LUCIANO WILLADINO ANDRADE DE OLIVEIRA

CULTIVO DE JUVENIS RECÉM NASCIDOS DO CAVALO-MARINHO *Hippocampus reidi* GINSBURG, 1933, COM DIFERENTES PROTOCOLOS DE ALIMENTAÇÃO E MANEJO

Recife, 2010

UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

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Dissertação apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco, como parte dos requisitos para a obtenção do grau de Mestre em Recursos Pesqueiros e Aquicultura.

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SUMÁRIO

RESUMO

Um dos principais limitantes para o sucesso na produção de cavalos-marinhos em cativeiro é a baixa sobrevivência dos juvenis nos primeiros dias de vida. Embora as causas dessa baixa sobrevivência não sejam totalmente entendidas, a alimentação é considerada crucial para a diminuição da mortalidade, uma vez que o sucesso do cultivo de larvas e juvenis de peixes marinhos depende, entre outros fatores, do conteúdo nutricional e do tamanho do alimento ofertado. O objetivo deste estudo foi avaliar o efeito de diferentes protocolos de alimentação e manejo no crescimento e sobrevivência de juvenis recém nascidos do cavalo-marinho *Hippocampus reidi* até o 14º dia de vida. Foram utilizados aquários de 38 litros de água do mar integrados a um sistema de recirculação, o qual contava com filtros mecânicos, filtro de radiação ultravioleta, filtro biológico e um fracionador de proteína ("*skimmer*"). Cada aquário possuía dois pontos de aeração próximos à superfície da água. O experimento contou com seis tratamentos: Art00h - *Artemia* recém eclodida (5 ind ml^{-1}); Art24h - *Artemia* enriquecida por 24 horas com a emulsão DHA Selco® (5 ind ml^{-1}); Rot+Art24h - rotíferos *Brachionus plicatilis* (10 ind ml^{-1}) até o setimo dia, seguido de *Artemia* enriquecida (5 ind ml^{-1}); Cop – naúplios e copepoditos de *Tisbe biminiensis* (2 ind ml^{-1}); e Cop+Art24h - dieta mista de copépodo *T. biminiensis* (2 ind ml^{-1}) mais *Artemia* enriquecida (3 ind ml^{-1}). No sexto tratamento (Jejum) não foi fornecido nenhum alimento. Todos os tratamentos, exceto o jejum, tiveram a adição da microalga *Nannochloropsis oculata* a cada dois dias em concentrações entre $2{,}2 \times 10^5$ até $3{,}2 \times 10^6$ células por ml. Foram realizadas quatro repetições por tratamento com 100 indivíduos por unidade experimental. Ao final do experimento, os indivíduos sobreviventes foram pesados e medidos (comprimento padrão, altura e peso). O tratamento Cop+Art24h apresentou sobrevivência significativamente maior (33,5 ± 5,4%), seguido pelos tratamentos Cop (6,6 ± 4,8%), Art00h (6,0 ± 8,3%) e Rot+Art24h (0,3 ± 0,5%), que não diferenciaram entre si. Nos demais tratamentos houve mortalidade total. Os parâmetros de crescimento foram significantemente maiores nos tratamentos Cop+Art24h e Art00h. Os resultados deste experimento sugerem que os copépodos *T. biminiensis* são um bom complemento na alimentação de juvenis do cavalo-marinho *H. reidi*.

Palavras chaves: peixes ornamentais, *Artemia*, rotífero, copépodo.

ABSTRACT

The main bottleneck for seahorse production is the low survival of the juveniles during the early stages of development. Although the causes for low survival are not totally understood, feeding is considered critical as success on marine fish larval breeding is dependent, among others factors, on the size and nutritional content of the prey organisms. This study evaluated the growth and survival of newborn seahorses *Hippocampus reidi* fed on different preys. The experiment was conducted on 38 liter aquaria connected to a seawater recirculation system, which contained mechanical and biological filters, UV sterilizer and protein skimmer. Each aquarium had two aeration points near the water surface. Six treatments were tested: Art00h – newly hatched *Artemia* nauplii (5 ind ml^{-1}); Art24h – 24 hours enriched *Artemia* metanauplii (5 ind ml^{-1}); Rot+Art24h - *Brachionus plicatilis* rotifers (10 ind ml^{-1}) from day 1 until day 7 followed by enriched *Artemia* metanauplii (5 ind ml^{-1}); Cop – *Tisbe biminiensis* (2 ind ml^{-1}); and Cop+Art24h – mixed diet with *T. biminiensis* (2 ind ml^{-1}) and 24 hours enriched *Artemia* metanauplii (5 ind ml^{-1}). In the treatment Starvation, no food item was provided. All the treatments, except the starvation, received microalgae *Nannochloropsis oculata* every other day at concentrations that ranged from 2.2×10^5 to 3.2×10^6. Each experimental unit received 100 newborn seahorses. At the end of the experiment, all remaining seahorses were measured and weighed. Treatment Cop+Art24h resulted in a significantly higher survival (33.5 ± 5.4%), followed by Cop (6.6 ± 4.8%), Art00h (6.0 ± 8.3%) and Rot+Art24h (0.3 ± 0.5%), which were not significantly different. No survivors were observed in the remaining treatments. Growth parameters were significantly higher in Cop+Art24h and Art00h. The results from this study suggest that the feeding copepods *T. biminiensis* increases growth and survival of the seahorse *H. reidi* during the first two weeks of life.

Key words: ornamental fish, *Artemia*, rotifer, copepod.

