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DEVELOPMENT OF ALTERNATIVE DIETS FOR THE MASS CULTURE OF THE EUROPEAN CUTTLEFISH *Sepia officinalis*

Dissertação apresentada à Universidade do Algarve para obtenção do grau de Doutor em Aquacultura, especialidade de Nutrição.

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RESUMO

O objectivo desta dissertação era o desenvolvimento de dietas económicas que permitissem o cultivo em grande escala do choco, *Sepia officinalis*. Esta foi dividida em duas grandes áreas de investigação, referentes aos hábitos alimentares das diversas fases da vida do choco, associados ao tamanho e à idade. A primeira parte focou os aspectos de cultivo de misidáceos para alimentar os juvenis de choco. A segunda parte consistiu em determinar os efeitos de dietas artificiais (em substituição de dietas naturais como camarão ou peixe congelado) no cultivo do choco, e determinar se estas poderiam substituir com sucesso as dietas naturais que são normalmente utilizadas.

O cultivo de misidáceos para determinar o potencial para a produção em grande escala foi realizado no National Resource Center for Cephalopods em Galveston, Texas (EUA). A espécie utilizada foi *Mysidopsis almyra* num sistema de cultivo estático. Este era composto por 4 tanques rectangulares (125 cm x 50 cm x 8 cm) com volume de 20 l cada. Estes tanques estavam ligados a um sistema de filtração biológica de 250 l com cascas de ostra partida, onde a degradação dos produtos de excreção era realizada. Mudanças de água e fornecimento de alimento eram realizadas duas vezes por dia e os tanques eram mantidos na escuridão. A produção diária durante 17 semanas foi de 139 $\pm$ 69 misidáceos.d^{-1}. A qualidade da água foi o factor mais importante para o sucesso do cultivo neste sistema. A reprodução foi consistente durante a experiência com concentrações até amónia e nitritos de 1.5 mg.l^{-1} e 0.250 mg.l^{-1}, respectivamente, desde que o pH fosse mantido acima de 7.5. A valores de pH inferiores, a reprodução foi suspensa e a mortalidade aumentou.

Depois de comprovada a viabilidade do sistema, as experiências seguintes testaram diversos tipos de dietas (vivas e artificiais) com o objectivo de reduzir os custos de produção relacionados com a alimentação. Foram testadas 6 dietas; 1) fitoplâncton (*Isochrysis* sp.), 2) uma dieta artificial (Liqualife®, Cargill, Minneapolis, MN), 3) uma dieta mista de zooplâncton (principalmente copepodes) e fitoplâncton, 4) 750 mg.g^{-1} de nauplios de *Artemia* enriquecidos e 250 mg.g^{-1} da dieta artificial, 5) nauplios de *Artemia* e, 6) nauplios de *Artemia* enriquecidos.

Os efeitos de nauplios enriquecidos comparados com nauplios sem enriquecimento na produção, sobrevivência e crescimento foram testados durante 17 semanas. Não se encontraram diferenças significativas ($p>0.05$) no crescimento e produção, mas a sobrevivência de misidáceos durante as duas primeiras semanas de

vida foi melhorada ($p<0.05$) quando alimentados com nauplios enriquecidos. Seguidamente foram testados os efeitos das 6 dietas na produção (em experiências de 30 dias) e sobrevivência de recém-nascidos (15 dias). A produção mais elevada foi obtida com nauplios enriquecidos, embora sem diferenças significativas ($p>0.05$) em relação às dietas 3, 4 e 5. Os misidáceos alimentados com as dietas 1 (fitoplâncton) e 2 (dieta artificial) produziram menos misidáceos ($p<0.05$) em relação às restantes dietas. A sobrevivência de misidáceos alimentados com as dietas 1 e 2 durante os primeiros 15 dias de vida foi também menor ($p<0.05$) em relação às restantes dietas. As dietas 1 e 2 revelaram-se inadequadas para o cultivo de *M. almyra*.

Seguidamente, os efeitos de diferentes densidades de misidáceos e temperaturas na produção, sobrevivência e crescimento foram testados. Até densidades de 100 misidáceos.l^{-1}, a produção ocorreu sem problemas a 26°C, com mudanças de água cada 12 horas. A produção foi de 273 ± 99 misidáceos.d^{-1}. No entanto, a densidades mais elevadas (200 misidáceos.l^{-1}) a cultura colapsou devido à deterioração da qualidade da água. Talvez com mudanças de água mais frequentes (por exemplo, cada 6 horas) estas densidades elevadas pudessem ser mantidas.

Os efeitos da temperatura (26°C, 22°C, 18°C) na produção, sobrevivência e crescimento foram testados posteriormente. A produção mais alta foi obtida a 22°C, embora sem diferenças significativas ($p>0.05$) em relação às restantes temperaturas. A sobrevivência de adultos foi mais elevada ($p<0.05$) às temperaturas mais baixas (22°C e 18°C). A sobrevivência de recém-nascidos até ao sétimo dia de vida seguiu um padrão inverso; foi mais elevada ($p<0.05$) à temperatura mais alta (26°C) e decresceu com o abaixamento de temperatura. A temperatura teve um efeito determinante no crescimento; misidáceos cresceram até 6.1 mm em 15 dias a 26°C, até 6.1 mm em 23 dias a 22°C e até 4.0 mm em 29 dias, a 18°C. Uma estratégia de cultivo desta espécie, para ser optimizada, deve tomar em consideração que adultos e juvenis devem ser cultivados a temperaturas diferentes.

Durante as experiências anteriores, o alimento que apresentou melhores resultados foram os nauplios de *Artemia* enriquecidos. No entanto, o elevado preço dos cistos aumenta consideravelmente os custos de produção. No entanto, os rotíferos (*Brachionus plicatilis*) envolvem custos de produção bastante inferiores. Os efeitos de uma substituição parcial e total de nauplios de *Artemia* por rotíferos foram testados num sistema de cultivo aberto em Tenerife (Espanha). Os misidáceos utilizados foram

identificados como pertencendo ao género *Leptomysis*. As 3 dietas testadas foram; 1) 33% nauplios de *Artemia* + 66% rotíferos, 2) 100% nauplios de *Artemia* e 3) 100% rotíferos. A produção de misidáceos alimentados com as dietas 1 e 2 foi idêntica ($p>0.05$) mas a dieta 3 (rotíferos) promoveu uma menor produção ($p<0.05$) em relação às restantes dietas. A sobrevivência de adultos foi idêntica ($p>0.05$) para as dietas 1 e 2, mas significativamente menor ($p<0.05$) para a dieta 3. Do mesmo modo, a sobrevivência de recém-nascidos durante os primeiros 7 dias foi idêntica ($p>0.05$) com as dietas 1 e 2, mas significativamente menor ($p<0.05$) para misidáceos alimentados exclusivamente com rotíferos (dieta 3). Surpreendentemente, o crescimento de misidáceos alimentados com rotíferos foi semelhante ao das duas restantes dietas durante os primeiros 20 dias. A partir dessa altura, o crescimento proporcionado por esta dieta (dieta 3) reduziu-se consideravelmente. De um modo geral, a produção, sobrevivência e crescimento de misidáceos alimentados exclusivamente com rotíferos foi menor ($p<0.05$) em relação às outras duas dietas.

Com base nestes resultados recomenda-se o seguinte regime alimentar; 1) alimentar com rotíferos durante os primeiros 20 dias e depois 2) alimentar com a dieta mista de rotíferos de nauplios de *Artemia*. Este regime reduz consideravelmente os custos de produção. O uso de rotíferos em substituição da *Artemia* (50%) reduziu os custos relacionados com a alimentação em cerca de 100 vezes. O custo de produção de um rotífero foi 99.5% menor em comparação com um nauplio de *Artemia*. O sucesso obtido ao substituir parte da *Artemia* por rotíferos foi o resultado mais importante obtido das experiências com misidáceos em relação a uma possível cultura em larga escala.

Estudos sobre nutrição com cefalópodes são relativamente recentes e estão directamente associados às dificuldades em manter cefalópodes em cativeiro durante largos períodos. Dietas artificiais enriquecidas com aminoácidos foram dadas a chocos para determinar os seus efeitos no crescimento, sobrevivência e condição. O sistema de cultivo usado era composto por 12 tanques de 500 l de volume cada, associados a um sistema de filtração biológica de 2 andares, com 6000 l de volume.

Apenas as dietas com suplemento integral de metionina e lisina promoveram crescimento ($p<0.05$). Nenhuma das restantes dietas proporcionou crescimento ($p>0.05$). Este facto é indicativo de que estes dois aminoácidos são essenciais para o metabolismo do choco e possivelmente de outros cefalópodes.

Desde um ponto de vista exclusivamente comercial, as dietas artificiais não promoveram bons resultados. As taxas de crescimento de chocos alimentados com estas

dietas, geralmente abaixo de 0.5% do seu peso por dia, foram consideravelmente menores comparadas com taxas de crescimento de chocos alimentados com filetes de peixe gato (até 3.8% do seu peso por dia), com a agravante de serem justamente os filetes de peixe gato que servem de base à elaboração das dietas artificiais. Estas também são bastante mais caras em comparação com os filetes de peixe gato, pois é necessário adicionar proteínas e aminoácidos a estas dietas (agravado por uma mão de obra considerável).

A composição (em aminoácidos) de vários tecidos de choco analisados sugere que os chocos alimentados com estas dietas artificiais estavam numa condição intermédia. Alguns aminoácidos essenciais (os aromáticos e os de cadeia longa) estavam presentes no sangue em concentrações menores, em comparação com chocos alimentados com camarão, mas em concentrações maiores quando comparados com chocos privados de alimento. Os aminoácidos prolina e alanina, usados para produzir energia, estavam também a ser utilizados intensamente, indicando que aminoácidos metabolicamente importantes estavam a desaparecer dos tecidos onde são normalmente armazenados mas estavam ainda presentes no sangue dos chocos alimentados com as dietas artificiais.

Contudo, a aceitação das dietas artificiais pelos chocos (e o crescimento moderado obtido) são passos significativos e importantes para futuramente se concretizar a elaboração de uma dieta artificial para cefalópodes que proporcione boas taxas de crescimento e sobrevivência e possa substituir as dietas naturais, reduzindo os custos de produção.

ABSTRACT

The objective of this dissertation research was to enable the mass culture of *Sepia officinalis* through the development of inexpensive diets. It was divided into two major areas concerning the feeding habits of *S. officinalis* according to their age and size. The first part focused on the culture of mysids to feed hatchling cuttlefish. The second part consisted on testing the effects of artificial surimi diets fed to cuttlefish and determine if they could successfully replace natural food (frozen shrimp or fish) that are normally used.

Mysid culture was evaluated at the National Resource Center for Cephalopods using *Mysidopsis almyra* and a static water system to determine the potential for large-scale culture. The system was composed of 4 rectangular trays (125 cm x 50 cm x 8 cm) with 20 l of water volume each. The trays were connected to a larger 250 l tank filled with crushed oyster shell where biological filtration occurred. Water exchange and feeding were performed twice a day, and the tanks were kept in the dark. Mysid production during 17 weeks was of 139 ± 69 hatchlings.d^{-1}. Water quality proved to be one of the most important factor in culture management when using a static water system. Reproduction occurred at ammonia-nitrogen and nitrite-nitrogen concentrations of 1.5 mg.l^{-1} and 0.250 mg.l^{-1}, respectively, provided that pH was maintained above 7.5. At lower pH values reproduction stopped and mysid mortality considerably increased.

This research demonstrated that this system was effective and the next experiments determined the effects of several food items (live and artificial) on mysid culture in order to reduce production costs related to feeding. In these experiments, 6 diets were used; 1) phytoplankton (*Isochrysis* sp.), 2) an artificial diet (Liqualife®, Cargill, Minneapolis, MN), 3) a mixed diet composed of zooplankton (mainly copepods) and phytoplankton, 4) 750 mg.g^{-1} *Artemia* nauplii and 250 mg.g^{-1} of the artificial diet, 5) *Artemia* nauplii and 6) HUFA enriched *Artemia* nauplii.

The effects of HUFA enriched *Artemia* nauplii and *Artemia* nauplii were tested on production, survival and growth during 17 weeks. Production and growth of mysids fed HUFA enriched nauplii and non-enriched nauplii was not significantly different ($p>0.05$), but survival of hatchlings fed the enriched nauplii was enhanced ($p<0.05$). Afterwards, the effects of 6 diets on mysid production and hatchling survival were tested. Each experiment lasted 30 days. Highest production was obtained when feeding

mysids the HUFA enriched *Artemia* nauplii, but no significant differences ($p>0.05$) were found on production between diets 3, 4, 5 and 6. Nevertheless, mysids fed diet 1 (phytoplankton) and diet 2 (artificial diet) produced significantly less ($p<0.05$) hatchlings than the remaining diets. In similar fashion, survival of hatchlings fed phytoplankton and the artificial diet was significantly lower ($p<0.05$) when compared to the remaining diets. The artificial diet (diet 2) and phytoplankton (diet 1) were not adequate for the culture of *M. almyra*.

The effects of mysid densities and temperatures on production, survival and growth were evaluated in the next experiments. Mysid culture with densities up to 100 mysids.l^{-1} was successful in this system at 26°C, with water exchanges performed every 12 h. Production at this density was 273 ± 99 hatchlings.d^{-1}. However, at higher densities (200 mysids.l^{-1}) the culture collapsed due to poor water quality. It is possible that by performing more frequent water exchanges and hatchling collection (e.g. every 6 h) such densities could be maintained in this system.

The effects of three temperatures (26°C, 22°C and 18°C) on mysid production, survival and growth were tested in the next set of experiments. Mysid production was higher at 22°C compared to 26°C, and 18°C, although there were no significant differences ($p>0.05$) between production at the three temperatures. Survival of adult mysids was significantly higher ($p>0.05$) at the lower temperatures (22°C and 18°C) than at 26°C. However, hatchling survival for 7 days followed a reverse pattern; it was significantly higher ($p<0.05$) at 26°C compared to the lower temperatures. Temperature had a marked effect on growth; mysids grew to 6.1 mm in 15 days at 26°C , to 6.1 mm in 23 days at 22°C and to 4.0 mm in 29 days at 18°C. A cost-effective strategy for the culture of *M. almyra* must address the fact that hatchlings and adults have different optimal temperatures for survival and growth.

During the previous experiments, *Artemia* nauplii was the best food item to grow *M. almyra*. Nevertheless, the high price of *Artemia* cysts added significantly to production costs. In contrast, rotifers (*Brachionus plicatilis*) are cheaper to produce. Therefore, the effects of a total and partial replacement of *Artemia* nauplii by rotifers were tested in a flow-through system. Mysids used in this study were identified as *Leptomysis* sp. Three diets were tested; 1) 33% *Artemia* nauplii + 66% rotifers, 2) 100% *Artemia* nauplii and 3) 100% rotifers. Mysid production was similar ($p>0.05$) for diets 1 and 2, but was significantly lower ($p<0.01$) for diet 3 (rotifers). Survival of spawning

adults fed diets 1 and 2 was similar ($p>0.05$) but spawning adults fed diet 3 had significantly lower survival ($p<0.05$). In a similar fashion, hatchling survival was similar for diets 1 and 2 ($p>0.05$), but hatchlings fed rotifers alone had significantly lower survival ($p<0.05$). Surprisingly, mysid growth was similar among the three diets for 20 days. After that, mysids fed 100% rotifers grew less than mysids fed the other diets. In general, production, survival and growth of mysids fed the rotifer-exclusive diet was significantly lower ($p<0.05$) than the other diets.

Based on the results, the following feeding regime is recommended; 1) feed rotifers during the first 20 days of the life cycle and then, 2) feed a diet of *Artemia* nauplii (33%) and rotifers (66%). This will enhance the large-scale culture of mysids by reducing production costs. The use of rotifers replacing part of the *Artemia* (50%) reduced costs related to feeding by about 100 times; in our laboratory, production costs for one rotifer were 99.5% lower compared to one *Artemia* nauplii. The sucessfull replacement of part of the *Artemia* nauplii used in the culture of *Leptomysis* sp. was the major advance obtained from experiments with mysids during this research and is a major step towards the large-scale culture of mysids.

Cephalopod nutrition is relatively recent and has been characterized by the difficulties of holding cephalopods for long periods of time. Surimi diets (catfish based) enriched with amino acids were fed to cuttlefish to determine the effects on growth, survival and body composition. A recirculating water system composed of 12 tanks (500 l each) connected to a two-tier filtration system (6000 l) was used.

The only prepared (surimi) diets that promoted significant growth ($p<0.05$) were the fully supplemented methionine and lysine diets. None of the other diets promoted growth ($p>0.05$). This indicates that methionine and lysine are indeed essential amino acids for the cuttlefish, and possibly other cephalopods.

From a commercial point of view, the surimi diets did not produce good results. Growth rates of cuttlefish fed these diets (generally below 0.5% $BW.d^{-1}$) were considerably lower compared to catfish fillets (up to 3.8% $BW.d^{-1}$) that serve as a base for their preparation. Furthermore, they are considerably more expensive to produce, due to the addition of proteins and amino acids, and the intensive labour that is required for their preparation.

The body composition indicated that cuttlefish fed the surimi diets were in intermediate condition. Several essential amino acids (from the aromatic and branch-chained groups) were present (in the blood) in lower concentrations compared to

cuttlefish fed shrimp but were present in higher concentration when compared to unfed cuttlefish. Proline and alanine, used for energy, were also being used intensively. This indicates that the metabolically important amino acids were desapearing from storing tissues but were still present in the blood of cuttlefish fed the surimi diets.

