, 1987), desempenhando importante papel no metabolismo intermediário e na reprodução de organismos aquáticos (CAMARA, 1994).

O uso de lipossomos como produtos de enriquecimento podem fornecer diferentes vantagens e possibilidades (HONTORIA et al., 1994; OZKIZILCIK e CHU 1994b; TOURAKI et al., 1995; TONHEIM et al., 2000). Os lipossomas são partículas discretas com um tamanho adequado para os náuplios filtrarem. É possível encapsular substâncias hidrossolúveis na fase aquosa entre a bicamada lipídica destas vesículas, bem como moléculas hidrofóbicas na fracção de hidrocarbonetos da cadeia dos fosfolipídios. Além disso, os lipídios polares que formam os lipossomas também podem ser facilmente introduzidos nas cadeias tróficas utilizadas na larvicultura (MONROIG et al., 2003).

Estas características permitem o encapsulamento em vesículas lipídicas de diferentes nutrientes de especial importância para o bom desenvolvimento das larvas

marinhas. Alguns exemplos de tais nutrientes são as vitaminas solúveis (MERCHIE et al., 1997) e fosfolípideos (COUTTEAU et al., 1997).

De acordo com Tolomei et al. (2004), a prática corrente para a realização do enriquecimento de artêmia durante seu processo de crescimento pode envolver a combinação de diatomáceas e emulsões comerciais (ex. Selco, INVE®). O enriquecimento de artêmia com preparações comerciais como Super Selco, DHA Selco, DHA Protein Selco, DC DHA Selco, Algamac 3050 e óleos marinhos que são ricos em HUFAs, aprimora o valor nutricional para o desenvolvimento larval (HAI et al, 2011). Ainda, segundo Tolomei et AL. (2004), componentes do A1 DHA Selco conseguem inibir o crescimento de bactérias durante o enriquecimento assim como outros tipos de emulsões avaliadas comercialmente.

## 3. Referências bibliográficas

AGUILAR-ÁGUILA, A.; TEJEDA-MANSIR, A.; RUIZ-MANR´IQUEZ, A. Using brine shrimp as a drug carrier for therapeutic applications in aquaculture. **Aquacult. Eng.,** v.13, p.301–309, 1994.

AKINBOWALE, O.L.; PENG, H.; BARTON, M.D. Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. **J. Appl. Microbiol.**, v.100, p.1103–1113, 2006.

ASSOCIAÇÃO BRASILEIRA DE CRIADORES DE CAMARÃO. Camarão à brasileira: censo 2003. Panorama da Aquicultura, v. 4, n. 82, p. 21-25, 2004.

AUSTIN, B. Marine Microbiology Cambridge Univ. Press, Great Britain, 1988. 222 pp.

AUSTIN, B.; BAUDET, E.; STOBIE, M. Inhibition of bacterial fish pathogens by *Tetraselmis suecica*. **J. Fish Dis.** v.15, p.55–61, 1992.

AUSTIN, B.; STUCKEY, L.F.; ROBERTSON, P.A.W.; EFFENDI, I.; GRIFFITH, D.R.W. A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida*, *Vibrio anguillarum* and *Vibrio ordalii*. **J. Fish Dis.** v.18, p.93–96, 1995.

BENAVENTE, G.P.;GATESOUPE, F.J. Bacteria associated with cultured rotifers and *Artemia* are detrimental to larval turbot, *Scophthalmus maximus* L. **Aquac. Eng.,** v.7, p.289–293, 1988.

BENGTSON, D.A.; LEGER, P.; SORGELOOS, P. Use of *Artemia* as a food source for aquaculture. In: *Artemia* biology (ed. by R. A. Browne, P. Sorgeloos & C.N.A. Trotman), CRC Press, Boca Raton, FL, USA. 1991. p. 255-285.

BERGH, O. The role of bacteria in marine fish larviculture. In: LAVENS, P., JASPERS, E., ROELANTS, I. (Eds.), Larvi'95-Fish and Shellfish Larviculture Symposium. European Aquaculture Society, Special Publication, vol. 24. European Aquaculture Society, Gent, Belgium, 1995. p. 491.

BIANCHI, M. Etude taxonomique et distribution écologique des bactéries vibrioıdes du milieu marin. 1976. 126 p. **Thése** Dr. Sc. Nat. Université Aix-Marseille II.

BOHN, J.A.; BeMILLER, J.N. (1-3)-β-D-Glucans as biological response modifiers: a review of structure-functional activity relationships. **Carbohydrate Polymers**. v. 28, p. 3-14, 1995.

BOYD, C.E., MASSAAUT, L. Risks associated with the use of chemicals in pond aquaculture. **Aquac. Eng.** v.20, p.113–132, 1999.

BURBOA-ZAZUETA, M.G. Efecto de la vitamina C bioencapsulada en *Artemia* sobre camarón Blanco *Penaeus vannamei* (Boone, 1931) durante el cultivo larvario. 1997. 66 p. **MS thesis**, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE)., Ensenada, México.

CÂMARA, M.R. Dietary phosphatidylcoline requirements of *Penaeus japonicus* BATE and *Penaeus vannamei* BOONE (Crustacea, Decapoda, Penaeidae). 1994. Gent: Universiteit Gent, 173p. **Tese** (Ph.D. in Applied Biological Sciences) - Faculty of Agricultural and Applied Sciences, Universiteit Gent, Belgium.

CAMPBELL, R.; ADAMS, A.; TATNER, M. F.; CHAIR, M.; SORGELOOS, P. Uptake of *Vibrio anguillarum* vaccine by *Artemia salina* as a potential oral delivery system to fish fry. **Fish Shellfish Immunol.,** v.3, p.451–459, 1993.

CHAIR, M.; DEHASQUE, M.; VAN POUCKE, S.; NELIS, H.; SORGELOOS, P.; LEENHEER, A. P. An oral challenge for turbot larvae with *Vibrio anguillarum*. **Aquac. Int.**, v.2, p.270–272, 1994.

CHRISTENSEN, A.M., INGERSLEV, F., BAUN, A. Ecotoxicity of mixtures of antibiotics used in aquacultures. **Environ. Tox. Chemistry** v.8, p. 2208-2215.

CHYTHANYA, R.; NAYAK, D. K.; VENUGOPAL, M. N. Antibiotic resistance in aquaculture. **Infofish International**, v. 6, p. 30-32, 1999.

COOK H.L.; MURPHY A. Rearing penaeid shrimp from eggs to post larvae. In: Proc. East Ass. Fish Comm. 19 Annual Conf., 1966. p. 283–288.

COSTA, R.A. Pesquisa de *Vibrio* no cultivo do camarão marinho *Litopenaeus vannamei* no estado do ceará. 2006. 101p. **Dissertação (mestrado)** – Universidade Federal do Ceará, Ceará.

COUTTEAU, P.; GEURDEN, I.; CAMARA, M.R.; BERGOT, P.; SORGELOOS P. Review on the dietary effects of phospholipids in fish and crustacean larviculture. **Aquaculture,** v.155, p.149–164., 1997.

DIETZE, J. E.; SCRIBNER, E. Occurrence of antibiotics in water from 13 fish hatcheries, 2001–2003, International Journal of Environmental Analytical Chemistry, v. 85, p. 1141-1152, 2005.

DIXON, B.A., VANPOUCKE, S.O., CHAIR, M., DEHASQUE, M., NELIS, H.J., SORGELOOS, P., DELEENHEER, A.P. Biocapsulation of the antibacterial drug sarafloxacin in naupliii of brine shrimp *Artemia franciscana*. **J. Aquat. Anim. Health,** v.7, p.42–45, 1995

DOUILLET, P. Effect of the bacteria on the nutrition of the brine shrimp *Artemia* fed on the dried diets. In: SORGELOOS, P., BENGTSON, D.A., DECLEIR, W., JASPERS, E. (Eds.), *Artemia* Research and its Applications. Ecology, Culturing, Use in Aquaculture, vol. 3. Universa Press, Wetteren, Belgium, 1987, p. 295– 308.

DUAN, D., XU, L., FEI, X., XU, H. Marine organisms attached to seaweed surfaces in Jiaozhou Bay, China. **World J. Microbiol. Biotechnol.,** v.11, p.351–352, 1995.

ESIOBU, N., ARMENTA, L., IKE, J. Antibiotic resistance in soil and water environments. **Int. J. Environ. Health Res.** v.12, p.133–144, 2002.

FAO. **SOFIA** .The state of fisheries and aquaculture, Roma, 2010. 218p.