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Introdução

Os cavalos-marinhos pertencem à família Syngnatidae e são geralmente encontrados em ambientes costeiros como recifes, baías e bancos de algas, além de habitarem ecossistemas estuarinos como manguezais e lagunas. No Brasil, duas espécies de cavalos-marinhos são naturalmente encontradas: *Hippocampus erectus (*Perry, 1810) e *Hippocampus reidi* (Ginsburg, 1933). O *H. reidi*, que é a espécie estudada no presente trabalho, tem distribuição natural restrita ao litoral do Atlântico ocidental, desde o Cabo Hatteras nos Estados Unidos até o litoral do Rio de Janeiro (Rosa et al., 2002).

Assim como ocorre com a maioria das espécies de peixe capturadas para fins ornamentais, a pesca de cavalos-marinhos não figura na maioria dos monitoramentos e estatísticas pesqueiras. No entanto, Vincent (1996) sugere que varias populações naturais de cavalos-marinhos estão ameaçadas de extinção, principalmente devido à pesca predatória. Este mesmo autor estimou que anualmente seriam comercializados mais de 20 milhões de exemplares em todo o mundo.

O crescente comércio internacional de cavalos-marinhos, aliado a degradação dos ambientes costeiros, chama a atenção para a necessidade de conservação do gênero *Hippocampus*. Todas as espécies de *Hippocampu*s estão incluídas no Apêndice II da CITES (Conservation on International Trade in Endangered Species of Wild Flora and Fauna). Desde 2004, os países signatários deste tratado devem tomar medidas a fim de que o comércio não represente uma ameaça às populações naturais (CITES, 2010). As duas espécies que ocorrem no Brasil, *H. reidi* e *H. erectus*, também figuram na lista vermelha da IUCN – *World Conservation Union* (IUCN, 2010).

O cultivo de cavalos-marinhos é visto por alguns especialistas como uma forma de garantir a conservação das populações naturais e atender à crescente demanda do mercado internacional (Vincent, 1996; Payne e Rippingale, 2000; Job et al., 2002). Além disso, os animais provenientes do cultivo apresentam características vantajosas para os aquariofilistas. Cavalos-marinhos selvagens capturados apresentam elevada mortalidade durante o transporte e a adaptação ao cativeiro e, muitas vezes, são vendidos em más condições de saúde. Um dos principais problemas na adaptação dos animais selvagens ao cativeiro está relacionado à dificuldade de aceitação de dietas inertes. O cultivo de juvenis, por sua vez, torna possível o treinamento alimentar, no qual se faz a transição gradual de alimentos vivos para alimentos congelados, mais convenientes aos aquariofilistas. A piscicultura de ornamentais permite também a rastreabilidade dos animais, além de um maior controle sanitário. Em vista disso, nos últimos anos houve um crescente interesse em fechar o ciclo de vida em cativeiro e desenvolver a criação comercial de cavalos-marinhos. A produção científica sobre o cultivo do gênero *Hippocampus* tem sido cada vez maior, assim como a quantidade de empreendimentos voltados para produção comercial (Koldewey e Martin-Smith, 2010).

Um dos principais fatores limitantes para o sucesso na produção de cavalos-marinhos em cativeiro é a baixa sobrevivência dos juvenis nos primeiros dias de vida (Scarratt, 1995). A razão para essa baixa sobrevivência não é totalmente entendida, mas a alimentação adequada é considerada crucial, pois o sucesso do cultivo de larvas e juvenis de peixes marinhos depende do conteúdo nutricional, digestibilidade e tamanho do alimento ofertado (Wilson e Vincent, 1998; Liao et al., 2001).

Objetivo

O objetivo deste estudo é avaliar o efeito de diferentes protocolos de alimentação e manejo no crescimento e sobrevivência do cavalo-marinho *H. reidi* nos primeiros dias de vida.

Revisão de Literatura

Os cavalos-marinhos são peixes teleósteos da família Syngnathidae, estando presentes em ecossistemas costeiros tropicais e temperados. Todas as espécies de cavalos-marinhos estão incluídas em um único gênero: *Hippocampus* (Lourie et al., 1999). Várias características morfológicas reforçam a monofilia (Teske e Matthee, 2004). Estas incluem a cabeça formando um ângulo reto em relação ao corpo, a bolsa incubadora e a adaptação da barbatana caudal em cauda preênsil (Fritzsche, 1980; Teske et al., 2005).

Resultados de análises moleculares comprovam que o gênero *Hippocampus* existe a mais de 15 milhões de anos. Portanto, os ancestrais teriam surgido antes da separação dos oceanos Indo-Pacifico e Atlântico na metade do Período Mioceno, explicando assim a ampla distribuição global destes peixes de reduzida capacidade natatória (Teske et al., 2004; Zalohar et al., 2009).

O comércio de cavalos-marinhos movimenta um mercado do qual participam pelo menos 46 países exportadores (CITES, 2010). Entretanto, apenas 11 destes países comercializam cavalos-marinhos cultivados (Koldewey e Martin-Smith, 2010). No início desta década, o Brasil despontava como um dos principais exportadores de cavalos-marinhos vivos da América Latina, comercializando milhares de indivíduos por ano, principalmente para a Europa e Estados Unidos (Baum e Vincent, 2005). Atualmente, as exportações destes peixes são regulamentadas pela Instrução Normativa Nº 202 do IBAMA, de 22 de outubro de 2008, a qual estabelece cotas anuais de captura e exportação concedidas a empresas ou associações de pescadores. Segundo o IBAMA , nos anos de 2006 e 2007 o Brasil exportou 1.517 e 2.745 cavalos-marinhos, respectivamente. A captura, entretanto, não é regulamentada ou contabilizada, não existindo dados sobre a pesca voltada para o mercado interno. No Brasil, os cavalos-marinhos são comercializados vivos em lojas de aquários, ou secos em mercados públicos para uso em artesanatos e medicina popular (Gasparini et al., 2005).