Nevertheless, the acceptance of artificial diets (and the moderate growth obtained) are significant steps towards the production of a successful artificial diet for cephalopods.

LIST OF FIGURES

Figures **Page**

LIST OF TABLES

$mg.l^{-1}$ and free amino acid composition of blood as $\mu g.l^{-1}$ (means, in bold; standard deviations below).

Table XIV - Comparison of cuttlefish fed the four methionine diets with shrimp-fed and unfed cuttlefish. The - or + indicates that the concentration of the amino acid in that tissue was significantly lower or higher ($p<0.05$) when compared with shrimp-fed or unfed cuttlefish.

Table XV - Comparison of cuttlefish fed the four lysine diets with shrimp-fed and unfed cuttlefish. The - or + indicates that the concentration of the amino acid in that tissue was significantly lower or higher ($p<0.05$) when compared with shrimp-fed or unfed cuttlefish.

Table XVI - Feeding rates, food conversions and Instantaneous Growth Rates for cuttlefish kept in isolated chambers and fed 4 different surimi diets.

Table XVII – Results obtained from the experiment testing the amino acid Leucine. Mean Instantaneous Growth Rates (MIGR) and feeding rates are expressed as $\%BW.d^{-1}$. Mortality is expressed as a percentage.

Table XVIII - Feeding rates, food conversions and Instantaneous Growth Rates for cuttlefish kept in individual chambers and fed thawed catfish.

Table XIX - Comparison between amino acid composition ($mg.g^{-1}$) of surimi diets used in the methionine experiment and amino acid composition in thawed catfish fillets.

TABLE OF CONTENTS

1. INTRODUCTION

Cephalopods are dynamic predators that occupy a dominant role in the trophic structures of the marine ecosystem and are an important food source for humans (Boletzky & Hanlon, 1983). All cephalopods are carnivores (Boucher-Rodoni *et al.*, 1987; Lee *et al.*, 1994). A market for cultured cephalopods has arisen for the past few years in two fields of biological sciences; one is the analysis of life cycles and the second is using cephalopods as experimental animals (Boletzky & Hanlon, 1983). Cephalopods have been used as research models in neuroscience, physiology, immunology, nutritional biochemistry, aging, oncology, molecular biology and ethology (Gilbert *et al.*, 1990; Oestmann *et al.*, 1997). Since cephalopods are regularly eaten in many regions of the world, they also have commercial importance. Cuttlefishes and other cephalopods are fished in many regions of the world (especially the Orient and Southern Europe) for human consumption (Lee *et al.*, 1998).

Due to their rapid growth rates (>10% Body Weight.d^{-1}) and protein dependant metabolism, cephalopods are remarkable among poikilotherms (Forsythe & Van Heukelem, 1987; Lee *et al.*, 1998) and their potential for mariculture has been recognized for many years (Hanlon *et al.*, 1991). When culturing cephalopods, several aspects have to be taken in consideration. Water quality and turnover rate are key factors. Temperature, salinity, pH, ammonia, nitrites, nitrates and dissolved oxygen are among those parameters that have to be closely monitored (Boletzky & Hanlon, 1983; Yang *et al.*, 1986; Lee, 1995; Lee *et al.*, 1995). However, the limited dietary alternatives for culturing cephalopods remains an important setback to achieve large cephalopod cultures (LaRoe, 1971; Segawa, 1987; DeRusha *et al.*, 1989; DiMarco *et al.*, 1993; Lee, 1994). The inability to grow cephalopods on an inexpensive and storable artificial diet has inhibited cephalopod mariculture on a commercial basis (O'Dor & Wells, 1987). Therefore, the formulation of such a diet is one of the primary and achievable goals needed for the successful large-scale culture of cephalopods (Lee, 1994).

The cuttlefish *Sepia officinalis* Linnaeus, 1758 (Figure 1), is one of the best known cephalopods of the old world, being very abundant in the Mediterranean Sea and Eastern Atlantic (Boletzky, 1983). The largest specimens can reach nearly half a meter in body length. *S. officinalis* inhabits coastal waters and the continental shelf at depths not greater than 150 m, and generally moves inshore to spawn (Boletzky, 1983). *S. officinalis* usually lay their eggs in shallow water at depths not greater than 30 to 40 m. Females become mature at mantle lengths between 11 to 25 cm. The number of eggs

vary with size; small females do not usually have more than 150 mature eggs while large females can have over 500 eggs (Mangold-Wirz, 1963). The main spawning season in nature covers spring and summer but spawning has also been observed in winter (Mangold-Wirz, 1963). Spawning normally begins soon after copulation and in nature a female can release all her mature eggs in a few days. However, in laboratory conditions, females tend to spawn over longer periods that could reach several months (Boletzky, 1975). Eggs are fixed individually to slender objects usually no more than 1 cm in diameter (cables, nets, soft plants, dead animals and tube worms, among others) through a ring shaped basal structure. Hatchlings usually measure between 6 and 9 mm and display basic behavior similar to adults (Boletzky, 1979). The anatomy of *S. officinalis* has been fully described by Tompsett (1939).

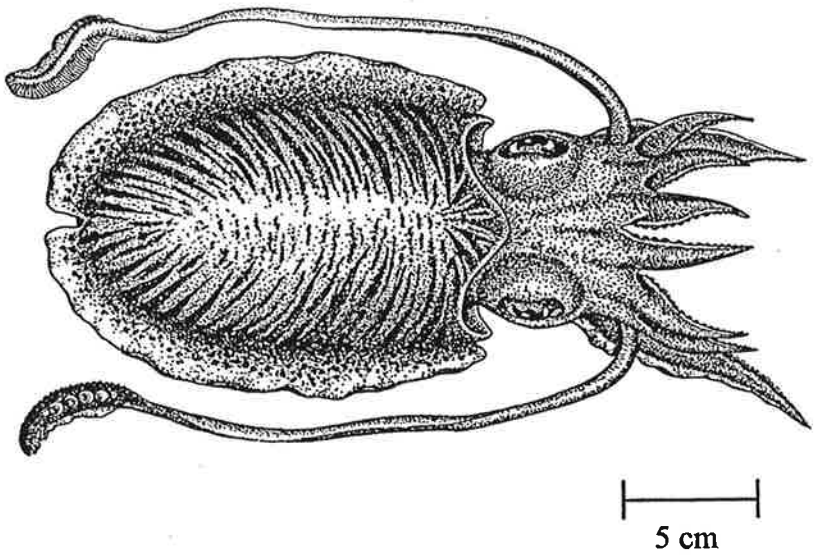

Figure 1 – Schematic representation (dorsal view) of the European cutllefish, *S. officinalis*. Adapted from Guerra, (1992).

There is a wide range of prey that *S. officinalis* feeds on (Boletzky, 1983; Boucher-Rodoni *et al.*, 1987) but culture in the laboratory has been successful using different prey animals (Pascual, 1978; Boletzky & Hanlon, 1983; DeRusha *et al.*, 1989; Lee *et al.*, 1991; Castro *et al.*, 1993; Castro & Lee, 1994; Forsythe *et al.*, 1994). Natural life span is variable, depending on several factors such as water temperature (Forsythe *et al.*, 1994) and it appears to vary from 1 to 2 years (Boletzky, 1983). Life span in laboratory conditions varied between 7.8 and 14 months (Forsythe *et al.*, 1994).

S. officinalis, is one of the most easily cultured of all cephalopod molluscs, having been maintained, reared and cultured in aquaria in both Europe and North America for several generations (Schroder, 1966; Richard, 1975; Pascual, 1978; Zahn, 1979; Boletzky & Hanlon, 1983; Hanlon, 1987; Forsythe *et al.*, 1991, 1994; Lee *et al.*, 1998) and has been the first cephalopod species to be cultured in an inland aquarium for several generations (Boletzky, 1983). This species seems to be the best candidate for life in captivity (Lee *et al.*, 1998) since it has: 1) large eggs, 2) voracious hatchlings, 3) high hatchling survival, 4) sedentary behavior, 5) tolerance to crowding, handling and shipping, 6) acceptance of nonliving foods 7) rapid initial growth rates (>6% $BW.d^{-1}$) and 8) most importantly, been shown to easily reproduce in captivity (Forsythe *et al.*, 1991, 1994). Physiological characteristics of *S. officinalis* are characterized by high adaptability, allowing young cuttlefish to survive under unfavorable conditions (Boletzky, 1979).

The availability of appropriate and economical feeds is one of the most important factors for promoting aquaculture (Chen & Long, 1991). Further, identification at large scale production of acceptable live feeds is of major importance for a successful culture of invertebrate larvae and fish fry (Chen & Long, 1991; Lim, 1991). Among some of the most important invertebrates used in larval culture are the rotifer, *Brachionus plicatilis* (Olsen *et al.*, 1993) and *Artemia* sp. (Sorgeloos *et al.*, 1983; Dhert *et al.*, 1993). Nevertheless, some high value aquatic animals (e.g., cephalopods) require alternative live prey for their culture during the first part of their life (Pascual, 1978; DeRusha *et al.*, 1989; Forsythe *et al.*, 1994).

Live mysids are frequently used as food for cephalopods (e.g. cuttlefish and squid) during the early stages of their life (Forsythe & Hanlon, 1980; Hanlon *et al.*, 1991; Forsythe *et al.*, 1994; Lee *et al.*, 1994, 1998). Mysids enabled the mass culture of cephalopods (e.g., cuttlefish) at the National Resource Center for Cephalopods (NRCC) in Galveston, Texas, USA (Yang *et al.*, 1986; Turk *et al.*, 1986; Hanlon *et al.*, 1991; Lee *et al.*, 1994, 1998). Every species of cephalopod cultured at the NRCC depend on mysids during their early growth stages. Feeding is extremely important, particularly at this stage of the life cycle. Boletzky & Wiedman (1978) reported that in underfed cuttlefish, certain differentiation can be greatly delayed independent of water temperature. Other live feeds such as *Artemia* nauplii have been fed to hatchling cephalopods, but results obtained for growth or survival were worse when compared to mysids (Pascual, 1978; DeRusha *et al.*, 1989; Hanley *et al.*, 1998). Mysid collection

requires extensive hand labor, as mysids have to be collected around Galveston Island using trawling plankton nets pulled by hand. Another problem is the mysid availability; during the winter months mysids are much harder to find than in the summer. Therefore, the ability to culture mysids in large numbers at low costs would be a significant step toward the successful commercial culture of cephalopods.

Mysids (Figure 2) are important prey organisms for many fish and crustacean species (Tattersall & Tattersall, 1951; Ogle & Price, 1976; Mauchline, 1980; Lussier *et al.*, 1988; Kuhn *et al.*, 1991; Fish & Fish, 1996) and therefore play an important role in the transfer of energy in the aquatic system (Carr & Adams, 1973; Fish & Fish, 1996).

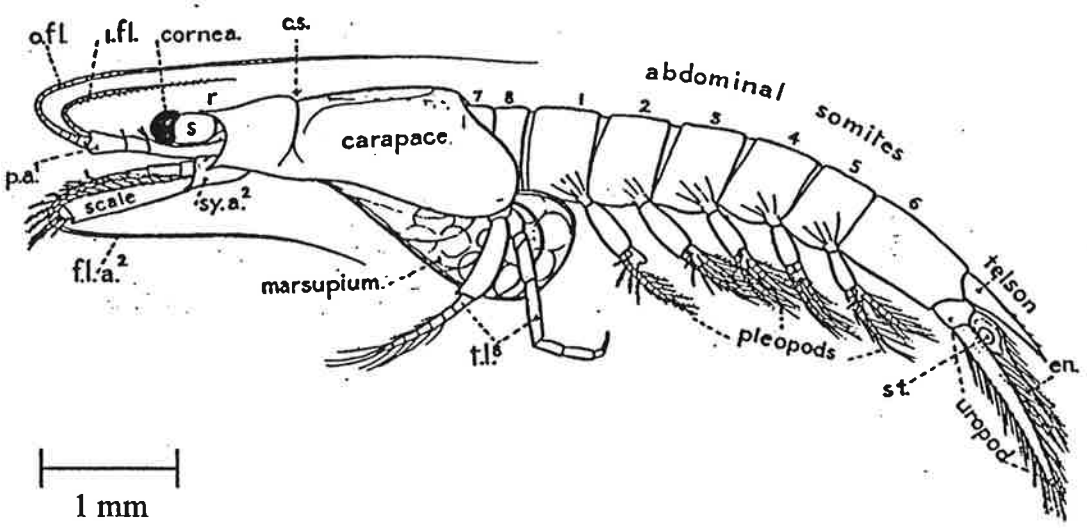

Figure 2 – Schematic representation of a typical female mysid shrimp; *p.a.*^{1}, peduncule of antennule; *o. fl.* and *i. fl.*, outer and inner flagella of antennule; *sy.* a^{2}, sympod of antenna; scale, antennal scale: *fl.* a^{2}, flagellum of antenna; *s*, stalk of eye; *r*, rostrum; *c.s.*, cervical sulcus; 7 and 8, 7th and 8th thoracic somites; 1-6, abdominal somites; *en.*, endopod of uropod; *st.*, statocyst; *t.l.*^{8}, 8th thoracic limb. Adapted from Tattersall & Tattersall, (1951).

Mysids are omnivorous and can feed on small planktonic organisms, such as diatoms or copepods, and organic detritus (Tattersall & Tattersall, 1951; Odum & Heald, 1972; Mauchline, 1980; Jerlin & Wooldridge, 1994; Dauby, 1995). Cannibalism is also present in some species (Reitsema & Neff, 1980). They usually present two different methods of feeding, either grabbing their food with the thoracic endopods or

feeding on fine suspended particles that they filter from the oncoming currents produced by movements of their thoracic limbs (Tattersall & Tattersall, 1951). Most species are filter feeders and do not select their prey (Dauby, 1995). Mysids usually swim close to the bottom by day but during the night they undertake vertical migrations upward, reaching the surface in some cases. Evidence of vertical migrations have been observed for several species (Schlacher & Wooldridge, 1994).

Recently, Price *et al.*, (1994) proposed a new genus (*Americamysis*) including 6 species, of which two were new. Among the species proposed to compose this new genus, 4 species (*Mysidopsis almyra, M. bahia, M. bigelowi* and *M. taironana)* were part of the genus *Mysidopsis* Sars, 1864. The new designation for the former species (*Americamysis almyra, A. bahia, A. bigelowi* and *A. taironana*) has already been accepted by some authors, although in the literature consulted from the year 1995 up to the present, others still include some of these species in the genus *Mysidopsis* (e.g., *M. bahia*). Therefore, during this dissertation, the designation of the species of the genus *Mysidopsis* used or cited in this study will be maintained.

Several species of *Mysidopsis* have been grown in the laboratory (Reitsema & Neff, 1980; McKenney, 1987; Lussier *et al.*, 1988; Ward, 1991). Although mysids have been cultured to be used extensively in acute and chronic toxicity tests (Nimmo *et al.*, 1978; Reitsema & Neff, 1980; Nimmo & Hamaker, 1982; McKenney, 1987; Kuhn *et al.*, 1991), production is usually low and not enough to sustain the culture of several hundreds of cuttlefish hatchlings during their early stages of their life.

After this initial first feeding period in which live feed is essential, there are several fresh or frozen foods on which juvenile or adult cephalopods can be maintained or grown (Boletzky & Hanlon, 1983; Toll & Strain, 1988; DeRusha *et al.*, 1989; Lee *et al.*, 1991; DiMarco *et al.*, 1993). Nevertheless, fifty percent of the labor required to culture cephalopods involves collecting, holding and feeding live prey (Lee, 1994). Collecting and immediately freezing the prey animals reduces labor costs by one-third. In contrast, the labor required to prepare an artificial food is significantly lower; for example, if an artificial diet is substituted for live food, an additional cost reduction of 20% is estimated. An even greater reduction can be obtained by using commercial production methods to produce larger quantities of diet (Lee, 1994).

The initial palatability of pelleted diets was tested on cuttlefish at the NRCC by Lee *et al.*, (1991). During this study, cuttlefish ate several diets, including turkey hot dog (an artificially prepared food composed of turkey and artificial pork flavoring) but

did not eat surimi (pollock with artificial crab flavoring). In the last few years, feeding experiments have been conducted with either moist or dry pellets (Lee *et al.*, 1991; Castro *et al.*, 1993) or surimi (fish myofibrillar protein concentrate) (Castro *et al.*, 1993; Castro & Lee, 1994), and demonstrated that cuttlefish readily accept different kinds of prepared diets. Castro *et al.*, 1993 showed that cuttlefish accepted a catfish-based surimi diet, although Instantaneous Growth Rates (IGR) were significantly lower ($p<0.01$) compared to cuttlefish fed raw shrimp. Further studies were conducted by Castro & Lee (1994) feeding artificial diets to cuttlefish. In this case, a surimi diet enriched with casein produced significant growth.