FERREIRA, C. F. G.; NUNES, B.A.; HENRIQUES-ALMEIDA, J.M.M.; GULHERMINO, L. Acute toxicity of oxytetracycline and florfenicol to the microalgae *Tetraselmis chuii* and to the crustacean *Artemia parthenogenetica*. **Ecotoxicology and Environmental Safety.** v. 67, p.452-458, 2007.

GATESOUPE, F.J. Lactic acid bacteria increase the resistance of turbot larvae, *Scophthalmus maximus*, against pathogenic *Vibrio*. **Aquat. Living Resour.,** v.7, p.277–282, 1994.

GATESOUPE, F.J. Probiotic and formaldehyde treatments of *Artemia* nauplii as food for larval pollack, *Pollachius pollachius*. **Aquaculture,** v.212, p.347–360, 2002.

GILMOUR, A., MCCALLUM, M.F., ALLAN, M.C. Antibiotic sensitivity of bacteria isolated from canned eggs of Californian brine shrimp (*Artemia salina*). **Aquaculture,** v.6, p.221–231, 1975.

GIMENEZ, G., PADROS, F., ROQUE, A., ESTEVEZ, A., FURONES, D. Bacterial load reduction of live prey for fish larval feeding using Ox-Aquaculture©. **Aquac. Res**, v.37, p.1130–1139, 2006.

GLOBAL AQUACULTURE ALLIANCE. Proposta de estratégia setorial sobre resíduos de antibióticos no camarão de cultivo. In: European Seafood Exhibition. Bruxelas, Bélgica: GAA, abr. 2002.

GOROSPE, J.N., NAKAMURA, K., ABE, M., HIGASHI, S. Nutritional contribution of *Pseudomonas sp*. in *Artemia* culture. **Fish. Sci.**, v.62, p.914– 918, 1996.

GOMEZ-GIL, B., ROQUE, A., TURNBULL, J.F. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. **Aquaculture** v.191, p.259–270, 2000.

GÓMEZ GIL, R.S.B. Estudio bacteriológico de quistes y nauplios de la población de *Artemia franciscana* (Kellogg 1906) del Gran Lago Salado. 1993. 75 p. **MS thesis**, Universidad Nacional Autónoma de México. México.

GOMEZ-GIL, B., ABREU-GROBOIS, F.A., ROMERO-JARERO, J., DE LOS HERRERA-VEGA, M. Chemical disinfection of *Artemia* nauplii. **J. World Aquac. Soc.**, v.25, p.579–583, 1994.

GREENBERG, A.E.; CLESCERI, L.S.; EATON, A.D. Standard Methods for the examination of water and wastewater (18th Ed.). Washington, USA: American Public Health Association, American water works Association and water Pollution Control Federation. 1992.

GRIFFITH, D.R.W. Microbiology and the role of probiotics in ecuadorian shrimp hatcheries. In: LAVENS, P., JASPERS, E., ROELANTS, I. (Eds.), Larvi'95-Fish and Shellfish Larviculture Symposium. European Aquaculture Society, Special Publication, vol. 24. European Aquaculture Society, Gent, Belgium, 1995, p. 478.

GRISEZ, L., M. CHAIR, P. SORGELOOS, AND F. OLLEVIER. Mode of infection and spread of *Vibrio anguillarum* in turbot *Scophthalmus maximus* larvae after oral challenge through live feed. **Dis. Aquat. Org**., v.26, p.181–187, 1996.

HAI, N.V., FOTEDAR, R., HAO, N. V. Penaeid Prawns. In: FOTEDAR, R., PHILIPS, B. (Eds), Recent advances and new species in aquaculture. Willey-Blackwell. UK, 2011, p. 142.

HAMEED, A.S.S., BALASUBRAMANIAN, G. Antibiotic resistance in bacteria isolated from *Artemia* nauplii and efficacy of formaldehyde to control bacterial load. **Aquaculture,** v.183, p.195–205, 2000.

HAMILTON, M.A.; RUSSO, R.S.; THURSTON, R.V. Trimmed Spearman Karber Method for estimating median lethal concentrations in toxicity bioassays. **Enviromental Sciense Technology,** v.11, p.714-719, 1977.

HAVENNAR, R., TEN BRINK, B., HUIS IN'T VELD, J.H.J. Selection of strains for probiotic use. In: FULLER, R. (Ed.), Probiotics, The Scientific Basis. Chapman & Hall, London, 1992. p. 209–224.

HIRSCH, R., TERNES, T., HABERER, K., HABERER, K., KRATZ, K. L. Occurrence of antibiotics in the aquatic environment. **Sci. Total Environ**., v. 225, p. 109-118, 1999.

HØJ, L.; BOURNE, D. G.; HALL, M. R. Localization, abundance and community structure of bacteria associated with *Artemia*: Effects of nauplii enrichment and antimicrobial treatment. **Aquaculture,** v.293, p.278–285, 2009.

HOLMSTRÖM, K.; GRASLUND, S.; WAHLSTROM, A.; POUNGSHOMPOO, S.; BENGTSSON, B.E.; KAUTSKY, N. Antibiotic use in shrimp farming and implications for environmental impacts and human health. **International Journal of Food Science and Technology,** v.38, p. 255-266, 2003.

HONTORIA, F.; CROWE, J.H.; CROWE, L.M.; AMAT, F. Potential use of liposomes in larviculture as a delivery system through *Artemia* nauplii. **Aquaculture,** v.127, p.255–264, 1994.

JOOSTEN, P. H. M.; AVILES-TRIGUEROS, M.; SORGELOOS, P.; ROMBOUT, J. H. W. M. Oral vaccination of the juvenile carp (*Cyprinus carpio*) and gilthead seabream (*Sparus aurata*) with bioencapsulated *Vibrio anguillarum* bacterin**. Fish Shellfish Immunol.,** v.5, p.289–299, 1995.

KELLAM, S.J., WALKER, J.M. Antibacterial activity from marine microalgae in laboratory culture. **Br. Phycol. J.** v.24, p.191–194, 1989.

KESKIN, M., KESKIN, M., ROSENTHAL, H. Pathways of bacterial contamination during egg incubation and larval rearing of turbot, *Scophthalmus maximus*. **J. Appl. Ichthyol.** v.10, p.1–9, 1994.

KOGURE, K., SIMIDU, U., TAGA, N. Effect of *Skeletonema costatum* (Grev.) Cleve on the growth of marine bacteria. **J. Exp. Mar. Biol. Ecol.** v.36, p.201– 215, 1979.

KUNGVANKIJ, P., TIRO, L.B.JR., PUDADERA, B.J.JR., POTESTAS, I.O., CORRE, K.G., BORLONGAN, E., TALEAN, G.A., BUSTILO, L.F., TECH, E.T., UNGGUI, A.; CHUA, T.E. Shrimp Hatchery Design, Operation and Management. NACA Training Manual Series 1, 1986. 88pp.

LALUMERA, G. M.; CALAMARI, D.; GALLI, P.; CASTIGLIONI, S.; CROSA, G.; FANELLI, R. Preliminary investigation on the environmental occurence and effects of antibiotics used in aquaculture in Italy. **Chemosphere,** v. 54, p. 661-668, 2004

LAVENS, P.; SORGELOOS, P. Manual on the production and use of live food for aquaculture. FAO Fish. Tech. Pap. 361, 1986. 295 p.

LAVILLA-PITOGO, C. R., BATICADOS, M. C. L.; CRUZ-LACIERDA, E. R.; DE LA PENA, L. D. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. **Aquaculture,** v.91, p.1–13, 1990.

LÉGER, P., BENGTSON, D.A.A., SIMPSON, K.L., SORGELOOS, P. The use and nutritional value of *Artemia* as food source. **Oceanogr. Mar. Biol.**, v.24, p.521–623, 1986.

LÉGER, P.; BENGTON, D.A.; SORGELOOS, P.; SIMPSON, K.L.; BECK, A.D. Requirements of food for larval organisms. **Artemia research and its applications**, v. 3,p.357-372, 1987.

LE ROUX, F., ZOUINE,M., CHAKROUN, N., BINESSE, J., SAULNIER, D., BOUCHIER, C., ZIDANE, N.,MA, L., RUSNIOK, C., LAJUS, A., BUCHRIESER, C., MÉDIGUE, C., POLZ, M.F., MAZEL, D., 2009. Genome sequence of *Vibrio splendidus*: an abundant planctonic marine species with a large genotypic diversity. Environ. Microbiol. doi:10.1111/j.1462-2920.2009.01918.x.

LIM, L.C.; CHO, Y.L.; DHERT, P.; WONG, C.C; NELIS, H.; SORGELOOS, P. Use of decapsulated *Artemia* cysts in ornamental fish culture. **Aquaculture Research**., v.33, p.575-589, 2002.