Grande parte dos estudos sobre cultivo de cavalos-marinhos tem como foco a alimentação inicial dos juvenis, uma vez que este é um fator decisivo para o cultivo destes animais. Como na maioria das espécies de peixes marinhos, o cultivo das fases iniciais de desenvolvimento do cavalo-marinho só é possível com o uso de alimento vivo. Um dos aspectos mais importantes na nutrição nas fases iniciais de desenvolvimento de peixes é proporcionar um nível adequado de ácidos graxos altamente insaturados (HUFA), principalmente o ácido eicosapentaenóico (EPA; 20:5n-3) e o ácido docosaexaenóico (DHA; 22:6n-3) (Sargent et al., 1997). Uma das fontes mais utilizadas de alimento vivo para as fases iniciais de organismos marinhos são os náuplios de *Artemia* sp. O valor nutricional de *Artemia* tem sido extensivamente estudado, variando enormemente entre as várias origens e estágios de desenvolvimento (Léger et al., 1986). A maioria das estirpes de *Artemia* apresenta baixos níveis de DHA, enquanto os níveis de EPA parecem ser específicos de acordo com a origem dos cistos (Navarro et al., 1993). Na tentativa de suprir tais ácidos graxos essenciais, alguns métodos de enriquecimento de *Artemia* têm sido desenvolvidos, utilizando microalgas, dietas microencapsuladas e emulsões de lipídios ricos em HUFA (Watanabe et al., 1983). Vários estudos têm demonstrado que o alimento vivo rico em HUFA melhora as taxas de crescimento, sobrevivência e metamorfose, além de aumentar a resistência a condições de estresse salino, térmico e químico das fases iniciais de desenvolvimento de peixes marinhos (Watanabe, 1993).

Diante da relativa facilidade do cultivo de rotíferos e da obtenção de náuplios de *Artemia*, a piscicultura marinha evoluiu nas últimas décadas baseada principalmente em espécies de peixes cujas formas jovens se adaptam a tais alimentos. Aparentemente, os juvenis de algumas espécies de cavalo-marinho podem ser cultivados apenas com a utilização de *Artemia*, tais como *Hippocampus abdominalis* (Woods, 2000; Woods, 2003, Martinez-Cardenas e Purser, 2007), *Hippocampus hippocampus* (Lenoir et al., 2008) e *Hippocampus withei* (Wong e Benzie, 2003). Por outro lado, em uma revisão sobre a alimentação de juvenis do gênero *Hippocampus*, Alexandre e Simões (2009) relataram que os rotíferos *Brachionus* spp. seriam muito pequenos e teriam composição nutricional inadequada para serem utilizados na primeira alimentação da maioria das espécies do gênero *Hippocampus.*

Atualmente, o cultivo de copépodos tem apresentado avanços significativos, tanto em termos de tecnologia como no uso de novas espécies. Isto tem permitido o cultivo de várias espécies de peixes marinhos antes considerados difíceis de cultivar, entre eles alguns cavalos-marinhos. O alto conteúdo de HUFA e a ampla faixa de tamanho dos copépodos (de 65 a 1000 μm) ao longo do seu ciclo de vida fazem deles um alimento adequado para larvas e juvenis de peixes (Watanabe et al., 1983). No caso dos cavalos-marinhos, várias espécies requerem o uso de copépodos ou apresentam resultados significantemente melhores quando estes são incluídos na alimentação dos juvenis. Wilson e Vincent (1998) fecharam o ciclo de vida em cativeiro de *Hippocampus fuscus* e

Hippocampus barbouri utilizando *Artemia* enriquecida e copépodos cultivados como alimento para os juvenis. Payne e Rippingale (2000) testaram o uso do copépodo *Gladioferens imparipes* e *Artemia* enriquecida no cultivo de *Hippocampus subelongatus*, com resultados significativamente superiores dos indivíduos alimentados apenas com o copépodo.

Outras espécies de cavalo-marinho, como *H. reidi*, são cultivadas utilizando plâncton capturado no ambiente natural, com predomínio de copépodos (Hora e Joyeux, 2009). Neste estudo, uma taxa de sobrevivência de 88,7% até o 109º dia de vida foi observada, sendo que a alimentação consistiu de plâncton coletado do primeiro ao sexto dia seguido por *Artemia* enriquecida até o 25º dia. Após este período, os animais foram alimentados com *Mysidium gracile* vivos ou congelados.

Carlos et al. (2009) realizaram um estudo piloto para avaliar a viabilidade técnica de produzir juvenis de *H. reidi* em tanques-rede colocados no interior de viveiros de camarão. A fase inicial do cultivo foi realizada em tanques plásticos de 200 litros e os juvenis foram alimentados com plâncton selvagem até 74 dias de vida com uma sobrevivência de 64% e tamanho médio de 6,2 cm.