The objective of this dissertation research was to enable the mass culture of *S. officinalis* through the development of inexpensive diets. It is divided into two major areas concerning the distinct feeding habits of *S. officinalis* according to their age and size. The first part concerns the culture of mysids to feed hatchling cuttlefish (up to two to three weeks old). Therefore, mysid culture was evaluated at the NRCC, using *M. almyra* and a small static water system. This system was tested initially to determine if it was adequate to produce mysids in large scale, using *Artemia* nauplii to feed the mysids. After determining that it was a viable system, other experiments were conducted to try and reduce production costs. First, *Artemia* nauplii enriched with HUFA (Selco®) were used to determine if it would increase production and survival. After these experiments, several other food items were evaluated to try and reduce production costs, since *Artemia* cysts are expensive and would not enable the commercial mass culture of mysids. Several experiments testing the effects of different temperatures and densities were conducted afterwards in this system in order to optimize mysid production. Another mysid (*Leptomysis* sp.) was used at the Spanish Oceanographic Institute of Tenerife (Canary Islands, Spain) to test the effects of a total or partial replacement of *Artemia* nauplii by rotifers on mysid production, survival and growth. If successful, the use of rotifers would greatly reduce production costs, increasing chances of culturing mysids in large scale. The second part of this research consisted on testing the effects of artificial surimi diets and determine if they could successfully replace natural food (frozen shrimp or fish) that are normally used in cuttlefish culture. During these experiments, surimi (catfish based) diets enriched with proteins and amino acids were fed to cuttlefish and their effects on growth, survival and body composition were evaluated.

The four chapters in this dissertation (2 through 5) dealing with mysid culture are identical to four papers submitted for publication. The first two papers (chapters 2 and 3; Domingues *et al.*, 1998, 1999a) were accepted and published in the journals "Aquaculture International" and "Aquaculture Research", respectively. The third paper (chapter 4; Domingues *et al.*, 1999b) was also accepted by the journal "Aquaculture Nutrition" and is currently "in press". Chapter 5 (Domingues *et al.*, 1999c), concerning mysid culture in Tenerife was submitted to the journal "Aquaculture Research". As for chapter 6, part of the data (experiments testing the amino acids methionine and lysine) was gathered in a paper (Domingues *et al.*, 1999d) submitted to the journal "Marine Biology" while the remaining data in that chapter was not submitted.

2. PILOT-SCALE PRODUCTION OF MYSID SHRIMP IN A STATIC WATER SYSTEM.

2.1 Introduction

The mysid shrimp *M. almyra* (Bowman, 1964) is a small crustacean that can grow to 1 cm in length (Bowman, 1964) and weigh approximately 0.5 g as an adult (Price, 1976). It inhabits estuarine waters and has been reported in Florida, Louisiana and Texas (Price, 1976); the probable distribution of this species is the northern part of the Gulf of Mexico (Reitsema & Neff, 1980; McKenney, 1994). According to Price (1976), this is the dominant mysid species off Galveston Island, comprising 82% of the mysids collected in the area. Brood sizes for *M. almyra* range from 3 to 42 and are directly related to the size of the female (Price, 1976). Although this is a relatively low reproduction rate, the fact that mysids have a short reproductive cycle (14-21 days) makes them a potentially good organism for large scale culture (Reitsema & Neff, 1980).

Because M*ysidopsis* species have a wide geographic distribution and they are sensitive to toxic substances, they have been used frequently for toxicity tests (Reitsema & Neff, 1980; Lussier *et al.*, 1988; Ward, 1991). Although *M. bahia* is the species most frequently used, *M. bigelowi* and *M. almyra* have also been used (Reitsema, 1981; Gentile *et al.*, 1982). Species of the genus *Mysidopsis* have been cultured in the laboratory (Nimmo *et al.*, 1977; Reitsema and Neff, 1980; Gentile *et al.*, 1982; Ward, 1984; Lussier *et al.*, 1988; Kuhn *et al.*, 1991). The mysid *M. bahia* became reproductively mature in 12 days at 29°C in laboratory trials (Nimmo *et al.*,1977); *M. almyra* has been raised successfully in a recirculating artificial seawater system over a 3 year period (Reitsema & Neff, 1980).

The objective of this research was to culture the estuarine opossum shrimp *M. almyra* in a static water system and to compare these results to earlier scientific reports that used flowing water. Preliminary trials were performed in a system composed of four small triangular trays (area of 1,750 cm^2, water depth of 6 cm and volume of 10 l each) placed outside during the summer months; these trays were not connected to any filtration system. Since the results obtained were satisfactory, a larger system connected to a biological filter was built inside. Conditions in this new system were based on the environmental conditions found in the estuaries of

Galveston Bay; temperature and salinity were maintained at average summer values for Galveston Bay. Two daily water exchanges resembled tidal cycles and they were scheduled to keep metabolites within limits. The tanks were kept dark, simulating very low light conditions characteristic of the bottom where mysids are found (e.g., extremely turbid water). The results demonstrated that mysids could be cultured effectively in the laboratory under these conditions and the emphasis of this research then focused on culture management, mysid production, survival and water quality.

2.2 Material and methods

Mysids were collected in marshes around Galveston Bay (Texas, USA) (Figure 3), using bottom trawl nets. Mysids were selected on the basis of species, condition and size. The reported sex ratio for *M. almyra* is 50:50 (Price, 1976) so selection by sex was not attempted.

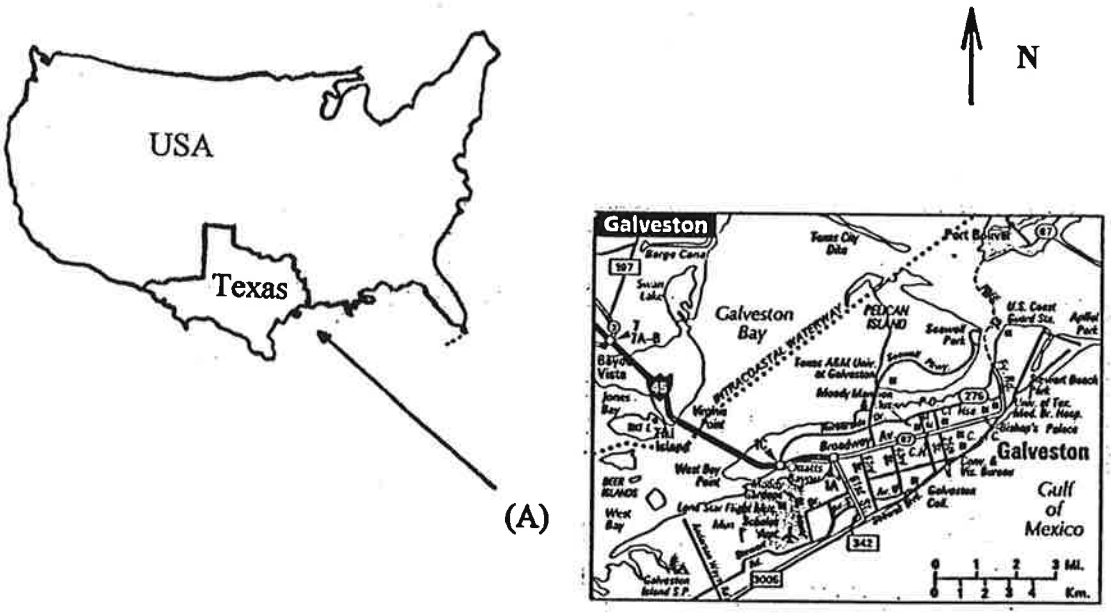

Figure 3 – Galveston Island (A), where mysids were collected and experiments were conducted (at the National Resource Center for Cephalopods; NRCC).

2.2.1 Preliminary trials

A total of 120 mysids were introduced in each of four shallow triangular trays with a total area of 1,750 cm^2 each. Water depth was 6 cm and total water volume was approximately 10 l per tray. There was no water circulation in the trays that were covered with black plastic in order to maintain the mysids in the dark. These trays were not connected to a pump or a filtration system, but about 30% of the water in all

four trays was replaced daily when siphoning uneaten food and wastes. The mysids were fed twice a day at 09:00 h and 17:00 h. Two trays were fed with *Artemia* nauplii (24 h incubation at 28°C and 15 ppt). Nauplii were collected after 24 h in hatching system and immediately fed to the mysids *ad libitum* after each water change. Density of *Artemia* nauplii being fed was not determined since the objective related to feeding in these preliminary trials was only to assure that mysids were fed in excess to prevent or reduce cannibalism. In culture systems, mysids will cannibalize their young or injured adults if sufficient food is not provided (Lussier *et al.*, 1988). The remaining two trays received dry pelleted shrimp feed (Rangen 35% protein commercial shrimp feed) manufactured by Rangen Inc, Angleton, Texas. Composition of this diet was approximately 35% crude protein, 8% crude fat, 4% crude fiber, 15% ash and 1% Phosphorous. The trial lasted 45 days and mysids were counted on days 1, 15, 30 and 45. The newly hatched mysids were allowed to grow in the trays.

2.2.2 Growth trials

Three sets of trials (15 days each) were conducted to determine growth rates of mysids from hatching to post reproduction. No replicates were used in each trial. The same trays used in the preliminary trials were used in these trials through the natural range of summer temperatures on Galveston Island. Water temperature in these trials was not controlled. It varied throughout the day and was different for the three sets of trials. This was due to the fact that the trays were outside in the shade. During the first, second and third trials, temperatures ranged from 24°C to 31°C, 25°C to 30°C, and 17°C to 28°C, respectively. During these three trials, ammonia-nitrogen remained below 0.5 $mg.l^{-1}$ and pH varied between 7.5 and 8.0; salinity was maintained between 22 and 24 ppt.

There was no water circulation in the trays and mysids were kept in the dark. A partial water change (30%) was performed every day when siphoning uneaten food and wastes from the trays. For each of these growth trials, 100 newly hatched mysids were placed in each tray. *Artemia* nauplii were hatched for 24 h and fed to the mysids. Concentration of *Artemia* nauplii being fed was not determined but excess feeding (to reduce cannibalism) was delivered to assure that *Artemia* nauplii were still present in the trays before siphoning and the next feeding period. Every second day, six mysids

were collected from each tray and measured. Each trial had duration of 15 days in order to assure that maturity and reproduction was achieved by mysids.

Statistical analysis (Zar, 1984) was performed using Kruskal-Wallis one-way ANOVA's to evaluate differences in growth between the three trials (as well as within each trial).

2.2.3 Pilot-scale trials

The static water system (Figure 4) was composed of four rectangular culture trays (125 cm Length x 50 cm Width x 8 cm Depth with a water volume of approximately 20 l), but during these experiments only one of the top and bottom trays were used.

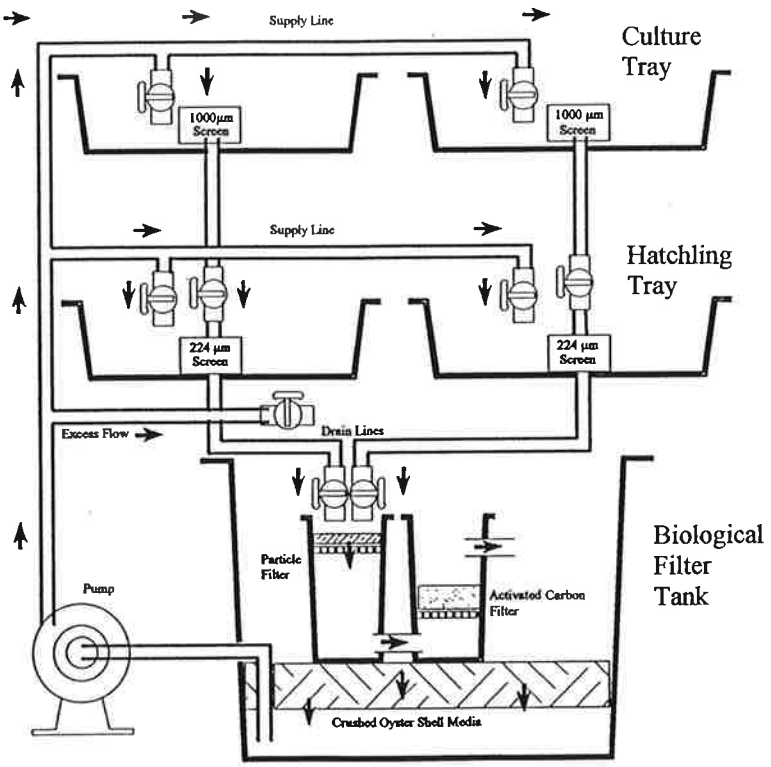

Figure 4 – The diagram represents a static water system used to culture mysids (*M. almyra*). The two top trays (culture trays) were used for holding broodstock, the two middle trays (hatchling trays) were used for the hatchlings, and the bottom tank was a biological filter tank, containing a particle filter, activated carbon and submerged, oyster shell biological filter. Water drained through the screened cores in the culture and hatchling trays into the biological filtration tank. It was then drawn from below the crushed oyster shell media, and pumped back into the culture and hatchling trays through supply lines.

The trays were plumbed to a third tank (water volume of about 250 l) containing crushed oyster shell (6x4 mm average dimensions) where biological filtration took place. One tray was placed directly above the other. The top tray (culture tray) had a 1 mm mesh core (10 cm high and 5 cm in diameter) connected to a valved outlet on the bottom. Temperature in the trays were maintained at 27 ± 2°C by heating the room; salinities were maintained at 20 ± 2 ppt. System water quality was determined twice a week; water samples were taken from both trays and the biological filter. Ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen were measured using HACH® reagents. Tests used were the salicylate, diazotization and cadmium reduction methods, respectively. Accuracy according to the manufacturer was 0.015 $mg.l^{-1}$, 0.0007 $mg.l^{-1}$ and 0.010 $mg.l^{-1}$ for the three tests, respectively. The pH was measured using a pH meter (Radiometer, Denmark). The room was kept dark; lights were turned on twice a day (for about 1 h) to collect hatchlings and exchange water. Light for maintenance was provided by two 30 W fluorescent bulbs covered with a red filter.

Water was exchanged twice a day between each tray and the biological filter at 09:00 h and 21:00 h. Most of the water drained to the bottom tray (hatchling tray) and newly hatched mysids were collected into a 224μm mesh sieve. Water exchange lasted 10 minutes at an average flow rate of 5 $l.minute^{-1}$ (two and a half tank volume exchanges) in order to promote a total water exchange in the culture and hatching trays. The 1 mm mesh core used in the culture tray prevented the adult mysids from escaping but allowed the mysid hatchlings (<2 mm in length) to pass into a collection sieve (224 μm mesh).

Optimal water flow for hatchling collection was found to be 8 $l.minute^{-1}$. At this flow rate, almost all of the hatchlings were collected into the sieve in good condition. Careful observations after collecting periods showed that the majority of the hatchlings had been collected at this flow rate, since very few hatchlings were present in the culture tray. Faster flow rates caused strong currents so that adult mysids could not swim, dragging them against the core while slower flow rates were not strong enough to overcome the swimming ability of newly hatched mysids. The hatchlings were counted and released into the hatching tray after each collecting period. Daily production was obtained by adding production from the two daily

collecting periods. Mysids of all sizes were retained in the hatching tray by a core of 224 μm mesh.

The top tray was stocked with 1,000 adult mysids. The mysids were fed twice a day after water exchange. *Artemia* cysts were hatched under fluorescent light at 28°C and 20 ppt for 24 h, harvested and fed immediately to the mysids. *Artemia* nauplii concentration in the culture tray in relation to water volume and mysid concentration was 10 nauplii.ml^{-1} and 200 nauplii.mysid^{-1} respectively. Older *Artemia* were not fed because older instar stages can contain up to 39% less energy and 34% less dry weight than freshly hatched nauplii (Vanhaecke *et al.*, 1983).

Mysid production both for every two weeks and for the total length of the experiment was expressed in number of hatchlings produced.d^{-1}. It was determined by adding the total number of hatchlings produced in those periods and dividing them by either 14 or by the total length of the experiment in days. Every 14 days the hatchling tray was drained and cleaned; the mysids in the hatchling tray were collected and counted in order to determine the survival rate. The survival trials had duration of 14 days to assure that mysids could reach sexual maturity.

Survival of adult mysids was determined after the first 5 weeks of the experiment and then every 4 weeks. The remaining mysids in the culture tray after these periods were counted to determine mortality and adult mysids were added to raise their numbers to 1000. Survival was expressed as a percentage.

2.3 Results

2.3.1 Preliminary trials

Results from the preliminary trials (Table I, Appendix) indicated that mysids would survive and grow in physical conditions similar to those found at their collection sites. Temperature varied between 26 °C and 32°C and salinity ranged from 17 ppt to 22 ppt. Ammonia-nitrogen levels in the trays ranged 0.4 mg.l^{-1} to 0.6 mg.l^{-1} and pH ranged 7.5 to 8.0. Hatchlings (<2 mm total length) were found in the trays on each sampling date, indicating that reproduction was taking place consistently under these experimental conditions.

At the end of 45 days, for mysids fed *Artemia* nauplii populations increased 323.3% in one tray and 256.6% in the second tray. About one-third of the mysids in each tray were less than 48 h old.

Mysid populations in the trays fed a commercial formulated dry pelleted shrimp feed (Rangen 35) decreased by half in the first 14 days and the trial was suspended after 30 days when mysid numbers declined to 20% of the original population. No juveniles were found in these trays.

2.3.2 Growth trials

Figure 5 shows mysid growth for the three trials. Mean body length at the beginning of the first, second and third trials was 2.0 ± 0.1 mm, 1.7 ± 0.1 mm and 1.9 ± 0.1 mm, respectively.