LÓPEZ-TORRES, M.A.; LIZÁRRAGA-PARTIDA, M.L. Bacteria isolated on TCBS media associated with hatched *Artemia* cysts of commercial brands. **Aquaculture,** v.194, p.11–20, 2001.

LYLE-FRITCH, L. P.; ROMERO-BELTRÁN, E.; PÁEZ-OSUNA, F. A survey on use of chemical and biological products for shrimp farming in Sinaloa (NW Mexico). **Aquacultural Engineering**, v. 35, p. 103-146, 2006.

MERCHIE, G., DEHASQUE, M., SORGELOOS, P. The use of DC *Artemia* minimize the risk of introducing potentially pathogenic microorganisms in shrimp and fish larviculture. In: Abstracts of the II Asia-Pacific Marine Biotechnology Conference and 8th Asia-Pacific Conference on Algal Biotechnology, May, Thailand, 1997, p. 10.

MERCHIE, G.; LAVENS, P.; RADULL, J.; NELIS, H.; DE LEENHEER, A.; SORGELOOS, P. Evaluation of vitamin C – enriched *Artemia* nauplii for larvae of the giant freshwater prawn. **Aquaculture International**, v.3, p.355-363, 1995.

MERCHIE, G.; LAVENS, P.; SORGELOOS, P. Optimization of dietary vitamin C in fish and crustacean larvae: a review. **Aquaculture,** v.155, p.165–188, 1997.

MOLINA-AJA, A., GARCIA-GASCA, A., ABREU-GROBOIS, A., BOLAN-MEJIA, C., ROQUE, A., GOMEZ- GIL, B. Plasmid proling and antibiotic resistance of

*Vibrio* strains isolated from cultured penaeid shrimp. **FEMS Microbiol. Lett.**, v.213, p.7–12, 2002.

MONROIG, O.; NAVARRO, J.C.; AMAT, I.; GONZÁLEZ, P.; AMAT, F.; HONTORIA, F. Enrichment of Artemia nauplii in PUFA, phospholipids , and water- soluble nutrients using liposomes. **Aquaculture International,** v.11, p.151–161, 2003.

MORIARTY, D.J.W. Control of luminous *Vibrio* species in penaeid aquaculture ponds. **Aquaculture** v.164, p.351-358, 1998.

MULLER-FEUGA, A. Microalgues Marines, Les Enjeux de la Recherche. Ifremer, Plouzané, France, 1997, 35 pp.

MULLER-FEUGA, A. The role of microalgae in aquaculture: situation and trends. **Journal of Applied Phycology,** v. 12, p.527–534, 2000.

MUNRO, P.D., HENDERSON, R.J., BARBOUR, A., BIRKBECK, T.H., Partial decontamination of rotifers with ultraviolet radiation: the effect of changes in the bacterial load and flora of rotifers on mortalities in start-feeding larval turbot. **Aquaculture,** v.170, p.229–244, 1999.

NAEGEL, L.C.A; RODRÍGUEZ-ASTUDILLO, S. Comparison of growth and survival of white shrimp postlarvae (*Litopenaeus vannamei*) fed dried *Artemia* biomass versus four commercial feeds and three crustacean meals. **Aquaculture International**, v.12, p.573–581, 2004.

NEELA, F.A., NONAKA, L., SUZUKI, S. The diversity of multi-drug resistance proles in tetracycline-resistant *Vibrio* species isolated from coastal sediments and seawater. **J. Microbiol.,** v.45, p.64–68, 2007.

NICOLAS, J.L., ROBIC, E., ANSQUER, D. Bacterial flora associated with a trophic chain consisting of microalgae, rotifers, and turbot larvae: influence of bacteria on larval survival. **Aquaculture,** v.83, p.237–248, 1989.

OESTMAN, D.J., LEWIS, D.H., ZETTLER, B.A. Clearence of *Amyloodinium ocellatum* dinospores by *Artemia salina*. **J. Aquat. Anim. Health,** v.7, p.257–261, 1995.

OLSEN, A.I., OLSEN, Y., ATTRAMADAL, Y., CHRISTIE, K., BIRKBECK, T.H., SKJERMO, J., VADSTEIN, O. Effects of short term feeding of microalgae on the bacterial flora associated with juvenile *Artemia franciscana*. **Aquaculture**, v.190, p.11 – 25, 2000.

OZKIZILCIK, S., CHU, F.E. Evaluation of omega-3 fatty acid enrichment of *Artemia* nauplii as food for striped bass *Morone saxatilis*_Walbaum.larvae. **J. World Aquacult. Soc.,** v.25, p.147–154, 1994a.

OZKIZILCIK, S.; CHU, F.L. E. Uptake and metabolism of liposomes by *Artemia* nauplii. **Aquaculture,** v.128, p.131–141, 1994b.

PEZZA, L., RÍOS, A., NOZAL, L., ARCE, L., VALCÁRCEL, M. Determinação simultânea de resíduos de cloranfenicol, tianfenicol e florfenicol em leite bovino por cromatografia eletrocinética micelar. **Quim. Nova**, v. 29, p. 926-931, 2006.

PONCE-PALAFOX J., MARTINE-PALACIOS C.A., ROSS L.G. The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei* Boone, 1931. **Aquaculture** v.157, p.107–115, 1997.

PONTES, C.S.; ANDREATTA, E.R. Efeito da Oferta de Náuplios de *Artemia franciscana* enriquecidos com Ácidos Graxos Poliinsaturados sobre o Desenvolvimento de Pós-Larvas do Camarão Marinho *Farfantepenaeus paulensis.* **R. Bras. Zootec**., v.32, p.1544-1550, 2003.

REITAN, K.I., RAINUZZO, J.R., ØIE, G., OLSEN, Y., Nutritional effects of algal addition in first feeding tanks of turbot (*Scophthalmus maximus)* L. larvae. **Aquaculture,** v.118, p.257–275, 1993.

RINGØ, E., SINCLAIR, P.D., BIRKBECK, H., BARBOUR, A., Production of eicosapentaenoic acid (20:5n-3) by *Vibrio pelagius* isolated from turbot (*Scophthalmus maximus)* L. larvae. **Appl. Environ. Microbiol.** v.58, p.3777–3778, 1992.

RODRIGUES, J. Carcinicultura marinha desempenho em 2004. Revista da ABCC, n.7 (2), p. 38-44, 2005.

RODRÍGUEZ, C., BHOSLE, N.B. Exopolysaccharide production by *Vibrio fisheri*, a fouling marine bacterium. **Biofouling**, v.4, p.301–308, 1991.

ROIHA, I.S., OTTERLEI, E., SAMUELSEN, O.B. Bioencapsulation of florfenicol in brine shrimp *Artemia franciscana,* naupli. **Journal of Bioanalysis & Biomedicine,** v. 2, p. 60-64, 2010.

ROQUE, A.; GOMEZ-GIL, B. Therapeutic effects of enrofloxacin in an experimental infection with a luminescent *Vibrio harveyi* in *Artemia franciscana* Kellog 1906. **Aquaculture,** v.220, p.37–42, 2003.

ROWE-MAGNUS, D.A., ZOUINE, M., MAZEL, D. The adaptive genetic arsenal of pathogenic *Vibrio* species: the role of integrons. In: THOMPSON, F., AUSTIN, B., SWINGS, J. (Eds.), The biology of vibrios. ASM Press, Washington DC, USA, 2006.

SHAO, Z.J. Aquaculture pharmaceuticals and biologicals: current perspectives and future possibilities. **Adv. Drug Deliv**. v. 50, p. 229–243, 2001.

SIQUEIRA, A. T.; CORREIA, E. S.; MOURA E. C. M.; SANTOS M. A. Efeitos de diferentes rações no cultivo do camarão cinza *Litopenaeus vannamei*. In: Congresso Brasileiro de Engenharia de Pesca, 11, Recife. Anais: Recife; AEP-BR, p. 785-791, 1999.

SKJERMO, J., VADSTEIN, O. Characterization of the bacterial flora of mass cultivated *Brachionus plicatilis*. **Hydrobiologia,** v. 255, p.185–191, 1993.

SKJERMO, J., VADSTEIN, O. Techniques for microbial control in the intensive rearing of marine fish larvae. **Aquaculture,** v.177, p.333–343, 1999.

SMITH L.L., BIEDENBACH J.M. & LAWRENCEA.L. Penaeid larviculture: Galveston method. In: Marine Shrimp Culture: Principles and Practices, (ed. byA.W. Fast & L.J. Lester), Amsterdam, the Netherlands. Elsevier Science Publishers, 1992. p.171-191.

SMITH, G.G., RITAR, A.J. The influence of animal density and water turbulence on growth and survival of cultured spiny lobster (*Jasus edwardsii*) larvae. **Aquaculture,** v.258, p.404–411, 2006.