Gomes-Jurado (2009) revisou os recentes avanços no cultivo de *H. reidi* em laboratório utilizando rotíferos e *Artemia* recém eclodida desde o primeiro dia, seguido de *Artemia* enriquecida após o sétimo dia. Nesta revisão, porém, não fica claro se o cultivo utilizando somente estes itens alimentares seria viável, uma vez que o próprio autor relata a ocorrência de copépodos *Tisbe* spp. nos tanques de cultivos. O autor acrescenta que se observa uma melhora nos índices de desempenho zootécnico de *H. reidi* quando há um grande crescimento populacional destes copépodos. Olivotto et al. (2008a) estudaram o uso do copépodo *Tisbe* spp. cultivado em laboratório como complemento à dieta de rotíferos e *Artemia* fornecida a juvenis de *H. reidi*. Os resultados deste estudo mostram que o crescimento e a sobrevivência são significativamente maiores com o uso de *Tisbe*, indicando o valor deste copépodo como complemento às dietas normalmente utilizadas.

Por outro lado, outros estudos indicam que *Tisbe* spp. não seria um alimento ideal na larvicultura de peixes marinhos, principalmente quando utilizado como único item alimentar. Ao testarem *Tisbe* spp. cultivado na alimentação do peixe palhaço *Amphiprion clarkii*, Olivotto et al. (2008b) obtiveram maiores sobrevivências com a combinação de *Tisbe* spp. com outros alimentos, enquanto que o uso exclusivo do copépodo resultou em mortalidade total. Resultados similares foram encontrados por Stottrup e Norsker (1997) quando testaram o copépodo *Tisbe holothuriae* na alimentação de larvas de linguado (*Hippoglossus hippoglossus*). Altas taxas de mortalidade foram observadas com o uso exclusivo deste copépodo, o que foi atribuído a diferenças de tamanho e conteúdo energético entre os náuplios de copépodos e rotíferos e à distribuição espacial dos náuplios de *Tisbe* spp. Como este copépodo tem hábito bentônico, ou seja, costuma ficar no fundo e nas paredes do tanque, estaria menos disponível para as larvas pelágicas do linguado.

Outro fator a se considerar na seleção de um alimento vivo a ser oferecido a uma espécie em uma determinada fase de seu cultivo são as mudanças na preferência alimentar durante o desenvolvimento desta espécie. Sheng et al. (2006) demonstraram que, à medida que o juvenil do cavalo-marinho *Hippocampus trimaculatus* se desenvolve, ele altera sua preferência alimentar. Assim, juvenis de um a três dias mostram um preferência por náuplios de copépodos. Para aqueles com 4 a 10 dias de vida, a preferência passa a ser copepoditos (fases mais adiantadas do desenvolvimento dos copépodos), enquanto os com idade entre 10 a 14 dias de vida preferem copepoditos e adultos dos copépodos e os náuplios de *Moina* spp. A partir do 14º dia, os alimentos preferidos são adultos de copépodos e *Moina* spp.

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Artigo Científico

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Rearing newborn juvenile seahorse *Hippocampus reidi* with different feeding protocols

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ABSTRACT

The main bottleneck for seahorse production is the low survival of juveniles during the early stages of development. Although the causes for low survival are not totally understood, feeding is considered critical. Rearing early stages of marine fish are dependent, among others factors, on the size and nutritional content of live prey organisms. This study evaluated growth and survival of newborn seahorses *Hippocampus reidi* reared with different feeding protocols. The experiment was conducted on 38 liter aquaria connected to a seawater recirculation system, which contained mechanical and biological filters, UV sterilizer and a protein skimmer. Each aquarium had two aeration points near the water surface. Six treatments were tested: Art00h – newly hatched *Artemia* nauplii (5 ind ml^{-1}); Art24h – 24 hours enriched *Artemia* metanauplii (5 ind ml^{-1}); Rot+Art24h – rotifers (*Brachionus plicatilis*; 10 ind ml^{-1}) from day 1 until day 7 followed by enriched *Artemia* metanauplii (5 ind ml^{-1}); Cop – Copepods (*Tisbe biminiensis*; 2 ind ml^{-1}); and Cop+Art24h –*T. biminiensis* (2 ind ml^{-1}) and 24 hours enriched *Artemia* metanauplii (5 ind ml^{-1}). In the treatment Starvation, no feed was provided. All the treatments, except Starvation, received the microalgae *Nannochloropsis oculata* every other day at concentrations ranging from 2.2 x 10^5 to 3.2 x 10^6. Each experimental unit received from 80 to 100 newborn seahorses. At the 14th day the experiment was finished and all remaining live seahorses were measured and weighed. Treatment Cop+Art24h resulted in a significantly higher survival (33.5 ± 5.4%), followed by Cop (6.6 ± 4.8%), Art00h (6.0 ± 8.3%) which were not significantly different from each other, but significantly higher than juveniles fed rotifers and *Artemia* (Rot + Art24h) with a survival of only 0.3% (± 0.5). No survivors were observed in the remaining treatments. Growth parameters were significantly higher in Cop+Art24h and Art00h. The results from this study suggest that the feeding of copepods *T. biminiensis* combined with enriched *Artemia* metanauplii increases growth and survival of the seahorse *H. reidi* during the first two weeks of life.

Keywords: ornamental fish, *Artemia*, rotifer, copepod.

1. INTRODUCTION

Seahorses belong to the Syngnatidae family and are usually found in coastal environments such as reefs, bays, seaweed banks, mangroves and lagoons. In Brazil, two species of seahorses are naturally found: *Hippocampus erectus* (Perry, 1810) and *Hippocampus reidi* (Ginsburg, 1933). The distribution of *H. reidi* is restricted to the western Atlantic coast from Cape Hatteras in the United States to the coast of Rio de Janeiro, Brazil (Rosa et al., 2002).