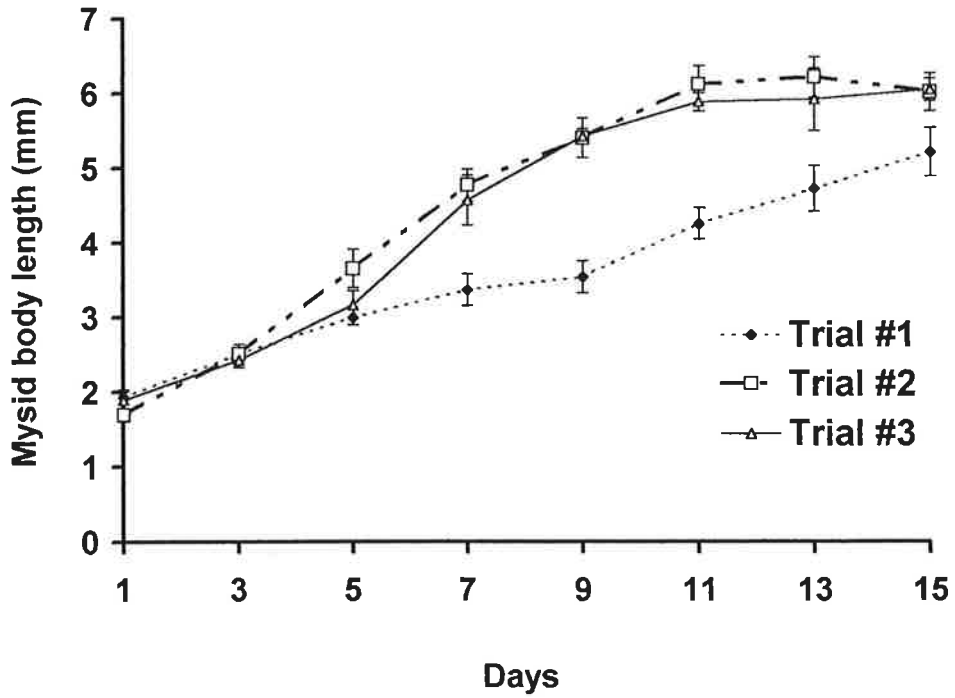

Figure 5 - Growth of mysids (mm) fed *Artemia* nauplii during 15 days.

At the end of the first second and third trials, the mean body length of mysids was 5.2 ± 0.3 mm, 6.0 ± 0.2 mm and 6.0 ± 0.1 mm. Growth was not significantly different ($p>0.05$) between the second and third trials, (except on day 5) but was significantly different ($p<0.05$) between the first and second trials from day 5 onwards, and for the first and third trial from day 7 until the end of the trials. In each trial, mysids released their first brood 13 days after being born.

2.3.3 Pilot-scale trials

2.3.3.1 Production and Survival

Daily mysid production (Figure 6) was very irregular. Highest production (303 hatchlings.d^{-1}) was obtained on day 84. No hatchlings were obtained on days 11, 108 and 109. The decrease on production on day 10 that led to the lack of hatchlings collected on day 11 was related to the lack of feeding. During those two days no *Artemia* nauplii were hatched due to technical problems. The bulbs that provided the light broke in one day and the ones used to replace them were not adequate. During those two days the mysids were not fed.

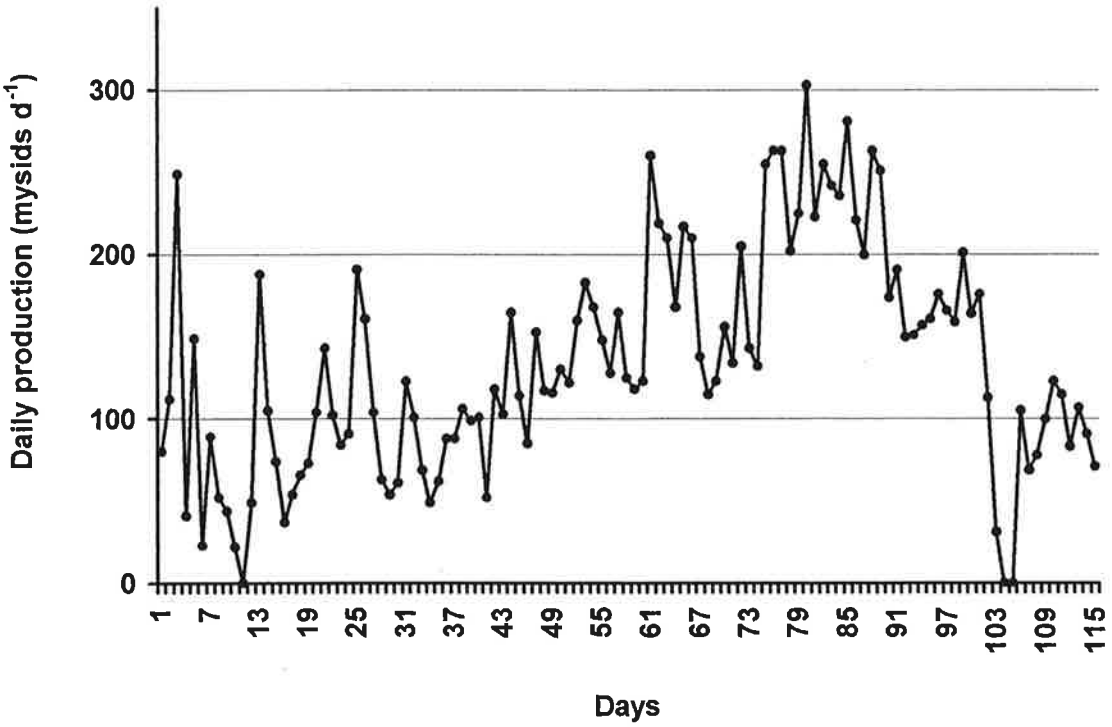

Figure 6 – Daily production from mysids fed *Artemia* nauplii for 17 weeks.

A total of 14,710 mysids were produced; a mean ± SD of 133 ± 69 mysids.d^{-1} were collected from the culture tray. Mean production was around 100 mysids.d^{-1} for the first 7 weeks (Figure 7) and gradually increased for the next 6 weeks. The highest production for a 14 day period was 3418 mysids or 244 ± 30 mysids.d^{-1} between weeks 11 and 13. After week 13, production decreased and the lowest was recorded during the two last weeks of the experiment (78 ±41 hatchlings.d^{-1}).

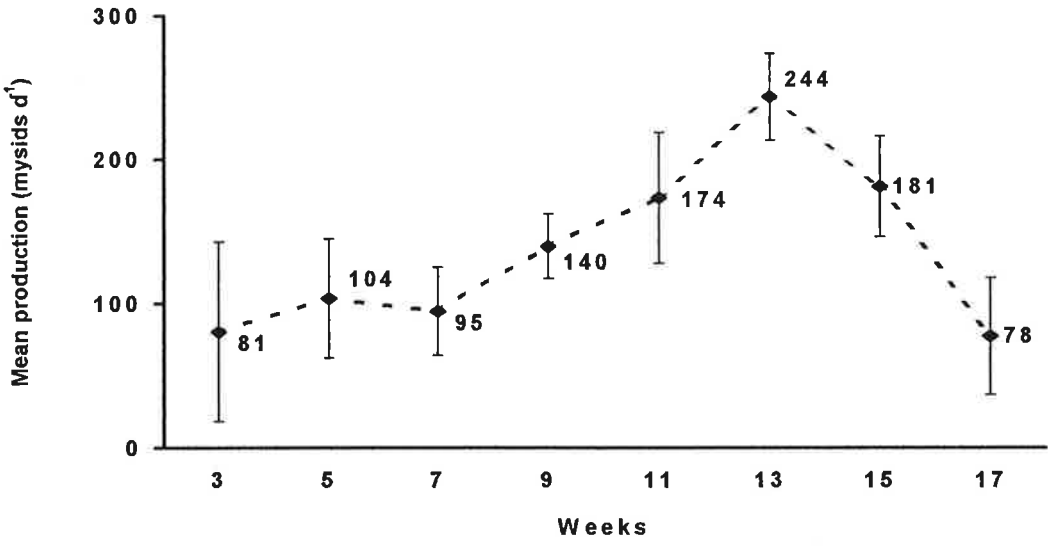

Figure 7 – Mean production (for the first three weeks and then every two weeks) from mysids fed *Artemia* nauplii for 17 weeks.

Of the 14,710 mysids produced, 6,250 survived the two weeks before harvesting (41.4 ± 11.3%). Survival of mysids after two weeks (Figure 8) in the hatchling trays ranged from 28.6% to 54.3%. Survival increased for the first 7 weeks, decreased between weeks 7 to 9 and increased gradually towards the end of the trial. The highest survival rates (over 50%) were obtained during the last six weeks of the experiment. Survival of adult mysids in the culture tray was 57% in the first 5 weeks, 60% between weeks 5-9, 66% between weeks 9-13 and 35% between weeks 13-17.

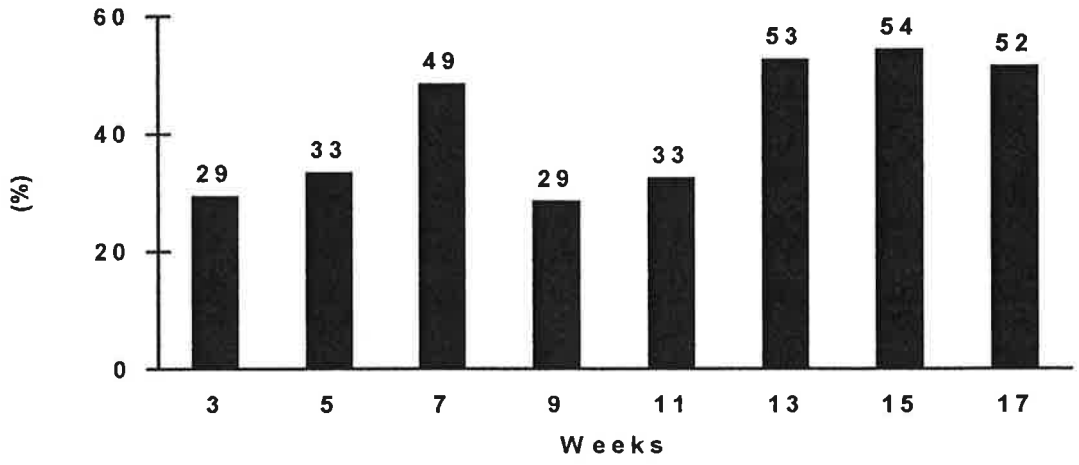

Figure 8 - Survival percentage of mysids fed *Artemia* nauplii. Survival was calculated for the first three weeks and afterwards, every two weeks.

Considering only the cost of *Artemia,* costs related to feeding for each adult mysid harvested after being grown for two weeks was about one cent each (US$0.01). This calculation is based on the spring 1996 price of *Artemia* sp. cysts (US$77 kg^{-1}), the use of 6 to 8 g $cysts.d^{-1}$ (to feed both adults and hatchlings), and a survival rate of mysids (after two weeks) of 42%.

2.3.3.2 Water Quality

The highest ammonia-nitrogen levels recorded during the trials were 1.68 $mg.l^{-1}$ in the culture tray and 1.25 $mg.l^{-1}$ in the hatchling tray. Ammonia-nitrogen concentrations were 0.00 $mg.l^{-1}$ in the biological filter before water exchanges were made with the trays. Values for ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen in both trays were highest and pH values in both trays were lowest immediately before each water exchange. Highest values recorded for nitrite-nitrogen in each tray were 0.240 $mg.l^{-1}$ in the culture tray and 0.302 $mg.l^{-1}$ in the hatchling tray. Highest nitrite-nitrogen concentrations in the biological filter before water exchanges were 0.005 $mg.l^{-1}$. Nitrate-nitrogen concentrations were low in all trays (<11 $mg.l^{-1}$) throughout the trial. The lowest pH recorded for each tray throughout the trial was 7.28 in the culture tray and 7.34 in the hatchling tray. The pH in the biological filter before water exchanges ranged from 8.15 at the start of the trials to 7.65 at the end of week 15.

At the end of week 15, mysids stopped reproducing and high mortality was observed in both trays. Water in the biological filter was changed and pH was raised to 8.1 in the biological filter with sodium bicarbonate. Hatchling production resumed two days later, but production (78 ± 41 $hatchlings.d^{-1}$) was the lowest for the total length of the experiment.

Figure 9 shows the variation in ammonia-nitrogen and nitrite-nitrogen concentrations and pH during a 12 h period on day 84 of the experiment, when production was highest (303 hatchlings). Ammonia-nitrogen levels at the end of the 12 h were 0.53 $mg.l^{-1}$ in the culture tray and 0.21 $mg.l^{1}$ in the hatchling tray (Figure 9A). These values were the lowest ammonia-nitrogen concentrations found in the tray after each 12 h period for the whole trial. Nevertheless during the trial, higher levels of ammonia-nitrogen (up to 1.0 $mg.l^{-1}$) were recorded in both trays while high

reproduction rates were maintained. Nitrite-nitrogen concentrations after 12 h of static flow were 0.237 $mg.l^{-1}$ and 0.225 $mg.l^{-1}$ in the culture and hatchlings tray, respectively (Figure 9B). The pH recorded at the end of this 12 h period was 7.92 in the culture tray and 7.98 in the hatchlings tray (Figure 9C).

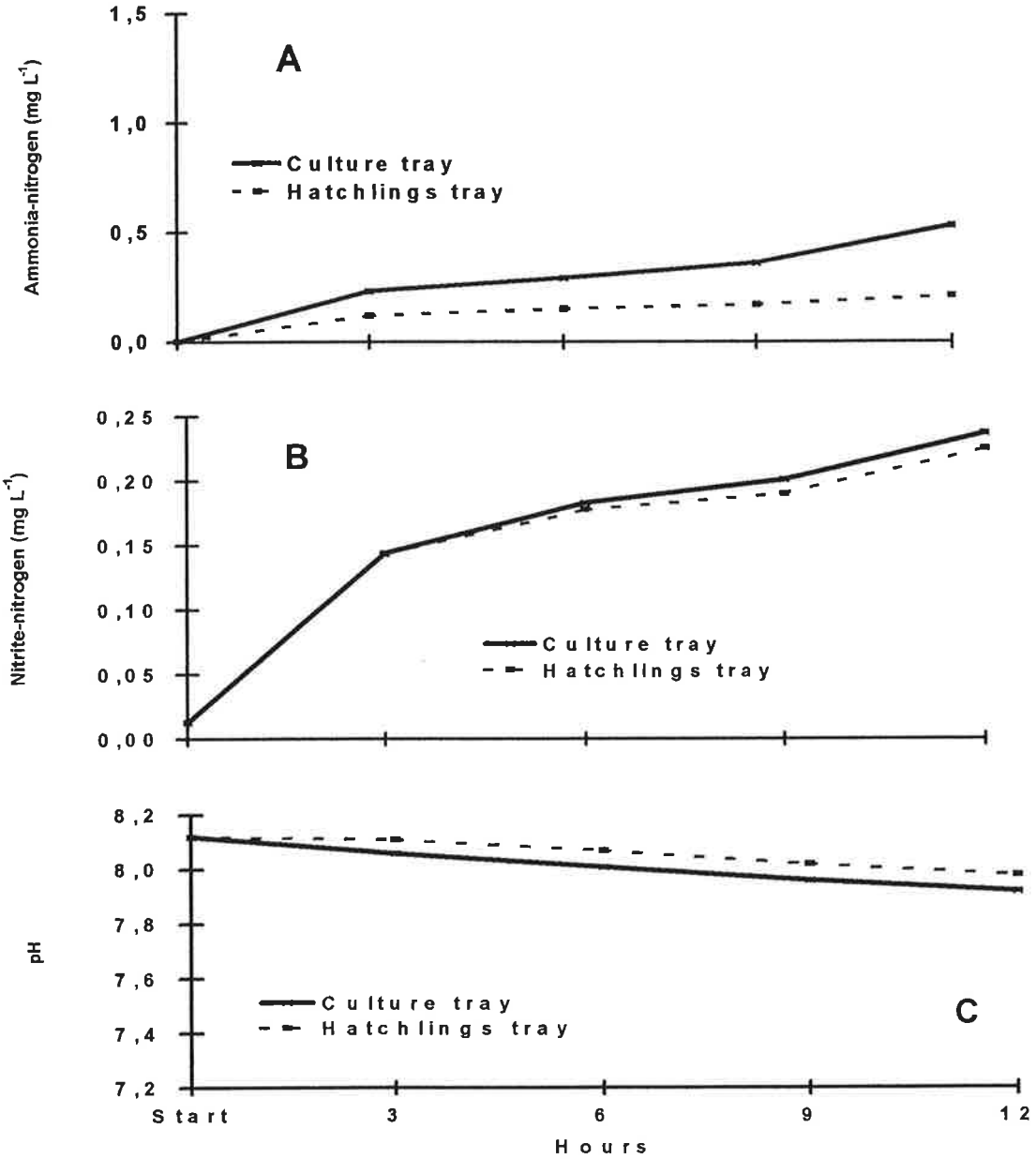

Figure 9 – Variation of A) Ammonia-nitrogen, B) Nitrite-nitrogen and C) pH in the culture trays during a 12 h period when mysid production was highest.

Figure 10 shows the change in concentration of ammonia-nitrogen and nitrite-nitrogen and the decrease in pH of water in the trays, during a 12 h period on the day before mysids stopped reproducing (day 107). Ammonia-nitrogen levels were 1.28 $mg.l^{-1}$ and 1.25 $mg.l^{1}$ in the culture and hatchlings trays, respectively (Figure 10A). Nitrite-nitrogen concentrations were 0.125 $mg.l^{-1}$ and 0.203 $mg.l^{-1}$ in the culture and hatchling trays, respectively (Figure 10B). These nitrite-nitrogen concentrations were lower than concentrations found in the trays when hatchling production was highest. The pH at the end of 12 h dropped to 7.38 in both culture and hatchling trays (Figure 10C). The slopes of these lines (*c.f.* Figure 10) appear to be greater than those of the period when reproduction was best (*c.f.* Figure 9).