SOARES, R.; PEIXOTO, S.; WASIELESKY, W.; D'INCAO, F. Effect of different food items on the survival and growth of *Farfantepenaeus paulensis* (Pérez-Farfante 1967) postlarvae. **Aquaculture Research**., v.37, p.1413-1418, 2006.

SORGELOOS, P., BOSSUYT, E., LAVIÑA, E., BAEZA-MEZA, M., PERSOONE, G. Decapsulation of *Artemia* cysts: a simple technique for the improvement of the use of brine shrimp in aquaculture. **Aquaculture,** v.12, p.311–315, 1977.

SORGELOOS, P.; BOSSUYT, E.; LAVENS, P.; LEGER, P.; VANHAECKE, P.; VERSICHELE, D. The use of brine shrimp *Artemia* in crustacean hatcheries and nurseries. In: CRC Handbook of mariculturre , Vol. 1. Crustacean Aquaculture (ed. by J.P. Moore), CRC Press, Boca Raton, FL, USA. 1983. p. 71-96.

SORGELOOS, P.; COUTTEAU, P.; DHERT, P.; MERCHIE, G.; LAVENS, P. Use of brine shrimp, *Artemia spp.*, in larval crustacean nutrition: A review. **Reviews in Fisheries Science**. v. 6, p. 55-68, 1998.

SORGELOOS, P.; DHERT, P.; CANDREVA, P. Use of the brine shrimp, *Artemia spp.*, in marine fish larviculture. **Aquaculture**. v. 200, p. 147-159, 2001.

SORGELOOS, P., LAVENS, P., LÉGER, P., TACKAERT, W., VERSICHELE, D. Manual for the culture and use of brine shrimp *Artemia* in aquaculture. The Belgian Administration for Development Cooperation. United Nations Food and Agriculture Organization, Belgium. 1986. 319 p.

SORGELOOS, P.; LÉGER, P. Improved larvicultures outputs of marine fish, shrimp and prawn. **J. World Aquacult. Soc**, v. 4, p. 251-264, 1992.

SORGELOOS, P., LÉGER, P., LAVENS, P. Improved larval rearing of European and Asian seabass, seabream, mahi-mahi, sigonid and milk fish using enrichment diets for *Brachionus* and *Artemia*. **World Aquacult**., v.19, p.78–79, 1988.

SPOLAORE, P.; JOANNIS-CASSEN, C.; DURAN, E.; ISAMBERT, A. Commercial applications of microalgae: Review. Journal of Bioscience and Bioengineering. v. 101, p. 87-96, 2006.

SUGITA, H., MIYAJIMA, C., DEGUCHI, Y. The vitamin B12-producing ability of the intestinal microflora of freshwater fish. **Aquaculture,** v.92, p.267–276, 1991.

TACON, A.G.J. The Nutrition and Feeding of Farmed Fish and Shrimp – A Training Manual 1. The Essential Nutrients. Food and Agriculture Organization of United Nations. Brasília, Brasil. Junho, 1987. 52p.

TOLOMEI, A., BURKE, C., CREAR, B., CARSON, J. Bacterial decontamination of on-grown *Artemia*. **Aquaculture,** v.232, p.357–371, 2004.

TONHEIM, S.K.; KOVENW; RØNNESTAD I. Enrichment of *Artemia* with free methionine. **Aquaculture,** v.190, p.223–235, 2000.

TOURAKI, M., MOURELATOS, S., KRARAMANLIDOU, G., KALAITZOPOULOU, S., KASTRITSIS, C. Biocapsulation of chemotherapeutics in *Artemia* as a means of prevention and treatment of infectious diseases of marine fish fry. **Aquacult. Eng.,** v.15, p.133–147, 1996.

TOURAKI, M.; RIGAS, P.; KASTRITSIS, C. Liposome mediated delivery of water soluble antibiotics to the larvae of aquatic animals. **Aquaculture,** v.136, p.1–10, 1995.

VERSCHUERE, L., ROMBAUT, G., SORGELOOS, P., VERSTRAETE, W. Probiotic bacteria as biological control agents in aquaculture. **Microbiol. Mol. Biol. Rev.,** v.64, p.655–671, 2000.

WATANABE, T.; OOWA, F.; KITAJIMA, C. Relationship between dietary value of brine shrimp *Artemia salina* and their content of w-3 HUFA. **Bulletin of Japanese Society of Scientific Fisheries**,v.46, p. 35-34,1980.

WILLIAMS A.S., DAVIS D.A., ARNOLD C.R. Density-dependent growth and survival of *Penaeus setiferus* and *Penaeus vannamei* in a semi-closed recirculating system. **J World Aquac Soc** v.27, p.107–112, 1996.

WURMANN C., MADRID, R.M., BRUGGER A.M. Shrimp farming in Latin America: currents status, opportunities, challenges and strategies for sustainable development. **Aquaculture Economic and Management**, v.8, p.117–141, 2004.

YFLAAR, B.Z.; OLIVERA, A. Utilização de náuplios de "branchoneta" *Dendrocephalus brasiliensis* (Pesta, 1921) na alimentação de larvas do "camarão cinza" *Litopenaeus vannamei* (Boone, 1931). **Acta Scientiarum. Biological Sciences,** v.25, p. 299-307, 2003.

# 4. Artigo científico

Artigo científico a ser submetido para publicação no periódico *Aquaculture Research*

## *Vibrio* spp. control in *Artemia* hatching and enrichment for *Litopenaeus vannamei* postlarvae feeding

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**Abstract**

The bacterial load in *Artemia* hatching and enrichment were evaluated in two experiments (I): newly hatched *Artemia* nauplii were exposed to *Chaetoceros calcitrans* microalgae, commercial probiotic (*Bacillus* spp.) and Florfenicol antibiotic added to hatching water of decapsulated and capsulated cysts. Presumptive *Vibrio* counts were recorded in hatching water and *Artemia*. Bacterial isolates from *Artemia* were identified. Additionally, *Artemia* nauplii were frozen and the presuntive *Vibrio* load evaluated after 48 hours. (II): *Artemia* metanauplius were enriched with *C. calcitrans*, commercial probiotic and emulsion DHA / EPA rich. Newly hatched nauplii represented the

Control. *Artemia* from the treatments were offered to *Litopenaeus vannamei* postlarvae ($PL_7$ to $PL_{19}$). Presuntive *Vibrio* were quantified in *Artemia*, postlarvae and rearing water. Results indicated that adding *C. calcitrans* in *Artemia* hatching water is an effective alternative to antibiotics but probiotic must be also considered to control the *Vibrio* spp. load in *Artemia* nauplii. The enrichment supplements increased the bacterial load in *Artemia* but they did not affect *Vibrio* concentration in postlarvae.

**Keywords**: *Artemia*, *Vibrio*, hatching, enrichment, *Litopenaeus vannamei*.

## Introduction

In fish and shellfish hatcheries the widespread use of *Artemia* as live food is due to their positive characteristics such as high protein content and ability to produce storable cysts (Léger, Bengton, Sorgeloos, Simpson & Beck 1987). The *Artemia* nutritional value can be further increased by the enrichment process (bioencapsulation). The enrichment technique exploits the fact that *Artemia* is a non-selective filter feeder organism in its second stage of development (instar II or metanauplius), which occurs eight hours after hatching (Campbell, Adams, Tatner, Chair & Sorgeloos 1993; Dixon , Vanpoucke, Chair, Dehasque, Nelis, De Leenheer & Sorgeloos 1995; Sorgeloos, Dhert & Candevra 2001). This feature also allows the use of *Artemia* in diseases control through the bioencapsulation of antimicrobial agents.

However, *Artemia* nauplii has been also reported as vector of pathogenic bacteria in shrimp hatcheries (López-Torres & Lizárraga-Partida 2001). The bacterial load associated with *Artemia* includes *Vibrio* spp. which are related to high mortality in penaeid shrimp rearings worldwide (Lightner & Lewis 1975; Baticados, Lavilla-Pitogo,

Cruz-Lacierda, dela Pena & Sunaz 1990, Gomez-Gil, Thompson, Thompson, Garcia-Gasca, Roque & Swings 2004).

Frequent and inappropriate use of antibiotics can induce the selection and proliferation of resistant bacterial strains. Therefore, alternative prophylactic measures to reduce *Vibrio* spp. spreading should be adopted as they are more cost-effective and less dependent on the chemicals use (Witte, Klare & Werner 1999; Planas & Cunha 1999).

In this context, this study evaluated different prophylactic methods to control the bacterial load in *Artemia* hatching and enrichment. *Litopenaeus vannamei* postlarvae fed with *Artemia* were also investigated.