The increasing international seahorse trade and the degradation of coastal environments have negatively impacted the natural populations of *Hippocampus* in various parts of the world. Currently, all species of the genus *Hippocampus* are listed in Appendix II of CITES (Conservation on International Trade in Endangered Species of Wild Flora and Fauna). Since 2004 all signatory nations must take measures to ensure that trade does not threat natural populations (CITES, 2010). *H. reidi* and *H. erectus* are also cited as vulnerable on the Red List of Threatened Species (IUCN, 2010). Therefore, there is a need to adopt practices that ensure the conservation of the genus *Hippocampus*.

Rearing seahorses in captivity is considered a way of ensuring the conservation of natural populations, at the same time that it meets the increasing international demand (Vincent, 1996; Payne and Rippingale, 2000; Job et al., 2002). As a result, in recent years there has been a growing interest in closing the life cycle in captivity and developing the commercial rearing of several seahorse species. The scientific literature on the biology and aquaculture of *Hippocampus* has also increased lately as has the number of commercial enterprises dealing with seahorse culture (Koldewey and Martin-Smith, 2010).

A major constraint on production of seahorses in captivity is the low survival of juveniles during the first days of life (Scarratt, 1995). The reason for this low survival is still not fully understood, but proper nutrition is considered crucial, since the successful rearing of the early development stages of marine fish depend, among other factors, on the nutritional content, digestibility and size of the food offered (Wilson and Vincent, 1998, Liao et al., 2001). The aim of this study was to assess the effect of different feeding protocols on growth and survival of newborn *H. reidi* juveniles under captivity.

2. MATERIALS AND METHODS

This study was conducted at the Laboratory of Marine Fish Farming, Universidade Federal Rural de Pernambuco - UFRPE, Recife, Brazil. The capture of wild broodstock and their maintenance in captivity were authorized by IBAMA, the Brazilian Environmental Agency (SISBIO 15213-1)

2.1 Rearing system

The experiment was run on 38L glass tanks integrated to a seawater recirculation system containing two mechanical filters (cartridges 25 and 5 mm), UV sterilizer (96 W), biological filter and a protein skimmer. The biological filter consisted of a 150L plastic box with layers of oyster shells, limestone and ceramic rings, which served as a substrate for nitrifying bacteria. Each rearing tank had two aeration points near the water surface, which not only maintained dissolve oxygen levels but also created a vibration in the air-water interface. Preliminary studies indicated that this vibration prevented newborn seahorses from getting stuck in the air-water surface tension.

Temperature and dissolved oxygen levels were measured daily with a digital oxygen meter (Yellow Springs Instruments, YSI model-550A), while pH and salinity were obtained with a pH meter (Tecnal, model TEC-2) and a hand-held optical refractometer, respectively. The photoperiod was maintained at 12 hours light and 12 hours of darkness per day. Means (± standard deviation) of temperature, salinity, pH and dissolved oxygen during the experimental period were 27.4 º C (± 1.2), 34.3 (± 1.2), 8.12 (± 0.14) and 6.67 mg / L (± 0.67), respectively.

2.2 Source of newborn juveniles

Newborns were obtained from wild caught broodstock as well as individuals born in captivity (F1). Captive broodstock were born and maintained in our laboratory for one year under conditions similar to those described in 2.1. When young, these animals were fed the copepod *Tisbe biminiensis* and *Artemia* nauplii, and subsequently received live and frozen *Litopenaeus vannamei* post-larvae and live 15 day-old *Artemia*.

Wild broodstock were caught in the Santa Cruz Channel, Pernambuco, Brazil (Lat 7 ° 48'40 "S 34 ° 53'03 Lon, 7" O). Five pregnant males with expanded pouches were selected. They were kept in captivity for 20 days when three births were obtained.

2.3 Live food culture

Nannochloropsis oculata and *Chaetoceros calcitrans* were used to feed rotifers and copepods. The culture medium for microalgae was Conway (Walne, 1974) to which silicate was added for *C. calcitrans* culture. *C. calcitrans* and *N. oculata* were grown at 30°C under natural photoperiod until reaching exponential growth (1.1×10^6 cells mL^{-1} and 1.4×10^6 cells mL^{-1}, respectively).

Rotifers (*Brachionus plicatilis*) were reared in 5 L cylindrical containers with an airstone and constant light (50 lux). Rotifers were fed *N. oculata* (1.4×10^6 cells mL^{-1}) and a commercial diet (Culture Selco ® Plus, INVE NV, Belgium). Population growth was estimated with a Sedgewick-Rafter chamber on samples fixed in 4% Lugol. The mean density during cultivation was 160 rot.mL^{-1}.

Copepods (*T. biminiensis*) were reared in 5 L containers filled with filtered (5 µm) and UV sterilized seawater. Every four days, nauplii, copepodites and copepods were separated with 250 and 63 µm mesh nets. Adults were retained in the 250 µm mesh, while nauplii and copepodites in the 63 µm mesh. Thereafter, rearing containers were washed, refilled with water, and adults were restocked, while nauplii and copepodites were offered to the seahorses or stored in 5 L containers to produce adults. Copepods, copepodites and nauplii were fed *C. calcitrans* and a commercial feed for ornamental fish (Alcon BASIC, Brazil). From 0.1 to 0.2 g of feed were offered per container, and the daily amount was adjusted in accordance with leftovers. The microalgae were added after each water exchange, maintaining an average concentration of 30 x 10^4 cells mL^{-1}. The water was gently aerated, salinity maintained between 30 and 33 and temperature ranged from 26.5 to 28.0°C.