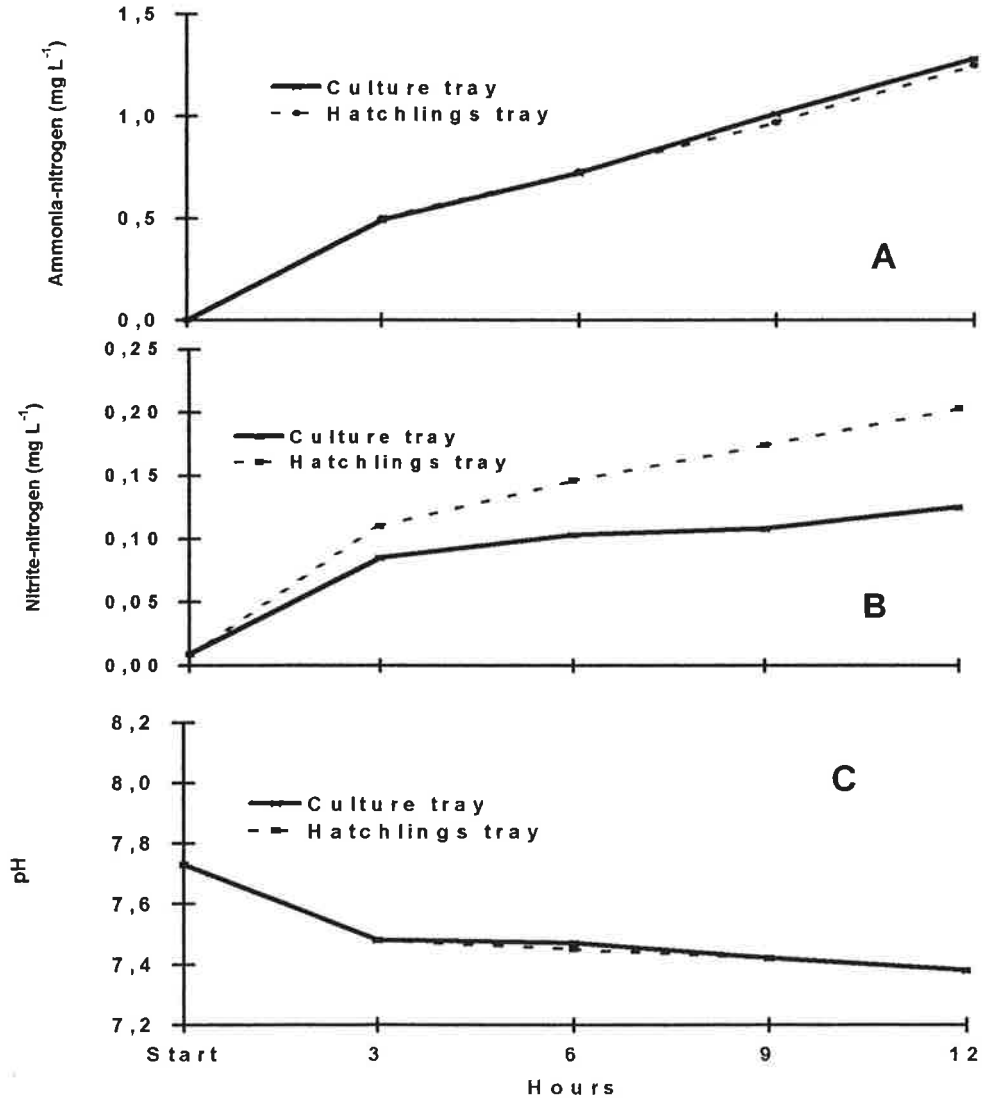

Figure 10 - Variation of A) Ammonia-nitrogen, B) Nitrite-nitrogen and C) pH in the culture trays during a 12 h period when mysid production ceased.

2.4 Discussion

A static water system presents several potential advantages and disadvantages when compared to mysid culture in flow-through systems. In flow-through systems mysids orient to a current (Mauchline, 1980). However, in static water systems, mysids do not have to expend as much energy to maintain their position, as there is no current. Another disadvantage of recirculating water systems is that *Artemia* nauplii are constantly dragged to and through the core where they become trapped or lost into the filterbed (Ward, 1991). In contrast, a static culture system will allow biological wastes to build up, resulting in poor water quality. Low dissolved oxygen and high concentrations of ammonia, nitrite and nitrate have been shown to increase mysid mortality (Brandt *et al.*, 1993). Therefore, culture management is the most important factor in achieving highly productive mysid cultures (Lussier *et al.*, 1988).

According to Lussier *et al.*, (1988), larger tanks with increased surface-to-volume ratios may be better for mysids because their epibenthic behavior requires large surface areas. Results from our research suggest that shallow trays with a large surface area to volume ratio appear to be effective for the large scale culture of mysids. Mysids in our culture system were mainly benthic and concentrated at the bottom when not feeding. Active swimming was rarely observed during our experiments, except when *Artemia* nauplii were added to the trays. Furthermore, newly hatched mysids have more area in which to escape from being cannibalized. Another potential advantage of trays in relation to aquaria is the higher number of mysids per unit of water volume that can be sustained. Comparison with literature indicates that mysid densities used in our trays (50 mysids.l^{-1}) were much higher than densities used by other researchers. Reitsema and Neff (1980) used about 6 mysids.l^{-1} (*M. almyra*); Leger *et al.*, (1989) used densities of 3 mysids.l^{-1} (*M. bahia*) and Ward (1991), also using *M. bahia* reports concentrations of 2.4 mysids.l^{-1}.

Acceptable water quality was maintained for the population of 1,000 mysids in 20 l of water, using two water exchanges a day. Mysids used in our experiment lived and reproduced in water with ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen below 1.5 mg.l^{-1}, 0.250 mg.l^{-1} and 12 mg.l^{-1}, respectively, provided that pH was above 7.5.

Perhaps if the water was exchanged more frequently, better results on production could be obtained, as newly hatched mysids would spend less time in the trays with cannibalistic adults. Furthermore, water quality would be improved. More

frequent water exchanges, feeding and hatchling collection could be implemented, using automation similar to other systems used at the NRCC laboratories (Lee, 1995).

Of the 37 papers that we reviewed on mysid culture, only one contained data on daily mysid production (Reitsema & Neff, 1980). These authors obtained up to 400 hatchlings.d^{-1} (using 350 adult *M. almyra* per aquaria) although the usual harvest was between 150 and 200 hatchlings.d^{-1}. The water volume in the aquaria was 57 l. Therefore, an average of 3.5 hatchlings.l^{-1}.d^{-1} were produced at an average harvest of 200 hatchlings.d^{-1}. Using the described static water system, we obtained an average of 6.5 hatchlings.l^{-1}.d^{-1}, almost double the previous report. Since no data were reported for the amount of food used or costs of production in any of the references, we have no means to compare the production costs of our static system to the recirculating water systems described earlier.

Between weeks 7 and 13, a gradual increase in mysid production was observed. Odum and Heald (1972) reported that the growth of algae in culture systems improved survival and productivity because mysids feed on phytoplankton, zooplankton and detritus. In the static tray system described here, the area around the valve was cleaned daily to prevent the build up of detritus that would clog the core, but unidentified attached epifauna and detritus accumulated in other areas of the tray. This might have provided a more suitable environment for mysids to live in as well as places for the hatchlings to hide. Production in the trays decreased from week 13 until the end of the trial. Reitsema & Neff (1980) found that the reproductive rate of cultured *M. almyra* decreased after 8 to 12 weeks.

Deterioration of water quality after 13 weeks was probably the most important factor affecting productivity. The pH of water in the filtration tank that was going to be used to replace the water in the trays after 12 h decreased continuously from 8.10 at the start of the experiment to 7.65 by the end of week 15. Toward the end of the experiment the starting pH was already very low (around 7.7), and after 12 h in the culture trays the pH decreased to values below 7.5. The drop in pH in the culture tray appears to be the main reason for the sudden decrease in hatchling production and the increase in mortality among adult mysids. Mortality in the culture tray was 43% in the first 5 weeks, 40% between weeks 5 and 9, 34% between weeks 9 and 13 and increased to 65% between weeks 13 and 17. Reproduction ceased during days 108 and 109 and resumed after sodium bicarbonate was added to the system to counteract the decrease in pH.

The decrease in pH is caused by bacterial oxidation of excretory ammonia-nitrogen by the nitrifying bacteria, *Nitrosomonas* and *Nitrobacter* sp. (Spotte, 1979). The use of sodium bicarbonate to buffer against a drop in the pH results in an increase in alkalinity (Ward, 1991). According to this author, the use of sodium bicarbonate did not cause mortality of adult *M. bahia*. Reproduction seemed to be affected especially at higher alkalinity values. In our system, reproduction and adult survival improved immediately after the addition of buffer and the subsequent increase in pH.

Reproduction occurred at ammonia-nitrogen and nitrite-nitrogen levels as high as 1.5 $mg.l^{-1}$ and 0.250 $mg.l^{-1}$ respectively, and at pH values as low as 7.6. These values seemed to have been well tolerated by mysids in our static water culture system. Leger *et al.* (1989) reported that nitrite-nitrogen strongly inhibits reproduction of *M. bahia* at levels as low as 0.05 $mg.l^{-1}$. However, Miller *et al.* (1990) found that concentrations up to 3.47 $mg.l^{-1}$ of ammonia-nitrogen did not affect the survival of *M. bahia* significantly. We obtained constant reproduction and low mortality rates for mysids kept in water with ammonia-nitrogen and nitrite-nitrogen concentrations that rose from 0.0 $mg.l^{-1}$ to 1.5 $mg.l^{-1}$ and 0.250 $mg.l^{-1}$, respectively in 12 h periods, when water was exchanged twice a day.

When pH decreased to 7.38, reproduction halted abruptly (days 108 and 109) and mortality increased sharply. Ammonia-nitrogen and nitrite-nitrogen concentrations in the trays were similar to periods of the trials when mortality was low and reproduction high. It is apparent from these data that pH falling below 7.4 was the major factor causing the sudden halt in reproduction and high mortality. Ward (1991) reported that in spite of a steady drop in pH from approximately 8.0 to 7.5 over a 24 week period a *M. bahia* population continued to increase. Miller *et al.* (1990) showed that ammonia-nitrogen concentrations are more toxic for mysids at lower pH values (7.0) than at higher values (8.0). Ammonia-nitrogen concentration in the static trays were fairly high throughout the experiment (0.6 to 1.4 $mg.l^{-1}$) and could have had a major impact on reproduction and survival as pH values fell to 7.38.

Survival rates of hatchlings grown for two weeks were low (41.4%). Mysids are cannibalistic (Lussier *et al.*, 1988). According to Reitsema & Neff (1980), mysids less than five days old cannot be maintained with mature ones. Since hatchlings were being introduced every day in the same tray for two weeks, it is possible that towards the end of each trial the larger mysids were cannibalizing the smaller ones.

Temperature accelerates the maturation of adult mysids. Several authors have described a direct relationship between water temperature and time to release of first brood for *M. bahia* (Nimmo *et al.*, 1977; McKenney, 1987; Lussier *et al.*, 1988; McKenney, 1996). Because of this, temperatures were maintained at the upper end of the natural range (25-29°C) in our system.

Feeding presented no technical problem to the culture of mysids in static water systems but the cost of *Artemia* represented the largest expense. Leger *et al.* (1987) reported that large *Artemia* nauplii could present a problem for hatchling mysids due to their size. For the static systems described here, the size of *Artemia* nauplii appears not to have been an inhibiting factor in hatchling or juvenile mysid culture as *Artemia* nauplii were fed upon by mysids immediately after hatching.

Although *Artemia* nauplii have been used in the last few years in mysid culture, the price of *Artemia* cysts is very high and increasing yearly, making it an expensive diet for large scale mysid culture. Considering only the cost of *Artemia* for the pilot-scale trials, the feeding cost for each adult mysid was about 1 cent each (US$0.01). As a result of these high feeding costs, alternative diets for the mass production of mysids should be tested. Microalgae (e.g., phytoplankton) should be tested since mysids have been reported to feed on phytoplankton (Webb *et al.*, 1987; Dauby, 1995) and the use of phytoplankton in substitution of *Artemia* nauplii would greatly reduce production costs.

3. EFFECTS OF DIFFERENT FOOD ITEMS ON THE CULTURE OF THE MYSID SHRIMP *M.almyra* IN A STATIC WATER SYSTEM.

3.1 Introduction

Consistent supply of food organisms is of utmost importance for continuous cultures (Leger *et al.*, 1987b). *Artemia* is a very useful food species in aquaculture because it is available as an off-the-shelf food in the form of dormant cysts that can be stored for many years (Watanabe, 1987). Cysts are hatched using simple procedures (Sorgeloos *et al.*, 1983). *Artemia salina* nauplii are fed as freshly hatched nauplii because older unfed nauplii lose their nutritional value (Watanabe *et al.*, 1978).

The content of omega-3 (n3) highly unsaturated fatty acids (HUFA) seems to be the most important factor determining the nutritional value of *Artemia* nauplii to marine organisms (Watanabe *et al.*, 1982; Leger, *et al.*, 1987a, 1987b, Lavens *et al.*, 1989; Kreeger *et al.*, 1991). The enrichment of *Artemia* nauplii with HUFA is a way to ensure that the nauplii supply the essential fatty acids (Robin *et al.*, 1987). The HUFA 22:6n3 is nearly absent in *Artemia* and 20:5n levels vary considerably between strains (Leger *et al.*, 1987b). These essential fatty acids can be incorporated in the *Artemia* (nauplii or adults) making them a suitable carrier for potential nutrients that are otherwise difficult to administer to crustacean larvae.

A disadvantage of using enriched *Artemia* is their larger size that may make them difficult to ingest for certain early larval stages of cultured species (Leger *et al.*, 1987a). Since the price of *Artemia* cysts is the major factor for the high cost of mysid production, a cheaper food would be necessary to successfully produce mysids in large scale (Chapter 2). Several species of mysids can feed on phytoplankton (Webb *et al.*, 1987; Dauby, 1995). The use of phytoplankton to grow mysids would be extremely beneficial since it is relatively cheap to produce and would greatly reduce production costs.

The objective of this research was to determine the effects of several live and artificial feeds and supplemented live feeds on production, growth and survival of the mysid shrimp *M. almyra*. Previous research (Chapter 2) had already demonstrated that mysids could be cultured effectively in a static water system that made feeding more efficient.

3.2 Material and methods

All the mysids used in these studies were collected from Galveston Bay (Texas, USA) (*c.f.* Figure 3) using bottom trawls. In the laboratory, test animals were selected for condition and species but not sexed. During all experiments, *Artemia* cysts used came from Ocean Star International Inc. (O.S.I. PRO 80), Snowville, Utah. Before the start of the experiments, estimates of hatching rate were made. Hatching rate was of 100,000 nauplii per gram of cyst.

3.2.1 Growth trials

Experiment one. Three sets of 15 day experiments were conducted to compare growth rates of mysids fed two diets (*Artemia* nauplii and HUFA enriched *Artemia* nauplii). Trays similar to those used in the previous trials (Chapter 2) were used in these experiments (shallow triangular trays with total area of 1,750 cm^2 each and water depth of 6 cm and 10 l of seawater). There was no water circulation in the trays and mysids were kept in the dark. A partial water exchange (30%) occurred every day due to siphoning of wastes and uneaten food. For each of these growth trials, 100 hatchlings were placed in each tray. The *Artemia* nauplii diet was fed to the mysids in one tray and a HUFA *Artemia* nauplii (SELCO®, INVE Inc., Gent, Belgium) diet was given to mysids in the second tray. Every two days 6 mysids were collected from each tray, killed with a solution of water and concentrated formalin at 20 $ml.l^{-1}$ and measured. Length was measured dorsally from the tip of the rostrum to the tip of the telson using an ocular micrometer. Statistical analysis was performed to determine differences in growth between mysids fed each diet (Zar, 1984).

Experiment two. After the initial trials, another experiment testing growth of mysids fed *Artemia* nauplii or HUFA enriched *Artemia* nauplii was conducted in a second culture system placed inside (*c.f.* Figure 4). This system was composed of 4 rectangular trays (125 cm x 50 cm and water depth of 8 cm) each with a water volume of approximately 20 l of sea water (Chapter 2). Feeding and water renewal were performed twice a day (09:00 h and 21:00 h) using the previously described method (Chapter 2). In this case, temperature was maintained at 27 ± 2°C. Salinity was 20 ± 2 ppt. During this experiment, 10 mysids were randomly collected, killed and measured every two days using the same method as for the previous trials. For all experiments, prey concentration of 100 *Artemia* $nauplii.mysid^{-1}$ were used. The experiment lasted

15 days. Statistical analysis was performed to determine differences in growth between mysids fed each diet (Zar, 1984).