## Materials and methods

*Experiment I*

The experiment evaluated three supplements to control *Vibrio* spp. during the hatching of *Artemia* cysts INVE® (INVE, Belgium, www.inve.be) Great Salt Lake (GSL-USA). A sequence of two trials tested capsulated and decapsulated cysts. A 12% sodium hypochlorite solution was used to decapsulate the cysts. According to the type of supplement four treatments were established: Antibiotic, Probiotic, Microalgae and Control. All supplements were applied directly into the hatching water. Antibiotic treatment received 300 mg $L^{-1}$ dose of Florfenicol (Roiha, Samuelsen & Otterlei 2010). A commercial probiotic consisting of *Bacillus subtilis*, *B. pumilus* and *B. licheniformis* ($2 \times 10^5$ CFU $mL^{-1}$) was used in Probiotic treatment. Microalgae treatment received *Chaetoceros calcitrans* ($8 \times 10^5$ cells $mL^{-1}$) collected during exponential growth phase. Only seawater was used in Control.

*Artemia* cysts (1g $L^{-1}$) were stocked in cylindrical-conical tanks filled with 20 L seawater previously disinfected with 15 ppm chlorine. Temperature, salinity, dissolved oxigen and pH were maintained at 29.9 ± 0.3 ºC, 27.7 ± 0.1 g $L^{-1}$, 5.7 ± 0.1 mg $L^{-1}$ e 8.1 ± 0.03 respectively, as recommended by Van Stappen (1996). The *Artemia* hatching tanks were illuminated (2000 lux) and aerated continuously. Three replicates were used for each treatment. After 24 h incubation, the hatched nauplli were harvested and counted. The nauplii hatching efficiency (HE) was calculated using the formula: HE = (mean number of nauplii hatched $mL^{-1}$ x water volume) / grams of cysts added.

Water and *Artemia* samples of all treatments were collected to quantify presumptive *Vibrio* colony forming units (CFU) using the agar Thiosulfate Bile Sucrose (TCBS agar; Himedia Laboratories Corporate Office, Mumbai, India, www.himediaslab.com). Water samples were serially diluted (1/10) in sterile saline solution (2.5% NaCl). Aliquots of 0.1 mL from three dilutions were spread plated on TCBS agar and incubated for 24 h at 30°C. After incubation the total colony forming units were enumerated.

The samples of *Artemia* were aseptically macerated, diluted and plated using the same methodology described for the water analysis. Additionally, samples of different *Artemia* treatments were frozen at a temperature of -25°C for 48 h and then the bacterial load was also determined. This analysis determined the temperature influence on the bacteria viability, since commercial hatcheries occasionally use *Artemia* in the frozen form.

From the nauplii samples, different bacterial morphotypes grown on TCBS agar were isolated and subjected to presumptive identification tests for the *Vibrio* genus (detection of glucose fermentation and oxidase). Further the morphotypes were identified through their biochemical profiles presented in the commercial bacterial

identification kit (API 20 E - Biomerieux, Marcy l'Etoile, France, www.biomerieux.com).

*Experiment II*

***Artemia* enrichment**

*Vibrio* concentration was evaluated in *Artemia* enriched with three different supplements: *C. calcitrans* (Microalgae treatment), commercial probiotic (Probiotic treatment) and a commercial emulsion rich in fatty acids (Selco treatment). Control was represented by newly hatched nauplii with no added supplements. Three replicates were used for each treatment.

Capsulated *Artemia* cysts (GSL, INVE®) were hatched under the same conditions of the Control in the first experiment. The newly hatched nauplii were washed with chlorine disinfected sea water and stocked (100 - 300 nauplii $mL^{-1}$) in 5 L containers filled with sea water and the different supplements. *Artemia* were submitted to enrichment process during 28 h under constant aeration.

Microalgae treatment was enriched with 8 x $10^5$ cells $mL^{-1}$ of *Chaetoceros calcitrans* and Probiotic treatments with 2 x $10^5$ CFU $mL^{-1}$ of commercial probiotic (*Bacillus subtilis*, *B. pumilus* and *B. licheniformis*). Selco treatment received 0.3 g $L^{-1}$ every 12 h (Merchie, Lavens, Radull, Nelis, De Leenheer & Sorgeloos 1995) of commercial emulsion rich in docosahexaenoic and eicosapentaenoic fatty acids besides substances to provide *Vibrio* control.

The *Artemia* hatching and enrichment processes were repeated during 12 days for *L. vannamei* postlarvae feeding. Average water temperature, salinity, pH and dissolved oxygen were maintained between 27-31°C, 28-30 g $L^{-1}$, 8.5 to 9.0 and 4.69 to 6.65 mg $L^{-1}$ respectively.

129

130 ***Litopenaeus vannamei* postlarvae rearing**

131 Shrimp postlarvae in $PL_7$ stage (7 days in the postlarval stage), with wet weight
132 mean of 0.843 mg, were randomly stocked in 12 plastic containers (10 L) at the density
133 of 50 PL $L^{-1}$. Water was previously desinfected with chlorine (15 ppm) for 24 h, and
134 then neutralized with ascorbic acid.

135 Enriched *Artemia* from the four treatments (Microalgae, Probiotic, Selco and
136 Control) were offered for postlarvae feeding during 12 days (three replicates each).
137 Shrimp were fed twice daily (07:00 and 19:00 h) at an initial rate of 5 nauplii $mL^{-1}$
138 reaching 12 nauplii $mL^{-1}$ at the end of rearing according to the larvae consumption.

139 The postlarvae were maintained under constant aeration at a temperature of 27-
140 28°C. Daily, 50% of water was exchange in the experimental units. Temperature,
141 dissolved oxygen, pH and salinity were monitored daily by a multiparameter sensor
142 (YSI 556).

143

144 **Bacteria analysis**

145 Presumptive *Vibrio* spp. were quantified in samples of enriched *Artemia*, water
146 of *Artemia* enrichment, postlarvae and water of postlarvae rearing.

147 The procedure for *Vibrio* analysis in water and *Artemia* samples followed the
148 methodology presented in the experiment I. The PL samples were weighted, macerated
149 and diluted (1/10) and 0.1 mL of three dilutions were plated in TCBS agar, incubated
150 and enumerated as described.

151 Additionally, the colonization of bacteria from commercial probiotic, was
152 verified by the quantification of *Bacillus* CFU in enriched *Artemia* and postlarvae from
153 Probiotic treatment. The samples were weighted, macerated and diluted (1/10) and 0.1

mL of three dilutions were plated in MYP agar (Mannitol Egg Yolk Agar polymyxin, Himedia®), incubated and enumerated.

**Data analysis**

The hatching efficiency data were submitted to ANOVA and Tukey's test. The Student's T-test was used to compare the bacterial count of *Artemia* natural biomass and frozen biomass. Bacterial counts data were submitted to ANOVA and Fisher tests. Differences were considered at the 5% significance level. All analyzes were performed using Statistica 7.0.

**Results**

**Experiment I**

The hatching efficiency was similiar between treatments and the mean (± SE) ranged from $1.9 \pm 0.1 \times 10^5$ to $2.7 \times 10^5$ nauplii $g^{-1}$.

The decapsulation process did not shown to be effective in reducing the presumptive *Vibrio* spp. load in nauplii and water in all treatments (Table 1).

The number of bacterial colonies in water was significantly lower in Antibiotic followed by Microalgae treatment of capsulated cysts (Table 1). Only the Probiotic treatment did not significantly differ from the Control.

A similar pattern was observed in the decapsulated cysts analysis, where the significant lower *Vibrio* counts in water were observed in Antibiotic and Microalgae (Table 1). However, the Microalgae did not differ significantly from the other treatments.

Insert Table 1

In the trial with capsulated cysts, *Vibrio* spp. load in *Artemia* was significantly lower in the supplemented treatments (Table 1). However, *Artemia* from decapsulated cysts showed significantly lower contamination in Antibiotic and Probiotic treatments (Table 1). The Microalgae did not differ from the other treatments.

The freezing nauplii process during 48 hours resulted in a reduction of 70.33 to 99.75% of the *Vibrio* spp. load (Table 2).

Insert Table 2

Of the 43 bacterial colony morphotypes isolated from nauplii of all treatments (capsulated cysts) 54% was identified as *Vibrio alginolyticus* and 36% as Gram-negative cocci oxidase-negative. *Vibrio parahaemolyticus*, *Pasteurella multocida*, *Aeromonas hydrophila*, *Aeromonas salmonicida* and *Ochrobactrum anthropi* each represented 2% of the total isolates. *V. parahaemolyticus* and *O. anthropi* were resistant to the Florfenicol.