The *Artemia* used here was AF 480 (INVE Aquaculture, Belgium). The newly hatched nauplii were offered to juvenile or enriched for 24 hours, depending on the experimental treatment. The enrichment was carried out with a commercial emulsion (DC DHA SELCO, INVE Aquaculture, Belgium) in cylindrical 10 L containers to which 0.6 g of emulsion was added per 200,000 nauplii.L^{-1}.

2.4 Experimental design

The experimental design consisted of six treatments with four replicates in time. Each replicate used a batch of newborn juveniles obtained from different males. After hatching, juveniles were gently collected and divided into six groups containing 80 or 100 individuals. The juveniles were then reared on six different feeds treatments until 14 days after birth. Treatments differed on the food type (rotifer, copepod and/or *Artemia*) and their combinations. As these live feeds present different biological characteristics, standardization of some experimental parameters, such as water exchange rates and prey density/biomass, was not possible. All feeding and water renewal protocols were previously tested. Treatments were as follows:

- Art00h. Juveniles were fed daily at 08:00 h with newly hatched *Artemia* at a concentration of 5 nauplii ml^{-1}. Approximately 300% of the water was renewed at night with a 600 µm mesh so that no uneaten *Artemia* would remain in the tank in the next morning;

- Art24h. Juveniles were fed enriched *Artemia* metanauplii at a concentration of 5 ml^{-1}. Feeding and water renewal protocols were identical to treatment Art00h;

- Rot + Art24h. Seahorse juveniles were fed a mixed diet containing rotifers and enriched *Artemia*. From day 1 to day 7, a concentration of 10 rotifers ml^{-1} was maintained with regular monitoring. From day 5 onwards enriched *Artemia* were added at an initial concentration of 0.5 ml^{-1}, which was gradually increased to 5 ml^{-1} on day 7 and maintained at this concentration until the end of experiment. In the first six days, 10% of the water was renewed with a 50µm mesh, but from day 7 onwards approximately 300% of the water was renewed during the night with a 600µm mesh;

- Cop. In this treatment, *T. biminiensis* was the sole feed offered to the seahorses. Due to the benthic habit of this copepod, it was not possible to carry out daily renewal of prey items (as performed in the *Artemia* treatments). However, in previous tests, it was determined that an initial concentration of 2.0 copepods ml^{-1} would be enough to maintain a stable concentration of copepods throughout the experimental period. A daily renewal of 10% of the water was applied to minimize the loss of microalgae in the tanks. Until day 6, a 50µm mesh was used, which was later replaced by a 600µm mesh;

- Art24h + Cop. This treatment consisted of an initial inoculation of 2.0 nauplii or copepodites of *T. biminiensis* per ml, followed by the addition of 1.0 copepod ml^{-1} every two days. Enriched *Artemia* metanauplii were also added daily at the concentration of 3.0 metanauplii ml^{-1}. The renewal of approximately 300% of water was carried out overnight with a 600µm mesh;

- Starvation. The juvenile seahorses received no food. Water was exchanged as in treatments Art00h and Art24h.

All treatments, except starvation, received the microalgae *N. oculata* every other day at concentrations ranging from 0.2 to 3.2 x 10^6 cells ml^{-1}. At the end of the experiment, seahorses were individually weighed and measured. The standard length (Ls) was defined as the sum of the lengths of tail, trunk and head, and the total height, as the sum of crown height, trunk length and tail length (Figure 1) (Lourie et al., 1999). These measures were determined with an ocular micrometer installed in a binocular microscope. The wet weight (mg) was determined using an analytical balance. The condition factor (K) was estimated by $K = 100\ W/Ls^3$, where W = weight (mg) and Ls = standard length (mm).

Throughout the experimental period, the feeding behavior of seahorses was observed. Dead individuals were removed daily from the rearing tanks in the early morning and late afternoon.

2.5 Data Analysis

Results of survival, length, height, weight and condition factor were subjected to one-way analysis of variance (ANOVA) after testing for normality and homogeneity of variance with Kolgomorov-Smirnov and Cochran tests, respectively. When ANOVA detected significant differences between treatments, Tukey's test was applied with a significance level of 5%. Results of survival rate were arcsine square root transformed before submitting to ANOVA. Results are presented as mean ± standard deviation (SD).

3. RESULTS

On day 14, a survival rate of 33.5% (± 5.4) was observed for seahorses fed *T. biminiensis* and *Artemia* metanauplii (Art24h + Cop), which was significantly higher than the other treatments (Table 1). Seahorse juveniles fed exclusively on *T. biminiensis* (Cop) and on newly-hatched *Artemia* nauplii (Art00h) had survival rates of 6.6% (± 4.8%) and 6.0 % (± 8.3), respectively, which were not significantly different from each other, but significantly higher than juveniles fed rotifers and *Artemia* (Rot + Art24h) with a survival of only 0.3% (± 0.5). Starved seahorses presented total mortality on day 7, while all juveniles fed on enriched *Artemia* (Art24h) were dead on day 9 (Figure 2).

Regardless of treatment, a significant number of seahorses fed copepods (Treatments Cop and Cop + Art24) were found dead on day 1 (Figure 2). This occurred especially in the Cop treatment, in which a mean 35.5% mortality were found on day 1. This contrasts with the other treatments where no mortality was observed during the initial 48 hours, even on the starvation group, although a large mortality may be observed between days 3 and 5.