3.2.2 Reproduction trials

Experiment three. A third set of experiments was conducted for 17 weeks, using a larger, indoor static water system (*c.f.* Figure 4). This system was composed of 4 rectangular trays (125 cm x 50 cm and water depth of 8 cm) each with a water volume of approximately 20 L of sea water. Connected to these 4 trays was a biofilter tank with submerged crushed oyster shell where biological filtration oxidized wastes produced by mysids and uneaten food. In this system, two upper culture trays containing mature, spawning adults were placed above two lower hatchling trays. Each of the culture trays was stocked with 1,000 adult mysids. The drain of the culture trays was covered with a 1,000 μm mesh core and it was connected to a valved outlet on the bottom. Hatchling collection and water renewal were performed twice a day (09:00 h and 21:00 h) using the previously described method (Chapter 2).

Water temperatures were kept at 27 ± 2°C, by heating the room with an electrical heater; salinities were maintained at 20 ± 2 ppt. Although there was no water circulation in the trays, water was completely renewed twice a day by recirculating it through the biofilter for nitrification for approximately 10 minutes. Ammonia-nitrogen, nitrites-nitrogen, nitrates-nitrogen and pH were measured at the end of each 12-h period using HACH® reagents (Loveland, Colorado) and a pinpoint pH meter respectively (Radiometer, Denmark). The room was kept dark and lights were turned on twice a day for about 1 h (for the mysid harvest and water renewal). Light for maintenance was provided by two 30 W fluorescent bulbs covered with a red filter.

Two diets were fed to adults and hatchlings in order to compare reproduction and survival rates. One diet consisted of newly hatched *Artemia* nauplii (24 h old). The second diet consisted of HUFA enriched *Artemia* nauplii. Enrichment with SELCO® has become a standard practice in many hatcheries that produce marine fish and shrimp (Leger *et al.*, 1989). Enrichment was accomplished by soaking 200,000 newly hatched *Artemia* nauplii in 8 l of sea water and 2 g of the SELCO® solution. In order to obtain a homogeneous emulsion, the SELCO® solution and water were

mixed in a blender for two minutes. The nauplii were kept in this solution at 28°C and 20 ppt for 12 h, then drained and fed to the mysids

Every two weeks the hatchling trays were drained and cleaned. The mysids were counted and differences in the broodstock production and survival of the offspring grown on the two diets were compared statistically with an unpaired student t-test, significance at $p<0.05$ (Zar, 1984). Mysid production was expressed in numbers of hatchlings produced.d^{-1}. Survival was expressed as a percentage calculated by dividing the number of mysids in the hatchling tray on day 14 by the total harvested from the culture tray during the 14 days and multiplying by 100.

Experiment four. Finally, three 30 days experiments were conducted, using six different diets. Two of the diets were the same as the ones tested in the 17-week experiment (*Artemia* nauplii and HUFA enriched *Artemia* nauplii). The other four diets were phytoplankton (*Isochrysis* sp.), an artificial diet (Liqualife®, Cargill, Minneapolis, MN), a diet composed of equal parts of phytoplankton and zooplankton (mainly copepods) and one consisting of 750 $mg.l^{-1}$ HUFA enriched *Artemia* nauplii and 250 $mg.l^{-1}$ of the artificial diet. Temperatures, salinities, water volumes, hatchling collection and water exchange intervals were the same as in the 17-week experiment. The room was kept dark with the exception of the experiment when phytoplankton was fed to the mysids; the lights were on 24 h a day. Mysid production and survival were determined as above. Production and survival of the offspring grown on the different diets were compared statistically with unpaired student t-test, significance at $p<0.05$ (Zar, 1984).

3.3 Results

3.3.1 Growth trials

Experiment one. Mysids fed the HUFA enriched *Artemia* diet were larger at the end of each experiment, but the differences were significant ($p<0.05$) only for the first trial from day 7 onwards (Table II, Appendix). The mean size of newly hatched mysids was 1.8 ± 0.2 mm and there was no significant difference ($p>0.05$) between hatchlings of mysids fed on the different feeds.

In all trials, ammonia-nitrogen was kept below 0.5 $mg.l^{-1}$ and pH dropped from 8.0 to 7.5. Salinity measured between 22 ppt and 24 ppt in all experiments.

Temperatures ranged from 24°C to 31°C, from 25°C to 30°C, and from 17°C to 28°C in the first, second and third experiments, respectively.

Experiment two. During the experiment conducted inside, mysids fed *Artemia* nauplii grew to 6.0 ± 0.4 mm while mysids fed HUFA enriched *Artemia* nauplii grew to 6.1 ± 0.2 mm (Figure 11). No significant differences ($p>0.05$) were found between size of mysids fed each diet at the end of the experiment. Size of hatchlings produced by mysids fed *Artemia* nauplii (1.9 ± 0.1 mm) and mysids fed HUFA enriched *Artemia* nauplii (1.9 ± 0.2 mm) was not significantly different ($p>0.05$).

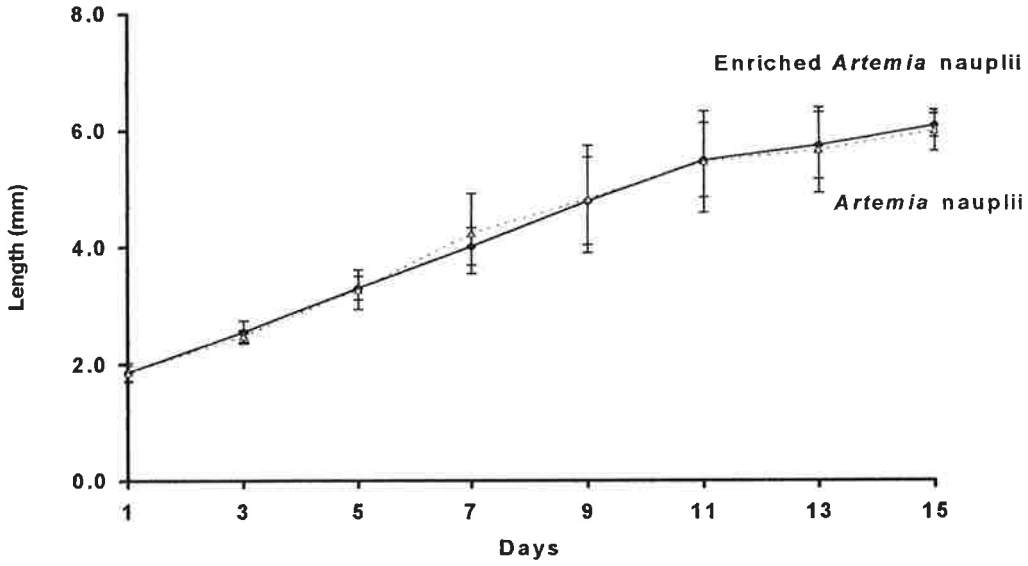

Figure 11 – Growth of mysids fed *Artemia* nauplii or HUFA enriched *Artemia* nauplii during 15 days.

3.3.2 Reproduction trials

During the 17-week long trial period a total of 30,461 hatchlings were produced (242 ± 161 hatchlings.d^{-1}; mean ± SD). Mysids fed HUFA enriched *Artemia* nauplii produced 15,751 hatchlings (139 ± 82 hatchlings.d^{-1}), while mysids fed *Artemia* nauplii produced a total of 14,710 hatchlings (133 ± 69 hatchlings.d^{-1}). Although hatchling production by mysids fed the HUFA enriched *Artemia* nauplii diet was consistently higher (Figure 12), no statistically significant difference ($p=0.459$; $T=0.741$) in hatchling production was found between the treatments.

Production in both treatments started to increase consistently after 35 days until approximately 90 days into the experiment. From that day onwards, production

decreased until day 106 when no mysids were produced. For two days (for mysids fed *Artemia* nauplii) and three days (for mysids fed the HUFA enriched *Artemia* nauplii) no production was obtained due to poor water quality (pH < 7.5). The highest production occurred between days 77 and 90. During this period, mysids fed the HUFA enriched *Artemia* nauplii produced an average of 262 ± 60 hatchlings.d^{-1} and mysids fed *Artemia* nauplii produced an average of 243 ± 32 hatchlings.d^{-1}. Hatchling production by mysids fed the HUFA enriched *Artemia* nauplii was significantly higher ($p<0.05$) during this period of highest production.

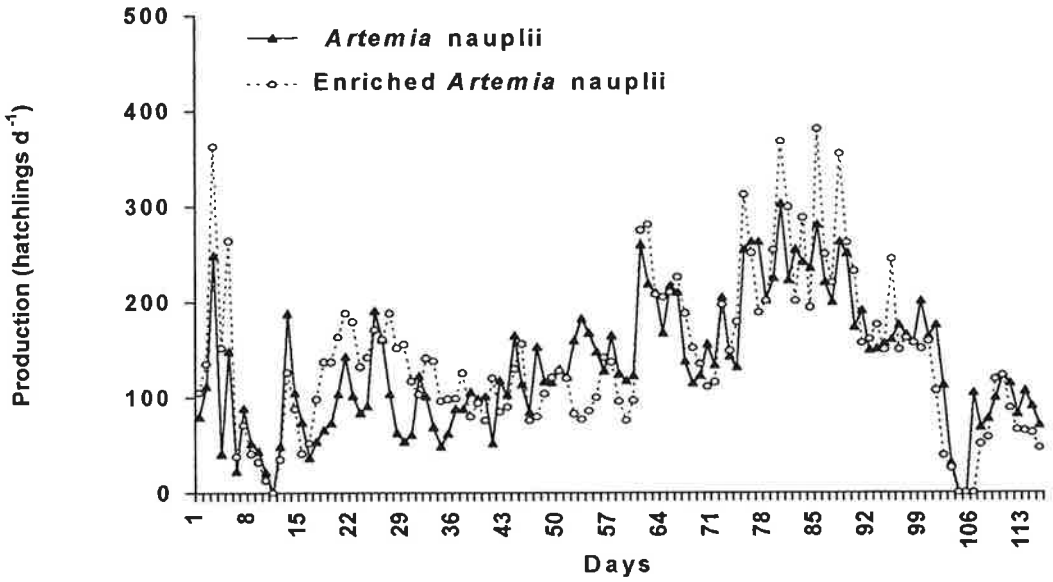

Figure 12 – Daily production by mysids fed *Artemia* nauplii and mysids fed HUFA enriched *Artemia* nauplii during 17 weeks.

Of the 30,461 hatchlings produced over 17 weeks, 15,760 (51.7%) survived the two weeks growth trials. The survival rates of mysids fed the HUFA enriched *Artemia* nauplii (59.1%) was significantly higher ($p=0.012$) than survival rates of hatchlings fed *Artemia* nauplii (41.4%). For all sampling periods of the experiment, survival percentage of mysids fed the HUFA enriched nauplii was higher in comparison to mysids fed *Artemia* nauplii (Figure 13). The survival percentage of hatchlings fed the HUFA enriched *Artemia* nauplii diet ranged between 43.2% and 74.3 %. The highest survival percentage was obtained at the end of week 13. Survival of hatchling mysids fed the *Artemia* nauplii without enrichment ranged between 28.6% and 54.3% with highest survival obtained between weeks 13 and 15.

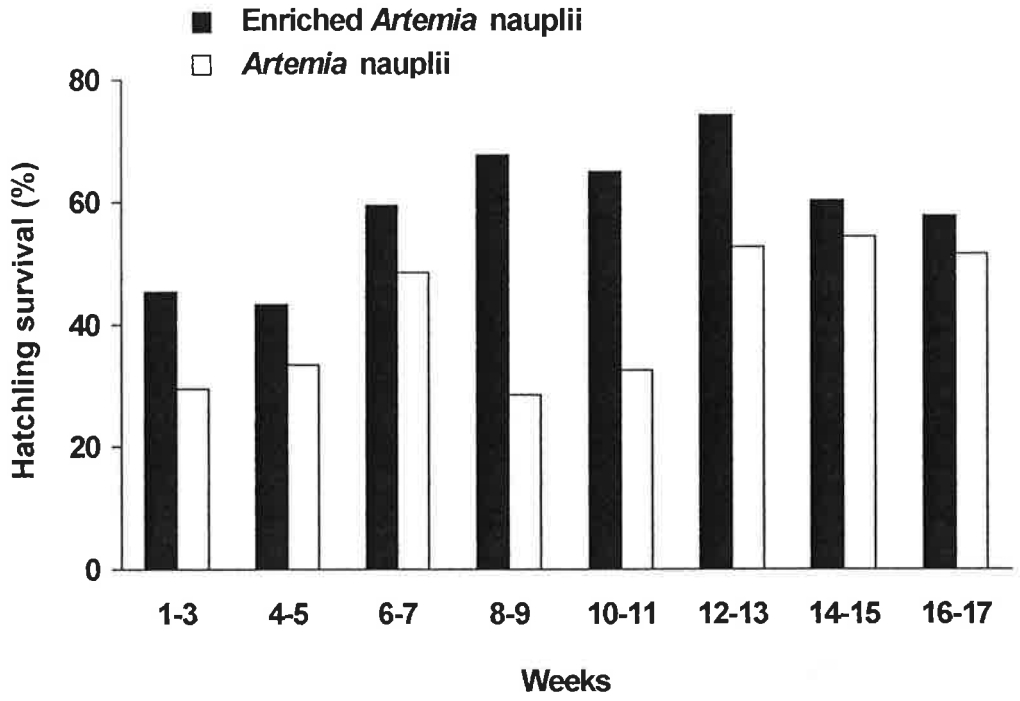

Figure 13 – Survival of mysids fed *Artemia* nauplii and mysids fed HUFA enriched *Artemia* nauplii during 17 weeks. Survival was determined at the end of the third week, and from then onwards every two weeks. Mysids fed HUFA enriched *Artemia* nauplii survived better during all the experiment.

The highest ammonia-nitrogen concentration levels recorded during the experiment were 1.68 $mg.l^{-1}$ in the culture tray fed *Artemia* nauplii. In the other 3 trays, ammonia-nitrogen as high as 0.75 $mg.l^{-1}$ occurred in the culture fed HUFA enriched *Artemia* nauplii; 1.25 $mg.l^{-1}$ occurred in the hatchling tray fed *Artemia* nauplii and 1.04 $mg.l^{-1}$ in the hatchling tray fed HUFA enriched *Artemia* nauplii. Ammonia-nitrogen was always 0.00 $mg.l^{-1}$ in the biofilter tank at the conclusion of 12 h periods between water changes. The highest nitrite-nitrogen values in each tray were 0.240 $mg.l^{-1}$ in the culture tray fed *Artemia* nauplii, 0.250 $mg.l^{-1}$ in the culture tray fed HUFA enriched *Artemia* nauplii, 0.302 $mg.l^{-1}$ in the hatchling tray fed *Artemia* nauplii and 0.410 $mg.l^{-1}$ in the hatchling tray fed HUFA enriched *Artemia* nauplii. Nitrite-nitrogen was always below 0.05 $mg.l^{-1}$ in the biofilter before the trays were flushed into it. Nitrate-nitrogen increased from 4.0 to 10.95 $mg.l^{-1}$ in the trays and biofilter over the 17 weeks.

The lowest pH recorded between flush cycles throughout the experiment varied between 7.38 and 7.75 in the culture tray fed *Artemia* nauplii, between 7.28 and 7.81 in the culture tray fed HUFA enriched *Artemia* nauplii, between 7.34 and

7.98 in the hatchling tray fed *Artemia* nauplii, between 7.34 and 7.95 for the hatchling tray fed enriched *Artemia* and between 8.0 and 7.64 in the biofilter.

On day 106 of the experiment, mysids fed both diets stopped reproducing and high mortality was observed in the tray. This abrupt end of reproduction coincided with the lowest pH values recorded throughout the experiment. Water in the biofilter was changed and pH was raised to 8.1 in the biofilter using sodium bicarbonate. Hatchling production resumed two days later on the tray where *Artemia* nauplii was being fed and three days later in the culture tray where mysids were fed HUFA enriched *Artemia* nauplii. Production at the end of this last 14 day period was the lowest (58 ± 42 hatchlings.d^{-1}) in the whole trial period for mysids fed the HUFA enriched diet, and the second lowest (78 ± 41 hatchlings.d^{-1}) for mysids fed *Artemia* nauplii, and was significantly different ($p<0.01$) when compared with production by mysids fed both diets in the two previous weeks.

Experiment four. Production by mysids fed the six different test diets are shown on Figure 14.

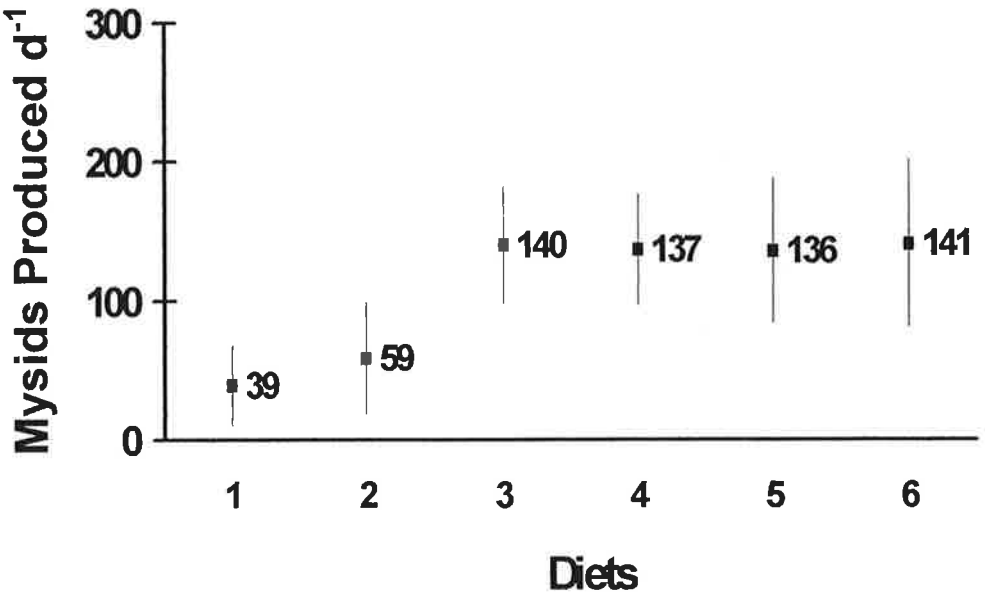

Figure 14 – Production by mysids fed six diets: 1) Phytoplankton, 2) Artificial diet (Liqua Life®), 3) Copepods+phytoplankton, 4) 75% HUFA enriched *Artemia* nauplii+25% Liqua life®, 5) *Artemia* nauplii and 6) HUFA Enriched *Artemia* nauplii.