From the nauplii of decapsulated cysts were obtained 31 isolates. Of these, 42% were identified as *V. alginolyticus*, 42% as Gram-negative cocci oxidase-negative and 16% as Gram positive isolates.

**Experiment II**

The *Vibrio* spp. concentrations in the *Artemia* enrichment water were significantly lower in the Control (newly hatched nauplii) and did not differ among other treatments (Table 3). In *Artemia* the lowest values of bacterial colonies were observed in Control. However, the Control did not differ from Microalgae and Probiotic

treatments. Selco presented the highest level of contamination differing significantly from Control.

The *Vibrio* spp. concentrations in the postlarvae rearing water from Probiotic treatment was significantly lower than Control (Table 3). No significant differences were observed in the number of bacterial colonies in postlarvae among treatments (Table 3).

Insert Table 3

*Bacillus* quantification (± SE) in metanauplius and postlarvae from Probiotic treatment was $8.7 \pm 4.2 \times 10^5$ and $1.4 \pm 0.8 \times 10^6$ CFU $g^{-1}$ respectively.

In the postlarvae rearing water, the mean values (± SE) of temperature (28.7 ± 0.04°C), OD (5.5 ± 0.1 mg $L^{-1}$), pH (8.50 ± 0.04) and salinity (29.5 ± 0.06 g $L^{-1}$) did not differ among treatments.

**Discussion**

Bacterial enumeration in TCBS medium shows that *Artemia* hold a high number of bacteria. According to Verdonck, Grisez, Sweetman, Minkoff, Sorgeloos, Ollevier and Swings (1994), *Artemia* are usually highly contaminated with bacteria (> $10^7$ CFU per gram) and mostly identified as *Vibrio* spp. Moreover it is necessary to control these bacterial loads before the use of *Artemia* in culture systems.

The use of Florfenicol resulted in the lowest *Vibrio* spp. load among the supplements tested in *Artemia* hatchery. The Florfenicol has been authorized in several countries for aquaculture activities (FAO 2005). In Brazil, it is the only antibiotic registered for this purpose in the Ministry of Agriculture, Livestock and Supply

(MAPA) (Schering Plough 2009). In fish farming, Florfenicol has potent activity against a broad range of pathogens (Samuelsen, Ervik & Bergh 2003), including microorganisms resistant to other antibiotics (Nordmo, Varma, Brokken & Sutherland 1994; Rangdale, Richards & Alderman 1997; Bruun, Schmidt, Madsen & Dalsgaard 2000; Thyssen & Ollevier 2001; Vue, Schmidt, Stehly & Gingerich 2002; Samuelsen & Bergh 2004). Our findings suggested that the Florfenicol dose (300 mg $L^{-1}$) was efficient in reducing *Vibrio* counts but some potentially pathogenic strains of *Vibrio* (*V. alginolyticus* and *V. parahaemolyticus*) remained in *Artemia* nauplii. Nevertheless, proliferation of these resistant bacteria could make possible infections more difficult to treat in hatchery systems.

The Microalgae treatment was the second most efficient in reducing *Vibrio* load in water and nauplii (capsulated). In accordance, Tolomei, Burke, Crear and Carson (2004), recommended the genus *Chaetoceros* to sanitize the external surface of *Artemia*. This effect may be related to the bacteriostatic or bactericidal microalgae activity (Kellam & Walker 1989; Olsen, Olsen, Attramadal, Christie, Birkbeck, Skjermo & Vadstein 2000). The microalgae antibacterial activity has been detected in microalgae extracts (Duff & Bruce 1966; Austin & Day 1990, Austin, Baudet & Stobie 1992; Tendencia & dela Peña 2003) and it may be related to the associated microflora, antimicrobial proteins, fatty acids and oxygen free radicals produced by microalgae cells (Marshall, Salas, Oda & Hallegraef 2005; Makridis, Alves Costa & Dinis 2006; Kokou, Ferreira, Tsigenopoulos, Makridis, Kotoulas, Magoulas & Divanach 2007).

Despite newly hatched *Artemia* nauplii are unable to bioencapsulate, probiotic bacteria can be active in the gills and body surface by competing with other bacteria for adhesion sites (Gatesoupe 1991; Verschuere, Rombaut, Sorgeloos & Verstraete 2000).

253 The present study has shown that probiotics reduced the *Vibrio* load in *Artemia* from
254 capsulated and decapsulated cysts.

255 Freezing *Artemia* for 48 h sharply reduced the *Vibrio* counts, but most values
256 were still over $10^7$ CFU. However, it has been suggested that *Vibrio* spp. are capable of
257 entering into a viable but non-culturable (VBNC) state when exposed to low
258 temperatures (Jiang & Chai 1996, Johnson & Brown 2002). Eventually, when
259 temperature rise bacterial cells are able to emerge from the VBNC state and become
260 culturable on bacteriological media. Thus, offering short-term frozen stored *Artemia* to
261 shrimp larvae may not reduce the potential of *Vibrio* spp. contamination, as cold-
262 induced death of bacteria only occur after several days to weeks (Oliver 1981).

263 The sodium hypochlorite used in the decapsulation process is able to totally
264 decontaminate *Artemia* cysts, but they can be quickly recolonized during the rupture
265 stage before hatching (Sorgeloos *et al*. 2001). At this stage, the organic substrate
266 glycerol is released from the cysts and offers an ideal culture medium for *Vibrio* spp. In
267 this study the decapsulation process did not effective reduced *Vibrio* concentration, but
268 decreased bacterial species in nauplii and may be regarded as an auxiliary prophylactic
269 treatment.

270 Hoj, Bourne and Hall (2009) characterized the bacterial community present in
271 *Artemia*, with more than half of *Vibrio* isolates being identified as *V. alginolyticus*.
272 López-Torres and Lizárraga-Partida (2001) observed that even when *Artemia* cysts were
273 hatched under sterile conditions *V. alginolyticus* was the dominant species. These
274 authors suggested that *V. alginolyticus* and *Vibrio* spp. isolated from *Artemia* hatching
275 tanks were associated with those isolated from tanks with zoea, mysis and postlarvae,
276 indicating that these *Vibrio* spp. remain associated with different development phases of
277 shrimp. Buglione, Vieira, Mouriño, Pedrotti, Jatoba and Martins (2010) observed that *V.*

*alginolyticus* strain caused high mortality of *L.vannamei* larvae. The *V. alginolyticus* virulence is correlated to enzyme collagenase activity, which can cause softening of shrimp muscle tissue (Brauer, Leyva, Alvarado & Sandez 2003; Yishan, Jiaming, Jichang & Zaohe 2011).

The presence of *V. parahaemolyticus* in samples of shrimp submitted to antibiotic treatment was reported by Verschuere *et al*. (2000). Likewise this bacteria was identified in *Artemia* from Antibiotic treatment. According to Gomez-Gil *et al*. (2004), *V. parahaemolyticus* attacks mainly shrimps at juvenile and adult stage.

*Ochrobactrum anthropi* was also resistant to the Florfenicol treatment. This species has been isolated from cryopreserved *Penaeus monodon* spermatophores (Nimrat, Bart, Keatsaksit & Vuthiphandchai 2008) and water samples from mangrove receiving shrimp farm effluents (Sousa 2006). However, this species has not been related to shrimp diseases in the literature.

*Pasteurella multocida* is also a bacteria usually not associated with disease in shrimp. However, Buglione *et al*. (2010) observed that after an experimental infection, this species increased mortality in *L. vannamei* larvae.

*Aeromonas* spp. compose the normal microflora of wild and reared crustaceans and can be considered an opportunistic pathogen (Lightner 1993). This genus has been associated to the soft shell syndrome in *Penaeus monodon* (Baticados, Coleso & Duremdez 1986; Uddin, Zafar Noman & Sharmin 2008).

The *Artemia* enrichment process increased bacterial load in water and *Artemia* specially in Selco treatment. This could be explained by the organic input from supplements addition and *Artemia* excretion, which allows a sudden increase of opportunistic bacteria (Igarashi, Sugita & Deguchi 1989; Skjermo & Vadstein, 1993; Verschuere, Dhont, Sorgeloos & Verstraete 1997; Olsen *et al*. 2000). Hoj *et al*. (2009)

and Haché & Plant (2011) also observed high bacterial abundance in *Artemia* enriched with microalgae and lipid emulsions in combination.