In treatments Cop, Cop + Art24h and Art00h, there was a gradual decrease in mortality between days 5 and 10, while little or no mortality from day 11 onwards.

The length, height and weight of seahorses from treatments Cop + Art24h and Art00h were significantly higher than those fed only with copepods (Table 1). The condition factor was not significantly different between treatments.

4. DISCUSSION

In nature, juvenile seahorse feed mainly on copepods (Payne and Rippindale, 2000). Several studies show that juveniles of *Hippocampus* fed a diet of copepods have significantly higher survival and growth rates than when fed rotifers and/or *Artemia* (Payne and Rippindale, 2000; Job et al., 2006; Olivotto et al., 2008a). Similarly, rearing *H. reidi* with wild plankton composed mainly of copepods usually results in comparatively higher survival rates (Carlos et al, 2009; Hora and Joyeux, 2009).

Although rotifers and *Artemia* are the most frequently used live feeds offered to the early development stages of marine fish, copepods present some advantages over them. The first one refers to the size ranges of copepods from nauplii to adult stage, which can range from 70 to 896 µm in *T. biminiensis* (Souza-Santos et al., 2006). Another advantage is that, unlike rotifers and *Artemia*, copepods are naturally rich in highly unsaturated fatty acids (HUFA), mainly eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic (DHA, 22:6 n-3) which are essential to early developmental stages of marine fish (Sargent et al. 1997; Fleeger, 2005). Even when enriched with n-3 HUFA rich emulsions, *Artemia* has a DHA: EPA ratio that is deemed suboptimal for the early stages of marine fish, mainly due to the instability of HUFA and their catabolism, particularly DHA (McEvoy et al., 1995). While the exact requirements of seahorses for HUFA is virtually unknown, it is likely that these may be similar to larvae and juveniles of other marine fish species (Sheng et al., 2006). In addition to the comparatively higher levels of essential fatty acids, copepods also exhibit higher levels of free amino acids than *Artemia* (Helland et al., 2003), which may be advantageous for juvenile seahorses as these do not present a fully developed digestive system.

From the present results, it was clear that the protocol combining *Artemia* and *T. biminiensis* resulted in significantly higher survival and growth. However, the use of *T. biminiensis* as a single food item was not as efficient as its combination with *Artemia*. Stottrup and Norsker (1997) tested the copepod *Tisbe holothuriae* as a substitute for rotifers on the rearing of *Hippoglossus hippoglossus* larvae. They observed high mortality with the exclusive use of copepods and attributed this to differences in size and energy content between the copepod nauplii and rotifers. Similar results were found by Olivotto et al. (2008b) when feeding clownfish *Amphiprion clarkii* with *Tisbe spp*. In the latter study, higher survival and growth were obtained with the combination of *Tisbe* spp. and other live feeds, while the exclusive use of the copepod resulted in an early mortality of all fish. These results were attributed to the spatial distribution of *Tisbe spp*., which, due to its benthic habit, usually stays at the bottom and the tank walls and thus becomes less available for fish larvae that are distributed preferentially in the water column. Nevertheless, *H. reidi* juveniles were observed feeding on *T. biminiensis* nauplii at the bottom and walls of our tanks on several occasions, confirming what was previously reported by Olivotto et al. (2008a). These authors used *Tisbe* combined with rotifer and *Artemia* in the feeding of *H. reidi* and found that the combination with *Tisbe* also resulted in higher survival and growth than those fed on rotifers and *Artemia* alone.

Juveniles fed *T. biminiensis* had a high mortality during the first 48 hours of life, whereas in the other treatments, including the starved group, juveniles were only found dead after day 3. Seahorse juveniles are born with nutritional reserves that allow survival for at least three days with no exogenous feeding. For instance, Sheng et al (2007) found that *Hippocampus trimaculatus* is able to survive from three to seven days in the absence of food. This is consistent with other studies with seahorse juveniles that report the non-occurrence of mortality during the first three days after birth, regardless of the food offered (Payne and Rippingale, 2000; Chang and Southgate, 2001; Alexander et al., 2009). The mortality of one-day old seahorses in the presence of *T. biminiensis* observed in the present study suggests that the cause would not be related to nutritional content or their spatial distribution. In previous studies in our laboratory, as well in this one, we observed that, in the presence of *T. biminiensis*, juvenile seahorses exhibited a behavior typical of fish affected by ectoparasites or attacked by small predators. Although we are unaware of any literature describing the predation or parasitism of *Tisbe* on fish, it is possible that they are attacking the weakest newborn seahorse juveniles, which can eventually lead to premature death. If this hypothesis is confirmed, we suggest that nauplii and copepodites of *T. biminiensis* should only be offered to seahorses after their third day of life.

5. CONCLUSIONS

The use of nauplii and copepodites of *T. biminiensis* combined with enriched *Artemia* increased the survival of newborn juveniles of *H. reidi*. This highlights the potential use of laboratory-reared *T. biminiensis* as a live feed in the initial culture of this seahorse species. The results also suggest the possibility that *T. biminiensis* predates on *H. reidi* juveniles during the first days of life.