Highest production was obtained from mysids fed the HUFA enriched *Artemia* nauplii, 141 ± 60 hatchlings.d^{-1}, although there were no significant differences ($p>0.05$) in production between treatment fed the HUFA enriched (diet 6) and *Artemia* nauplii (diet 5) (136 ± 55 hatchlings.d^{-1}), the treatment fed equal parts of phytoplankton and zooplankton (diet 3) (140 ± 42 hatchlings.d^{-1}) and the one with 750 mg.g^{-1} HUFA enriched *Artemia* nauplii and 250 mg.g^{-1} of the artificial diet (diet 4) (137 ± 40 hatchlings.d^{-1}).

Production by mysids fed the phytoplankton, diet 1 (39 ± 29 hatchlings.d^{-1}) and the artificial diet Liqualife® (59 ± 40 hatchlings.d^{-1}) were significantly lower ($p<0.05$) than production by mysids fed the other four diets; the former cultures then collapsed (production gradually decreased until no hatchlings were obtained) at the end of two weeks. Ammonia-nitrogen and nitrite-nitrogen levels were always kept below 1.5 mg.l^{-1} and 0.290 mg.l^{-1} and pH was always above 7.5 for all the 30 day experiments.

Survival of mysids grown for two-week periods on these diets is shown in Figure 15.

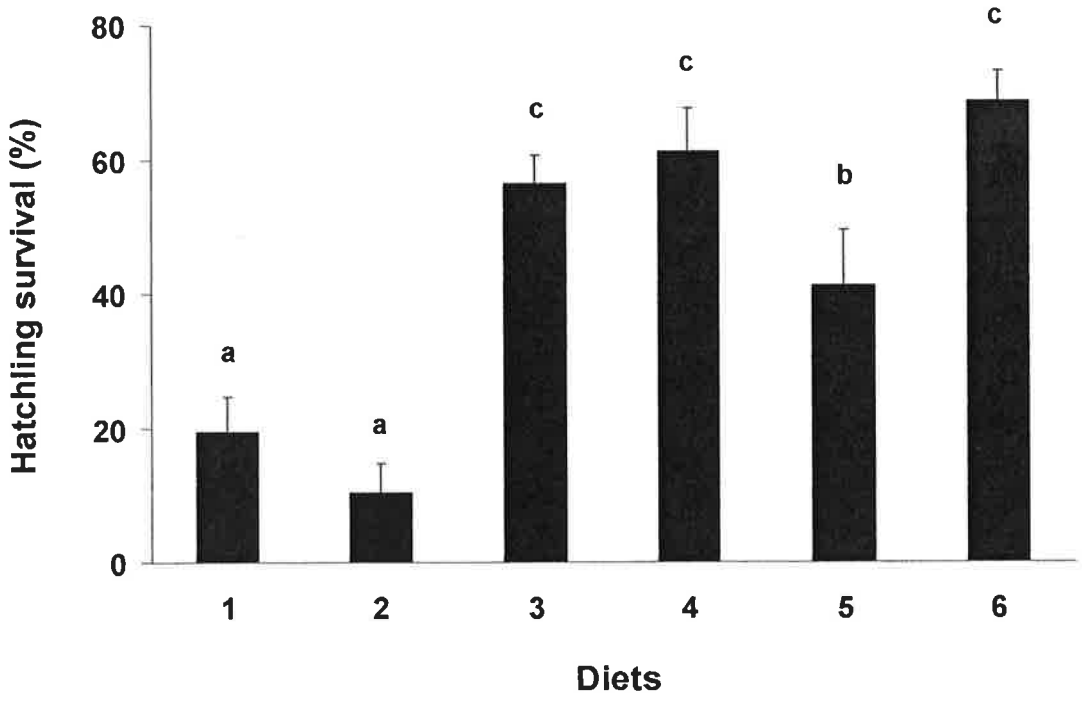

Figure 15 – Survival (after 15 days) of mysids fed six diets: 1) Phytoplankton, 2) Artificial diet (LiquaLife®), 3) Copepods+phytoplankton, 4) 75% HUFA enriched *Artemia* nauplii+25% Liqua life®, 5) *Artemia* nauplii and 6) HUFA Enriched *Artemia* nauplii. The "a", "b" and "c" indicate that the values were significantly different ($p<0.0001$).

The highest survival rates ($p<0.0001$) were obtained when mysids were fed HUFA enriched *Artemia* nauplii, diet 6 (69%) and a combination of 750 $mg.g^{-1}$ HUFA enriched *Artemia* nauplii and 250 $mg.g^{-1}$ of the artificial diet, diet 4 (61%). The lowest survival rates ($p<0.0001$)were obtained when mysids were fed the artificial diet, diet 2 (10%) and phytoplankton, diet 1 (19%). Survival of mysids fed *Artemia* nauplii (diet 5) was 41% and mysids fed a mixed diet of zooplankton and phytoplankton (diet 3) had a survival rate of 56% ($p<0.0001$).

3.4 Discussion

The importance of essential fatty acids for the early stages of cultured marine fish and crustacean larvae is well documented (Cahu *et al.*, 1986; Leger *et al.*, 1989; Kuhn *et al.*, 1991). *Artemia* are commonly used as food for larval forms of many cultured species. The great variability in essential fatty acid content of *Artemia* makes it essential to determine good stains for mariculture (Leger *et al.*, 1987b). In fact, different sources of *Artemia* or even different batches from the same source resulted in significant variations in nutritional effectiveness for marine larval organisms (Leger *et al.*, 1987a). This unpredictability of essential fatty acid levels or even their absence from certain *Artemia* strains is a major restraint for the successful use of *Artemia* as a suitable prey in larviculture of marine crustaceans (Leger *et al.*, 1987c). The concern for nutritionally complete larval diets led to the concept of "packing" or value-added strategies using *Artemia* as the container. Leger *et al.*, (1987a) reported that 99% of the variability in biomass of the mysid, *M. bahia*, was correlated with 20:5n-3 and 22:6n-3 fatty acid content of *Artemia* nauplii.

During these growth trials mysids fed both diets grew to 4 mm in 7 days and to reproductive size in 13 days at temperatures that ranged from 19°C to 31°C. This indicates that in this temperature range mysids can be grown to full size in a week and start reproducing in less than two weeks on *Artemia* nauplii. Mysids fed HUFA enriched *Artemia* nauplii grew significantly larger ($p<0.05$) than mysids fed *Artemia* nauplii only in the first trial and from day 7 onwards (*c.f.* Table II, Appendix). These results were not replicated in succeeding experiments. Cripe (1989) using *Artemia* nauplii deficient in fatty acids and *Artemia* nauplii rich in fatty acids did not find significant differences in growth of young *M. bahia*. Kuhn *et al.*, (1991) obtained better growth and reproduction rates on *M. bahia* fed *Artemia* enriched with fatty

acids (SELCO®). Kreeger *et al.*, (1991) obtained a greater number of viable offspring of *M. intii* fed *Artemia* enriched with lipid microspheres. Leger *et al.*, (1987b) found that A*rtemia* enriched with n3-highly unsaturated fatty acids (n3-HUFA) were a better food source for *M. bahia* than *Artemia* nauplii, resulting in better survival and growth.

Results obtained during these experiments indicate that HUFA enriched *Artemia* nauplii did not cause significantly better reproduction than *Artemia* nauplii without any enrichment ($p>0.05$). However, hatchling survival was significantly increased ($p<0.05$) by feeding hatchlings with HUFA enriched *Artemia* nauplii. These results indicate that HUFA enriched *Artemia* nauplii are a better food item for mysid survival, especially during the early stages. Leger *et al.*, (1989) also found that enriched *Artemia* nauplii did not significantly enhance daily production of *M. bahia.* In contrast, Kuhn *et al.*, (1991) working with *M. bahia* obtained twice as many young when feeding enriched *Artemia* nauplii in comparison to *Artemia* nauplii alone.

Leger *et al.*, (1987b) found that the size of *Artemia* nauplii could present a problem to juveniles (< 24 h old mysids). In our experiments, *Artemia* nauplii were enriched for 12 h and were therefore larger than the newly hatched *Artemia.* The enriched nauplii alone or combined with an artificial diet fed to juvenile mysids promoted better survival than the non-enriched smaller nauplii. Hence, the small difference in nauplii size appears not to be an inhibiting factor in hatchling mysid culture.

The improved survival of juvenile mysids fed HUFA enriched *Artemia* nauplii may be the result of a more nutritionally complete food for the early stages of the life cycle and/or spawning through enrichment with fatty acids. Kreeger *et al.*, (1991) showed that enrichment of *Artemia* nauplii with fatty acids greatly increased the number of viable offspring of *Metamysidopsis enlongata* and *M. intii.* The current experiments confirm that hatchling survival but not production were enhanced with supplementation. Leger *et al.*, (1989) demonstrated that when *Artemia* nauplii enriched with fatty acids were fed to mysids, larger juvenile were produced. This was not observed in these experiments since size of mysids less than 12 h old was the same regardless of the treatment.

Results from these experiments indicate that HUFA enriched *Artemia* nauplii were the food that produced the best survival for mysid hatchlings However, special attention must be devoted to water quality when using it, since it appears to cause a

more rapid deterioration of water quality. The use of HUFA adds organic matter to the system, compromising water quality, particularly buffering capacity by making the pH drop more rapidly in the culture tray when compared to *Artemia* nauplii alone. This drop in pH in the culture trays appears to be the main reason for the halt in production and increased mortality. The same drop was not observed in the hatchling tray. Reproduction in all experiments occurred at ammonia-nitrogen and nitrite-nitrogen concentrations up to 1.68 $mg.l^{-1}$ and 0.410 $mg.l^{-1}$ respectively, as long as pH was kept above 7.5. Water quality measurements suggest enhanced development of ammonia nitrifiers, resulting in lower levels of ammonia-nitrogen, higher levels of nitrite-nitrogen and lower pH values in the treatment fed HUFA enriched *Artemia* nauplii than the trays where *Artemia* nauplii were fed.

The artificial diet (diet 2) was eaten by mysids, but uneaten particles sank rapidly. Since we were using a static water system, these were probably not the ideal experimental conditions to test this artificial diet. We have been testing live feeds in this system and wanted to see how an artificial diet would do. A system where water would be flowing or the food re-suspended into the water column through aeration might have allowed the food to be in constant movement and more attractive to the mysids. Nevertheless, the results obtained from this experiment strongly indicate that in these culture conditions, the artificial diet is not suitable to grow mysids. Two weeks after the start of the experiment, a rapid deterioration in water quality in the trays was noticed. Although ammonia-nitrogen and nitrite-nitrogen values recorded were similar to the ones found when feeding other diets, pH reached values of 7.1, resulting in high mysid mortality.

The phytoplankton (diet 1) and artificial diet (diet 2) used alone were not appropriate for the culture of *M. almyra*. The water quality in the culture and hatchling trays when feeding phytoplankton was the best recorded during all experiments. Therefore, high mortality of mysids fed this diet cannot be associated to poor water quality. However, phytoplankton was the diet that clearly produced the worse production of all diets. As for survival of hatchlings, it was the second worse result obtained, after the artificial diet. Some *Mysidopsis* species can feed on diatoms and prey on small crustaceans such as copepods (Odum & Heald, 1972; Mauchline, 1980). According to Venables (1986), juvenile of the related species *M. bahia* have been grown on diatoms (*Skeletonema* sp. and *Nitzschia* sp.) or the blue-green algae (*Spirulina subsalsa*). Nevertheless, the algae used in this study were clearly

inadequate for the culture of *M. almyra*. Ward (1987) reports that *Mysidopsis* species may prefer moving prey such as *Artemia* nauplii. Observations by Hallberg (1977) and Fulton (1982) on feeding behavior of *Mysidopsis* species indicated that they present typical activity of organisms with good visual acuity. This may also be the case of *M. almyra*. This could explain the bad results obtained when feeding phytoplankton and the artificial diet in our experiments. Mauchline (1970) found that some *Mysidopsis* species are dominantly carnivores. It is possible that *M. almyra* is mainly carnivorous (which is supported by the observations of cannibalism when insufficient food was present).

Copepods combined with phytoplankton promoted good results both on production and survival. Nevertheless, it was very time consuming and expensive to collect and sort zooplankton with plankton nets; this treatment also involved the culture of microalgae. Therefore, the use of copepod and phytoplankton became even more expensive than using HUFA enriched *Artemia* nauplii.

Water quality in the trays fed phytoplankton was always the best when comparing all the experiments. Ammonia-nitrogen and nitrite-nitrogen were always lower than 0.5 $mg.l^{-1}$ and 0.1 $mg.l^{-1}$ respectively and pH was always above 7.7 at the end of 12 h. The mixed diet of 750 $mg.g^{-1}$ *Artemia* nauplii and 250 $mg.g^{-1}$ artificial diet promoted similar production rates but slightly lower survival rates than enriched nauplii alone. Since mysids were always fed in excess during the 17-week experiment, the 75% *Artemia* nauplii concentration might have been adequate nutrition for the mysid populations.

In conclusion, the use of SELCO® did enhance *Artemia* nauplii for mysid survival ($p<0.05$), although mysid production and growth was not significantly greater. In contrast, phytoplankton and artificial diets had significantly lower production and survival rates.

4. THE CULTURE OF *M. almyra* IN A STATIC WATER SYSTEM: EFFECTS OF MYSID DENSITY AND TEMPERATURE ON PRODUCTION, SURVIVAL AND GROWTH.

4.1 Introduction

A vast number of continually changing environmental factors (e.g. salinity, dissolved oxygen, suspended particles, temperature and currents) modifies the physiological performance of estuarine organisms. Tolerance limits for these environmental parameters are controlled genetically and for each species there is an optimal range where physiological performance is the most efficient (McKenney, 1987). Since *M. almyra* is an estuarine organism, it must be able to adapt to the constantly changing estuarine environment, and it must exhibit different physiological performances according to the different environmental parameters. Among these parameters, temperature and salinity represent the dominant ecological factors within the estuarine environment (McKenney, 1987).

Temperature has an important effect on growth, maturation and reproduction rates of *M. almyra*. Reitsema & Neff (1980) reported that reproduction in *M. almyra* almost ceased at 20°C compared to 25°C; reproduction was increased at 30°C. *M. almyra* reached sexual maturity in 17-21 days at 25°C (Reitsema & Neff, 1980) while the related species *M. bahia* reached sexual maturity in 14 days at 23°C (Leger *et al.*, 1989). Mysid density in a culture tank affects reproduction. According to Lussier *et al.*, (1988), a culture that is overcrowded will cease reproduction, resulting in high proportion of adults and females with empty brood sacs. This author indicates optimal densities of 15 adult mysids.l^{-1} in flow-through systems and 10 adult mysids.l^{-1} for static cultures.

The ability to culture mysids in large numbers at low production costs would enable the successful aquaculture of several economically important marine species, including cephalopods (Hanlon *et al.*, 1991). The goal of this research project was to determine the possibility of growing the mysid shrimp *M. almyra* in large scale using a static water system. The effectiveness of this static system to culture *M. almyra* has already been determined (Chapter 2) and several food items were evaluated in order to reduce production costs (Chapter 3). The following experiments were conducted to determine the optimum mysid density and temperature for this static water system in order to optimize production, survival and growth of *M. almyra*.

4.2 Material and methods

Mysids used in all experiments were collected from Galveston Bay (Texas, USA) (*c.f.* Figure 3) using bottom trawls. Animals selected for this study were unsexed adults in good condition. The culture system (*c.f.* Figure 4) was composed of 4 rectangular trays (125 cm x 50 cm and water depth of 8 cm). Approximately 20 l of natural sea water was added to each tray. According to Lussier *et al.*, (1988), natural seawater is generally a better culture medium for mysids than artificial seawater. Connected to these trays was a tank with submerged crushed oyster shell where biological filtration occurred. The two upper trays (culture trays) contained mature, spawning mysids and the two lower trays (hatchling trays) were used to collect the hatchlings. These hatchling trays were used for the growth and survival trials. Each experiment had a duration of 30 days. The drain of the upper culture trays was covered with a 1,000 μm mesh core and connected to a valved outlet in the bottom. Hatchling collection and water exchange were performed at 09:00 h and 21:00 h every day using the previously described method (Chapter 2). During each water exchange, water was recirculated with a biofilter for 10 minutes. Between water exchanges, there was no water circulation in the trays except during the temperature experiments, when gentle aeration was supplied to both the culture and hatchling trays, using air stones.