The bacterial load in postlarvae and rearing water were not associated with the concentrations of *Vibrio* observed in newly hatched nauplii (Control) and enriched *Artemia*. The daily renewal (50%) of postlarvae rearing water probably reduced the abundance of *Vibrio* spp. Krishnika and Ramasamy (2012) also recorded a significant reduction of *Vibrio* after the water exchange of *Artemia* rearing tanks. Silva, Soares, Calazans, Vogeley, Valle, Soares and Peixoto (2011), observed a presumptive *Vibrio* load of 7.9 x $10^7$ CFU $g^{-1}$ in *L.vannamei* postlarvae ($PL_{10}$) fed newly hatched *Artemia* nauplii. In the present study this load was slightly lower for $PL_{19}$ (0.17 x $10^7$ CFU $g^{-1}$).

Overall results of this study indicated that adding *C. calcitrans* in *Artemia* hatching water is an effective alternative to antibiotics. Additionally, the use of probiotic must be also considered to control the *Vibrio* spp. load in *Artemia* nauplii. The enrichment supplements increased the bacterial load in *Artemia* but they did not affect *Vibrio* concentration in postlarvae.

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## References

Austin B. & Day J.G. (1990) Inhibition of prawn pathogenic *Vibrio* spp. by a commercial spray-dried preparation of *Tetraselmis suecica*. *Aquaculture* **90**, 389-392.

Austin B., Baudet E. & Stobie M. (1992) Inhibition of bacterial fish pathogens by *Tetraselmis suecica*. *Journal of Fish Diseases* **15**, 55-61.

Baticados M.C.L., Lavilla-Pitogo C.R., Cruz-Lacierda E.R., dela Pena L.D. & Sunaz N.A. (1990) Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. *Disease of Aquatic Organisms* 9, 133– 139.

Baticados C. L., Coleso R. M. & Duremdez R. S. (1986) Studies on the chronic soft shell syndrome in the tiger prawn, *Penaeus monodon* Fabricius from brackish water ponds. *Aquaculture* 56, 271-285.

Brauer J. M. E., Leyva J. A. S., Alvarado L. B. & Sandez O. R. (2003) Effect of dietary protein on muscle collagen, collagenase and shear force of farmed white shrimp (*Litopenaeus vannamei*). *European Food Research and Technology* **217**, 277–280.

Bruun M.S., Schmidt A.S., Madsen L. & Dalsgaard I. (2000) Antimicrobial resistance patterns in Danish isolates of *Flavobacterium psychrophilum*. *Aquaculture* **187**, 201-212.

Buglione C.C., Vieira F.N., Mouriño J.L.P., Pedrotti F.S, Jatoba A. & Martins M.L. (2010) Experimental infection with different bacterial strains in larvae and juvenile *Litopenaeus vannamei* reared in Santa Catarina State, Brazil. *Acta Scientiarum. Biological Sciences Maringá* **32**, 291-296.

Campbell R., Adams A., Tatner M., Chair M. & Sorgeloos P. (1993) Uptake of *Vibrio anguillarum* vaccine by *Artemia salina* as a potential oral delivery system to fish fry. *Fish and Shellfish Immunology* **3**, 451–459.

Dixon B.A., Vanpoucke S.O., Chair M., Dehasque M., Nelis H.J., Sorgeloos P. & De Leenheer A.P. (1995) Biocapsulation of the antibacterial drug sarafloxacin in naupliii of brine shrimp *Artemia franciscana*. *Journal of Aquatic Animal Health* **7**, 42–45.

Duff D.C.B. & Bruce D.L. (1966) The antibacterial activity of marine planktonic algae. *Canadian Journal of Microbiology* **12**,877-884.

FAO, Food and Agriculture Organization of the United Nations. (2005) Responsible use of antibiotics in aquaculture. FAO Fisheries Technical Paper, Rome, pp.97.

Gatesoupe F.J. (1991) Managing the dietary value of *Artemia* for larval turbot, *Scophthalmus maximus*; the effect of enrichment and distribution techniques. *Aquacultural Engineering* **10**, 111-119.

Gomez-Gil B., Thompson F.L., Thompson C.C., Garcia-Gasca A., Roque A. & Swings J. (2004) *Vibrio hispanicus* sp. nov., isolated from *Artemia* sp. and sea water in Spain. *International Journal of Systematic and Evolutionary Microbiology* **54**, 261-265.

Haché R. & Plante S. (2011) The relationship between enrichment, fatty acid pro files and bacterial load in cultured rotifers (*Brachionus plicatilis* L-strain) and *Artemia* (*Artemia salina* strain *Franciscana*). *Aquaculture* **311**, 201-208.

Hoj L., Bourne D.G. & Hall M.R. (2009) Localization, abundance and community structure of bacteria associated with *Artemia*: Effects of nauplii enrichment and antimicrobial treatment. *Aquaculture* **293**, 278-285.

Igarashi M.A., Sugita H. & Deguchi Y. (1989) Microflora associated with eggs and Nauplii of *Artemia salina* . *Nippon Suisan Gakkaishi* **55**, 2045-2045.

Jiang X. & Chai T. (1996) Survival of *Vibrio parahaemolyticus* at low temperature under starvation conditions and subsequent resuscitation of viable, nonculturable cells. *Applied and Environmental Microbiology* **62**, 1300-1305.

Johnson M.D. & Brown M.H. (2002) An investigation into the changed physiological state of *Vibrio* bacteria as a survival mechanism in response to cold temperature and studies on their sensitivity to heating and freezing. *Journal of Applied Microbiology* **92**, 1-12.

Kellam S.J. & Walker J.M. (1989) Antibacterial activity from marine microalgae in laboratory culture. ***British Phycological Journal*** **24**, 191-194.

Kokou F., Ferreira T.,Tsigenopoulos C.S., Makridis P., Kotoulas G., Magoulas A. & Divanach P. (2007). Identification of bacteria growing in association with batch cultures of the microalgae *Chlorella minutissima*. In 8th International Marine Biotechnology Conference, pp.105 Eilat, Israel.

Krishnika A. & Ramasamy P. (2012) Effect of water exchange to eliminate *Vibrio* sp. during the naupliar development of *Artemia franciscana*. *Journal of Fisheries and Aquatic Science*, doi: 10.3923/jfas.2012.

Léger P., Bengton D.A., Sorgeloos P., Simpson K.L. & Beck A.D. (1987) Requirements of food for larval organisms. *Artemia research and its applications,* **3**, 357-372.

Lightner D.V. (1993) Diseases of cultured penaeid shrimp. In: Handbook of mariculture (ed. by Mc Vey, J. P.), pp. 393-486. CRC press Ink, Boca, Raton.

Lightner D.V. & Lewis D.H. (1975) A septicemia bacterial disease syndrome in penaeid shrimp. *Marine Fisheries Review* **37**, 25– 28.

López-Torres M.A. & Lizárraga-Partida M.L. (2001) Bacteria isolated on TCBS media associated with hatched *Artemia* cysts of commercial brands. *Aquaculture* **194**, 11-20.

Makridis P., Alves Costa R. & Dinis M.T. (2006) Microbial conditions and antimicrobial activity in cultures of two microalgae species. *Tetraselmis chuii* and *Chlorella minutissima*, and effect on bacterial load of enriched *Artemia* metanauplii. *Aquaculture* **255**, 76-81.

Marshall J.A., de Salas M., Oda T. & Hallegraef G. (2005) Superoxide production by marine microalgae-I. Survey of 37 species from 6 classes. *Marine Biology* **147**, 533-540.

Merchie G., Lavens P., Radull J., Nelis H., De Leenheer A. & Sorgeloos P. (1995) Evaluation of vitamin C – enriched *Artemia* nauplii for larvae of the giant freshwater prawn. *Aquaculture International* **3**,355-363.

Nimrat S., Bart A.N., Keatsaksit A. & Vuthiphandchai B. (2008) Microbial flora of spermatophores from black tiger shrimp (*Penaeus monodon*) declines over long-term cryostorage. *Aquaculture* **274**, 247-253.

Nordmo R., Varma K.J., Sutherland I.H. & Brokken E.S. (1994) Florfenicol in Atlantic salmon, *Salmo salar* L. Field evaluation of efficacy against furunculosis in Norway. *Journal of Fish Diseases* **17**, 239-244.

Oliver J. D. (1981) Lethal cold stress of *Vibrio vulnificus* in oysters. *Applied and Environmental Microbiology* **41**, 710-717.

Olsen A.I., Olsen Y., Attramadal Y., Christie K., Birkbeck T.H., Skjermo J. & Vadstein O. (2000) Effects of short term feeding of microalgae on the bacterial flora associated with juvenile *Artemia franciscana*. *Aquaculture* **190**, 11 – 25.

Planas M. & Cunha I. (1999) Larviculture of marine fish: problems and perspectives. *Aquaculture* **177**, 171–190.

Rangdale R.E., Richards R.H. & Alderman D.J. (1997) Minimum inhibitory concentrations of selected antimicrobial compounds against *Flavobacterium psychrophilum* the causal agent of rainbow trout fry syndrome (RTFS). *Aquaculture* **158**, 193-201.