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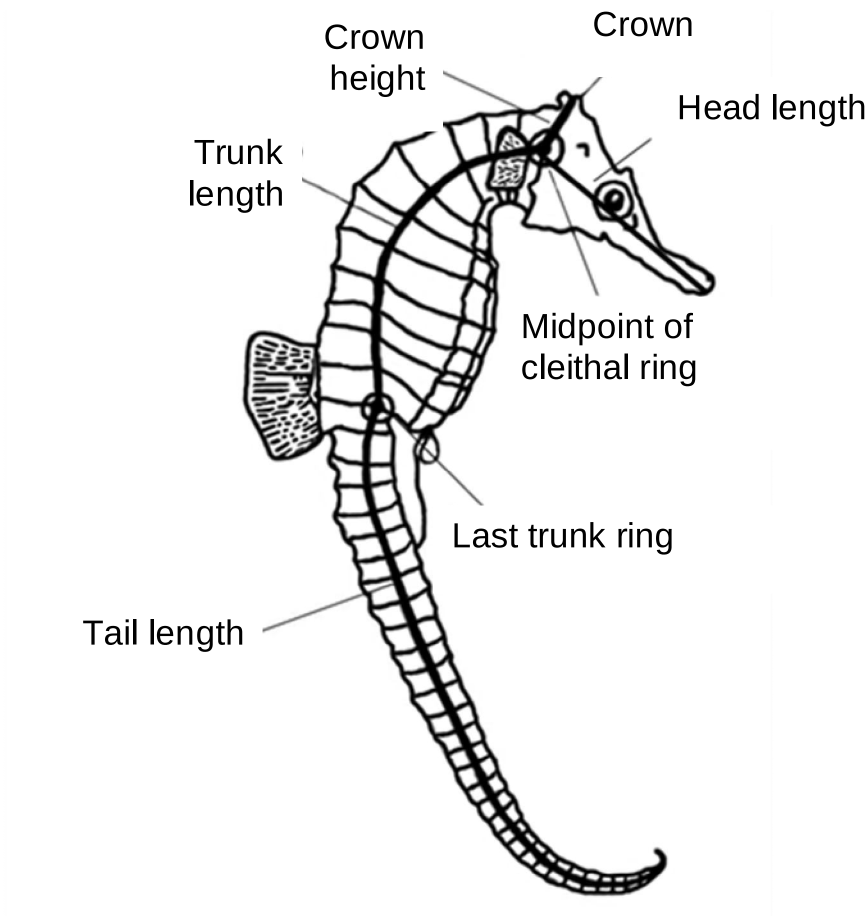

Figure 1 – Measures: total length (sum of tail length, trunk length and head length) and height of the seahorse (the sum of crown height, trunk length and tail length) (Modified from Lourie et al., 1999)

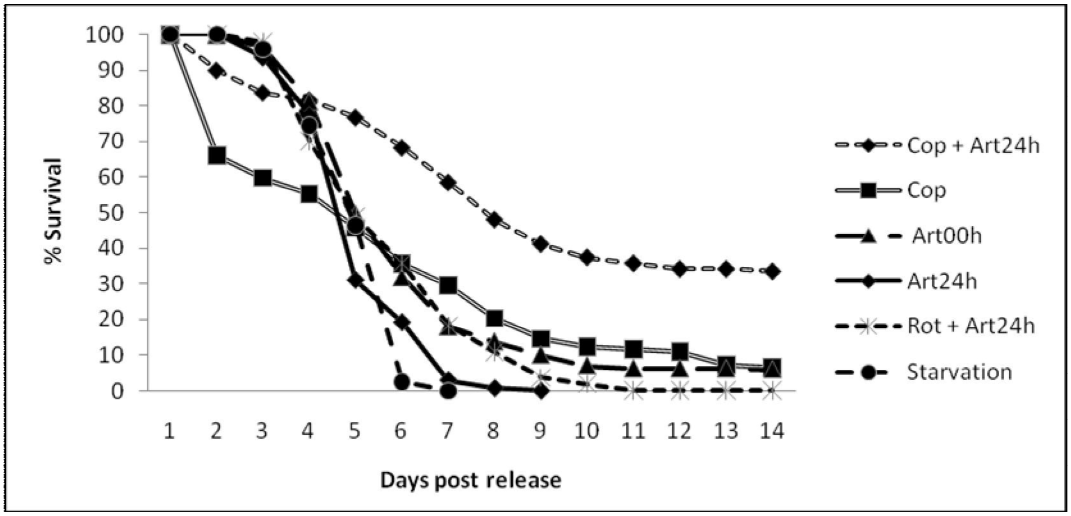

Figure 2. Survival rate (%) of juvenile *Hippocampus reidi* reared under different protocols over the first 14 days of life.

Table 1. Mean (± SD) survival (%), length (mm), height (mm), weight (g) and condition factor (x10^3) of juvenile seahorse *Hippocampus reidi* reared with different feeding protocols during the first 14 days of life.

Treatment	Survival	Length	Height	Weight	Condition factor
Cop+Art24h	33.5^a (± 5.4)	16.89 ^{a} (± 3.14)	14.85 ^{a} (± 2.86)	0.018 ^{a} (± 0.009)	0.346 ^{a} (± 0.084)
Cop	6.6 ^{b} (± 4.8)	14.01 ^{b} (± 2.36)	12.14 ^{b} (± 2.23)	0.010 ^{b} (± 0.006)	0.328 ^{a} (± 0.061)
Art00h	6.0 ^{b} (± 8.3)	17.31 ^{a} (± 2.82)	15.10 ^{a} (± 2.42)	0.019 ^{a} (± 0.007)	0.350 ^{a} (± 0.045)
Art24h	0.0 (± 0.0)	-	-	-	-
Rot+Art24h	0.30 ^{c} (± 0.50)	-	-	-	-
Starvation	0.0 (± 0.0)	-	-	-	-

The letters represents the results of Tukey test.

6. Anexo

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