Roiha I.S., Otterlei E. & Samuelsen O.B.(2010) Bioencapsulation of florfenicol in brine shrimp *Artemia franciscana,* naupli. *Journal of Bioanalysis & Biomedicine* **2**, 60-64.

Samuelsen O. B., Bergh O. & Ervik A. (2003) Pharmacokinetics of Florfenicol in cod *Gadus morhua* and in vitro antibacterial activity against *Vibrio anguillarum*. *Diseases of Aquatic Organisms* **56**, 127-133.

Samuelsen O. & Bergh O. (2004) Efficacy of orally administered Florfenicol and oxolinic acid for the treatment of vibriosis in cod (*Gadus morhua*). *Aquaculture* **235**, 27-35.

Schering Plough Animal Health. (2009) Aquaflor. Florfenicol. Technical Monograph for catfish health professionals, U.S., pp. 36.

Silva E.F., Soares M.A., Calazans N.F., Vogeley J.L.; do Valle, B.C., Soares R. & Peixoto S. (2011) Effect of probiotic (*Bacillus* spp.) addition during larvae and postlarvae culture of the white shrimp *Litopenaeus vannamei. Aquaculture Research*, doi: 10.1111/j.1365-21 09.20 11.03 001.x.

Skjermo J. & Vadstein O. (1993) Characterization of the bacterial flora of mass cultivated *Brachionus plicatilis*. *Hydrobiologia* **255**, 185-191.

Sorgeloos P., Dhert P. & Candevra P. (2001) Use of brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture* **200**, 147 – 159.

449 Sousa O.V. (2006) Avaliação do perfil da comunidade microbiana de ecossistemas de
450 manguezal receptores de efluentes da atividade de cultivo de camarão no estado do
451 Ceará. Ph.D. thesis, Universidade Federal do Rio de Janeiro, Ceará, Brasil.
452 Tendencia E.A. & dela Peña M. (2003) Investigation of some components of the
453 greenwater system which makes it effective in the control of luminous bacteria.
454 *Aquaculture* **218**,115-119.
455 Thyssen A. & Ollevier F. (2001) In vitro antimicrobial susceptibility of *Photobacterium*
456 *damselae* subsp. *piscicida* to 15 different antimicrobial agents. *Aquaculture* **200**,
457 259–269.
458 Tolomei A., Burke C., Crear B. & Carson J. (2004) Bacterial decontamination of on-
459 grown *Artemia*. *Aquaculture* **232**, 357-371.
460 Uddin S. A., Zafar M., Noman A. S. M. & Sharmin A. (2008) Isolation and
461 Identification of *Vibrio* spp. and *Aeromonas* spp. in wild and hatchery reared
462 *Penaeus monodon* postlarvae from Cox's Bazar, Bangladesh. *Pakistan Journal of*
463 *Marine Sciences* **17**, 161-167.
464 Van Stappen G. (1996) Introduction, biology and ecology of *Artemia.* In: *Manual on the*
465 *production and use of live food for aquaculture.* (ed. by Lavens P.& Sorgeloos P.)
466 FAO Fisheries Technical paper 295pp.
467 Verdonck L., Grisez L., Sweetman E., Minkoff G., Sorgeloos P., Ollevier F & Swings J.
468 (1994) Variability of the microbial environment of rotifer *Brachionus plicatilis* and
469 *Artemia* production systems. Journal of World Aquaculture Society **25,** 55–59.
470 Verschuere L., Dhont J., Sorgeloos P. & Verstraete W. (1997) Monitoring Biology
471 patterns and r/K-strategists in the intensive culture of *Artemia* juveniles. *Journal of*
472 *Applied Microbiology* **83**, 603-612.

Verschuere L., Rombaut G., Sorgeloos P. & Verstraete W. (2000) Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews* **64**, 655-671.

Vue C., Schmidt L.J., Stehly G.R. & Gingerich W.H. (2002) Liquid chromatographic determination of Florfenicol in the plasma of multiple species of fish. *Journal of Chromatography B* **780**, 111-117.

Witte W., Klare I., Werner G. (1999) Selective pressure by antibiotics as feed additives. *Infection* **27**, 35–38.

Yishan L., Jiaming F., Zaohe W. & Jichang J. (2011) Genotype Analysis of Collagenase Gene by PCR-SSCP in *Vibrio alginolyticus* and its Association with Virulence to Marine Fish. *Current Microbiology* **62**, 1697-1703.

**Table 1**. Average values (± SE) of presumptive *Vibrio* count in *Artemia* hatching water and *Artemia* nauplii (capsulated and decapsulated cysts) from different treatments.

| | *Vibrio* count | | | |
|---|---|---|---|---|
| | Capsulated cysts | | Decapsulated cysts | |
| | Water ($10^5$ CFU $mL^{-1}$) | *Artemia* ($10^7$ CFU $g^{-1}$) | Water ($10^5$ CFU $mL^{-1}$) | *Artemia* ($10^7$ CFU $g^{-1}$) |
| Control | $620.0 \pm 450.0^{c}$ | $120.0 \pm 90.0^{b}$ | $200.0 \pm 150.0^{b}$ | $45.0 \pm 24.0^{b}$ |
| Antibiotic | $0.008 \pm 0.007^{a}$ | $0.02 \pm 0.007^{a}$ | $7.50 \pm 2.90^{a}$ | $3.10 \pm 0.70^{a}$ |
| Microalgae | $37.0 \pm 21.0^{b}$ | $0.40 \pm 0.30^{a}$ | $44.0 \pm 1.90^{ab}$ | $29.0 \pm 10.0^{ab}$ |
| Probiotic | $590.0 \pm 230.0^{c}$ | $15.0 \pm 15.0^{a}$ | $320.0 \pm 190.0^{b}$ | $15.0 \pm 9.0^{a}$ |

Different superscript letters in the same column indicate significant differences between treatments ($P < 0.05$).

**Table 2**. Average values (± SE) of presumptive *Vibrio* count in *Artemia* nauplii (capsulated and decapsulated cysts) from different treatments, before and after freezing.

| | *Vibrio* count | | | |
|---|---|---|---|---|
| | Capsulated cysts | | Decapsulated cysts | |
| | Before ($10^7$ CFU $g^{-1}$) | After ($10^7$ CFU $g^{-1}$) | Before ($10^7$ CFU $g^{-1}$) | After ($10^7$ CFU $g^{-1}$) |
| Control | $120.0 \pm 90.0^a$ | $25.0 \pm 23.0^b$ | $45.0 \pm 24.0^a$ | $2.60 \pm 1.00^b$ |
| Antibiotic | $0.02 \pm 0.007^a$ | $0.00005 \pm 0.00003^b$ | $3.10 \pm 0.70^a$ | $0.92 \pm 0.43^b$ |
| Microalgae | $0.40 \pm 0.30^a$ | $1.30 \pm 1.10^a$ | $29.0 \pm 10.0^a$ | $6.00 \pm 1.60^b$ |
| Probiotic | $15.0 \pm 15.0^a$ | $1.40 \pm 0.90^b$ | $15.0 \pm 9.0^a$ | $2.50 \pm 0.70^b$ |

Different superscript letters in a row indicate significant differences ($P < 0.05$) between before and after freezing.

**Table 3.** Average values (± SE) of presumptive *Vibrio* count in *Artemia* rearing water, *Artemia*, *Litopenaeus vannamei* postlarvae (PL) and postlarvae rearing water from different treatments.

| | *Vibrio* count | | | |
|---|---|---|---|---|
| | *Artemia* Water ($10^6$ CFU $mL^{-1}$) | *Artemia* ($10^7$ CFU $g^{-1}$) | PL Water ($10^6$ CFU $mL^{-1}$) | PL ($10^7$ CFU $g^{-1}$) |
| Control | $3.4 \pm 3.0^{a}$ | $6,5 \pm 3,0^{a}$ | $0.25 \pm 0.00^{b}$ | $0.17 \pm 0.07$ |
| Selco | $100.0 \pm 30.0^{b}$ | $160.0 \pm 140.0^{b}$ | $0.11 \pm 0.03^{ab}$ | $0.87 \pm 0.86$ |
| Microalgae | $17.0 \pm 5.0^{b}$ | $40.0 \pm 7.0^{ab}$ | $0.15 \pm 0.09^{ab}$ | $0.04 \pm 0.02$ |
| Probiotic | $67.0 \pm 23.0^{b}$ | $23.0 \pm 2.0^{ab}$ | $0.05 \pm 0.02^{a}$ | $0.06 \pm 0.02$ |

Different superscript letters in the same column indicate significant differences between treatments ($P < 0.05$).

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