Water quality was carefully monitored in each experiment. Ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen were measured twice a week at the end of 12 h periods using HACH® reagents (Loveland, Colorado) (Chapter 2). The pH was measured every day at the beginning and the end of a 12 h cycle, using a pinpoint pH meter (Radiometer, Denmark). The room was kept in the dark with the exception of the hatchling collecting and water renewal periods. During these periods, light was provided by two 30 W fluorescent bulbs covered with a red filter.

Enrichment has been shown to be an effective way to ensure good quality *Artemia* nauplii for the mysids (Kuhn *et al.*, 1991; Ward, 1987). Previous experiments in this static system indicated that enriched *Artemia* nauplii were the best food item for mysids (Chapter 3). Therefore, in these experiments both adult and hatchling mysids were fed *Artemia* nauplii enriched with HUFA (Selco®) for 12 h. *Artemia* cysts were hatched for 24 h under intense fluorescent light at temperatures of 28°C and salinities between 18 ppt and 22 ppt and then collected on a 53 μm mesh screen.

Enrichment of the newly hatched *Artemia* nauplii was accomplished by soaking them in a solution of Selco® and sea water at 20 ppt (0.25g of Selco®.l^{-1} of sea water) for 12 h. To obtain a homogeneous solution, Selco® and sea water were mixed in a blender for one minute prior to adding *Artemia* nauplii.

4.2.1 Experiments with different mysid densities

During the experiments testing different mysid densities, temperatures were kept at 26 ± 2°C; salinities were maintained at 20 ± 2 ppt. A total of five densities were tested (500, 750, 1,000, 2,000 and 4,000 mysids) per 20 l tray, corresponding to densities of 25, 37.5, 50, 100 and 200 mysids.l^{-1}, respectively. Hatchlings were collected and counted twice a day. Mysid production was expressed in the number of hatchling.d^{-1}. Mean production was obtained by dividing the number of hatchling harvested during the experiment by 30 (the number of days). Unpaired student t-tests were used to compare differences on daily production from all mysid densities tested (Zar, 1984). Adult mysid survival was expressed as a percentage and determined by counting the mysids remaining at the end of each experiment and dividing then by the initial number of mysids for each population.

4.2.2 Experiments testing production at different temperatures

A total of 1,000 adult mysids (50 mysids.l^{-1}) were used in each experiment testing the effects of different temperatures on production, growth and survival. The test temperatures were 18 ± 1°C, 22 ± 1°C and 26 ± 2°C; salinity was maintained at 20 ppt. As for the previous experiments, mysids were fed *Artemia* nauplii enriched with Selco®. Hatchlings were collected and counted twice a day. Mysid production was expressed in the number of hatchling.d^{-1} and mean production for the experiment was also determined. Unpaired student t-tests were used to compare daily production from mysids cultured at different temperatures (Zar, 1984). Percent adult mysid survival was determined by counting the mysids in the culture trays at the end of each experiment and dividing it by 1,000 (the initial density) and multiplying it by 100.

4.2.3 Hatchling survival experiments

To determine survival of hatchlings, a total of five-7 day experiments were conducted at each of the three different temperatures, 18°C, 22°C and 26°C . For each

experiment, a total of 200 hatchlings were placed in one or both hatchling trays. Water exchanges and feeding were performed twice a day. At the end of 7 days, mysids left in the trays were counted; survival was expressed as a percentage by dividing the final number of mysids by the initial number of mysids and multiplying by 100. The non-parametric Kruskal-Wallis one-way ANOVA was used to determine differences in hatchling survival at the different temperatures (Zar, 1984).

4.2.4 Growth experiments

For each temperature tested, a total of 200 hatchlings were placed in one of the hatchling trays. Feeding and water renewal was performed twice a day following the protocol for the survival experiments. Each experiment was terminated two days after the mysids began to reproduce. Every two days, 10 mysids were randomly collected, killed with alcohol (70%) and measured for growth using a dissecting microscope with an ocular micrometer. The time of release of the first brood was registered during each growth experiment with the exception of mysids kept at 18°C. In this case the experiment ended after 29 days before any reproduction was observed. Statistical analysis using a Kruskal-Wallis one-way ANOVA was used to determine significant differences in growth of mysids related to temperature as well as time (Zar, 1984).

4.3 Results

4.3.1 Mysid production at different densities

Production at different mysid densities is shown in figure 16. There was a positive correlation between mysid densities and hatchling production up to 100 mysids.l^{-1}; production was 84 ± 35 hatchlings.d^{-1} at densities of 25 mysids.l^{-1}, 134 ± 46 hatchlings.d^{-1} at 37.5 mysids.l^{-1}, 133 ± 69 hatchlings.d^{-1} at 50 mysids.l^{-1} and 273 ± 99 hatchlings.d^{-1} at 100 mysids L^{-1}. Production was not significantly different ($p=0.943$) between densities of 37.5 and 50 mysids.l^{-1}, but was significantly different ($p=0.000$) between all other mysid densities. The 200 mysids.l^{-1} population ceased producing any new hatchlings after day 5. Statistical comparison between mysid densities is shown in Table III (Appendix). Survival of spawning mysids at the end of each experiment was 76%, 72%, 73% and 58% for densities of 25, 37.5, 50, and 100 mysids.l^{-1}, respectively.

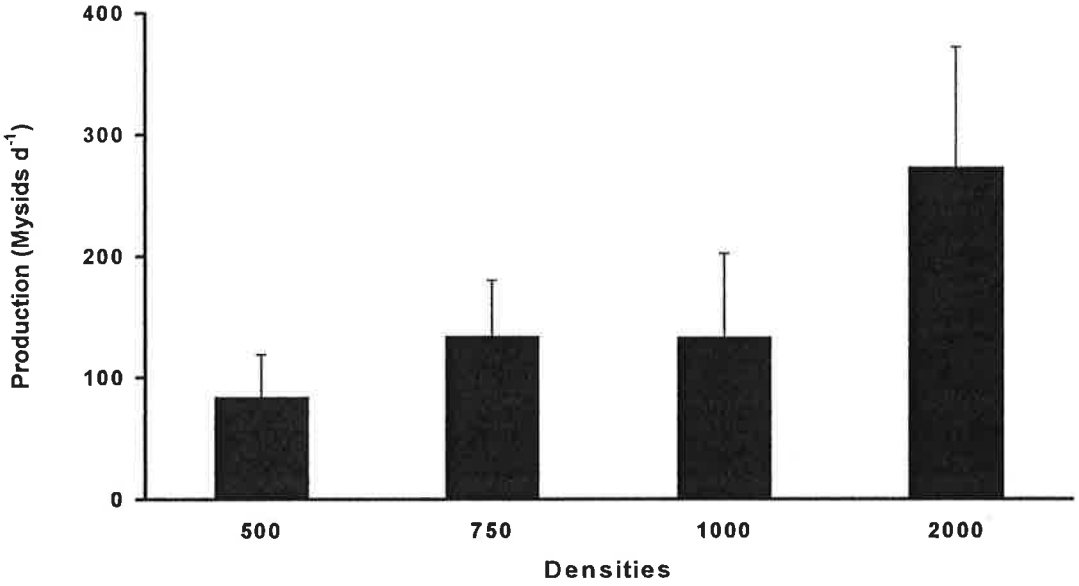

Figure 16 – Production by populations of 500, 750, 100 and 2000 mysids (densities of 25, 37.5, 50 and 100 mysids.l^{-1}) during 30 days. Bars indicate standard deviations.

Water quality was maintained at acceptable levels. Ammonia-nitrogen and nitrite-nitrogen never exceeded 1.65 mg.l^{-1} and 0.250 mg.l^{-1}, respectively. The lowest pH value registered was 7.41 in the tray with density of 100 mysids.l^{-1}; pH values were always above 7.5 for all other mysid densities. When testing densities of 200 mysids.l^{-1}, production was very low and mortality increased rapidly by the fourth day of the experiment. Between water changes (every 12 h), pH in the water decreased to 7.1 and ammonia-nitrogen and nitrite-nitrogen increased to 2.3 mg.l^{-1} and 0.320 mg.l^{-1} respectively, causing high mysid mortality.

4.3.2 Mysid production at different temperatures

Hatchling production at different temperatures is shown in Figure 17. Mean hatchling production was 225 ± 80, 232 ± 121, and 192 ± 66 hatchlings.d^{-1} at 26°C, 22°C and 18°C, respectively; there were no significant differences ($p>0.05$) in hatching production at these three temperatures. Survival of spawning mysids in the culture trays at the end of each experiment (30 days) was 69%, 82% and 80% at 26, 22 and 18°C, respectively.

Ammonia-nitrogen in the trays after 12 h were always below 1.2 mg.l^{-1} at 18°C and 22°C and below 1.4 mg.l^{-1} at 26°C. Highest nitrite-nitrogen values recorded

after a 12-h period were 0.190 $mg.l^{-1}$ at 18°C, 0.215 $mg.l^{-1}$ at 22°C and 0.250 $mg.l^{-1}$ at 26°C. The pH values were never below 7.6 for any temperature.

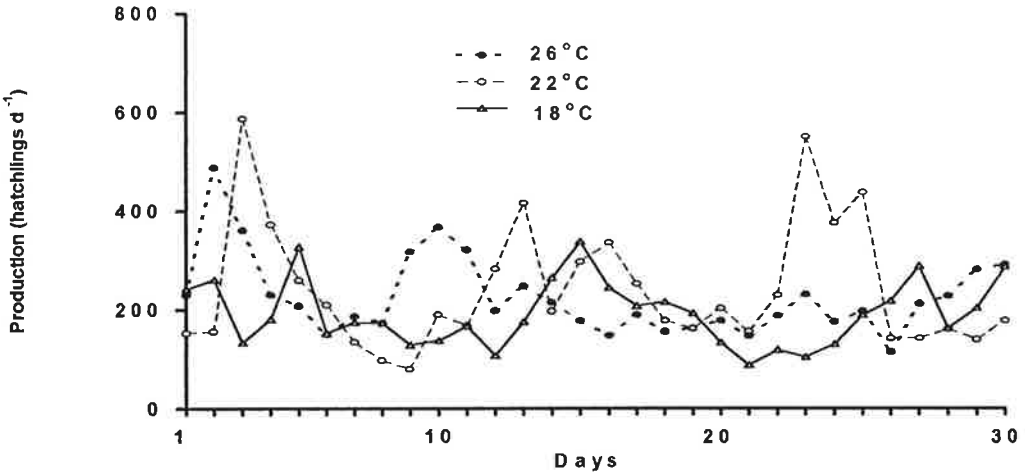

Figure 17 – Hatchling production by 1000 mysids cultured at three different temperatures (26°C, 22°C and 18°C) during 30 days.

4.3.3 Hatchling survival at different temperatures

Percent survival of newly hatched mysids after 7 days at the three temperatures tested is shown in Figure 18.

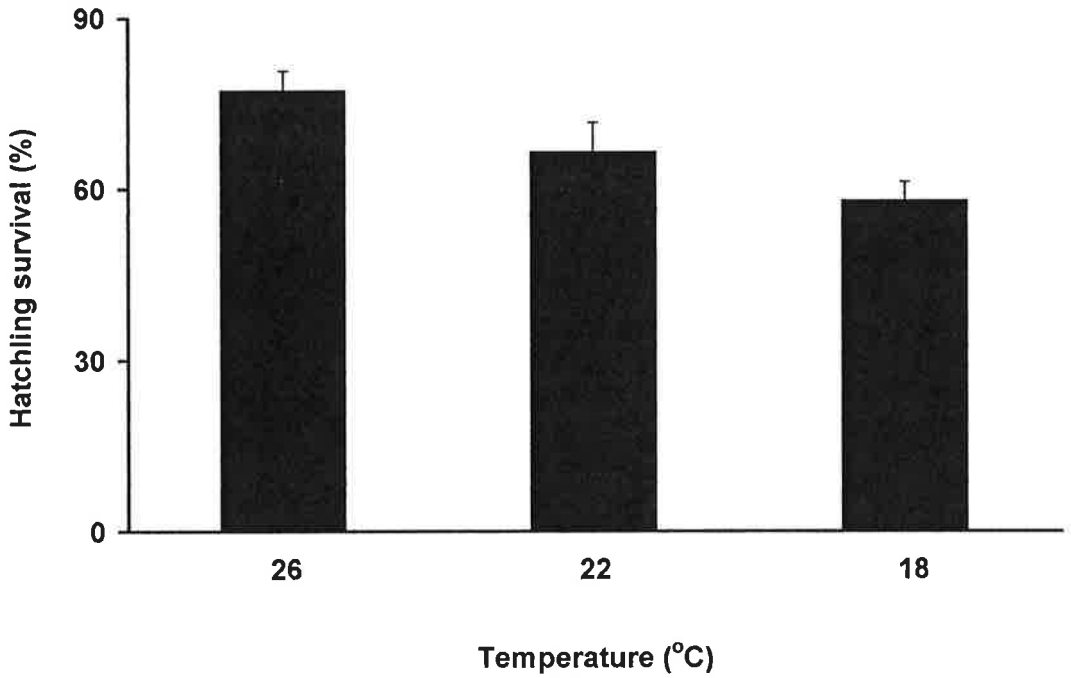

Figure 18 – Hatchling survival at three different temperatures (26°C, 22°C and 18°C) cultured for 7 days. For each temperature, 4 replicates were made. Bars indicate standard deviations.

Survival was 77.3 ± 3.5% at 26°C, 66.6 ± 5.1% at 22°C and 58 ± 3.3% at 18°C. Survival was directly correlated to temperature and significantly different for mysids kept at 26 and 22°C (DF=5, p=0.012), at 26 and 18°C (DF=5, p=0.009) and at 22 and 18°C (DF=5, p=0.028).

4.3.4 Growth at different temperatures

Mysid growth at different temperatures is shown in Figure 19. Mysids grew to 6.1 ± 0.1 mm in 15 days at 26°C, to 6.1 ± 0.1 mm in 23 days at 22°C and to 4.0 ± 0.23 mm in 29 days at 18°C. There was a positive correlation between growth and temperatures since at higher temperatures mysids grew significantly ($p<0.05$) faster.

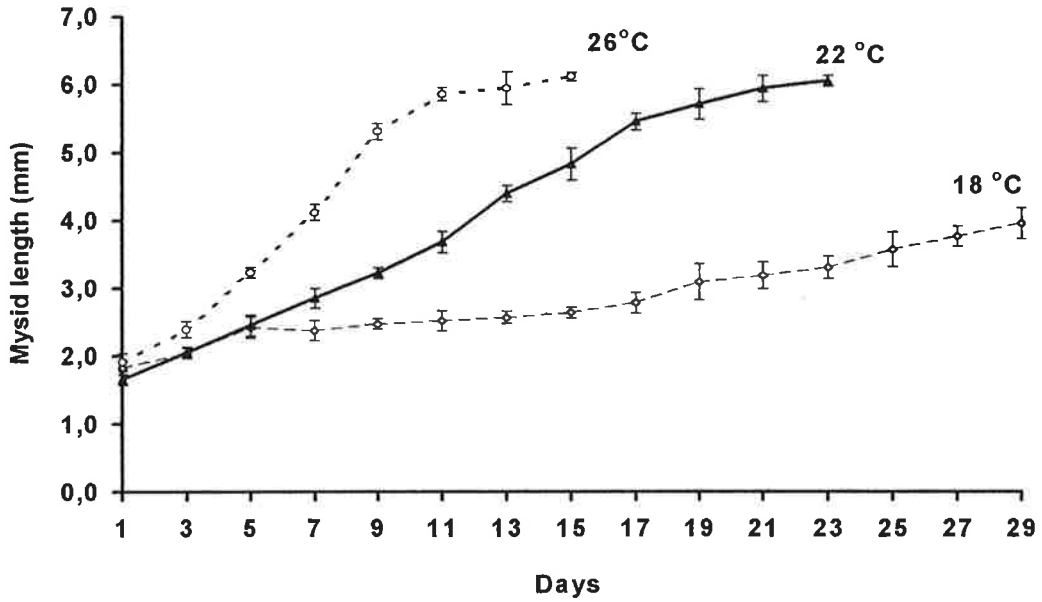

Figure 19 – Growth of mysids cultured at three different temperatures (26°C, 22°C and 18°C) during 30 days. Every two days, 30 mysids were collected from each tray and measured. Bars indicate standard deviations.

No significant differences were identified in the size of hatchlings from spawning adults kept at 26°C and 18°C (p=0.065) but mysids hatched at 26°C and 18°C were significantly larger than the ones hatched at 22°C (p=0.000 and p=0.014, respectively). Mysids reared at 26°C grew significantly larger from day 3 onwards in relation to mysids grown at either 22°C and 18°C. Although mysids hatched at 18°C were initially larger than the ones hatched at 22°C, mysids cultured at each temperature were the same size by day 3. However after day 5, mysids reared at 22°C grew significantly larger than mysids reared at 18°C.

Mysids in the hatchling trays cultured at 26°C released their first brood 13 days after hatching, while mysids cultured at 22°C released their first brood 8 days later, at 21 days. Size of mysids at the time of release of first brood during these growth trials in the hatchling trays; 5.9 ± 0.2 mm at 22°C and 5.9 ± 0.3 mm at 26°C, was not significantly different (p=0.139). At the end of 29 days, mysids cultured at 18°C had not released or produced any hatchlings in their pouches.

4.4 Discussion

Determining the optimum mysid densities is one of the most important factors for the success of mysid culture. If the culture system is densely populated, there is a higher possibility of adult mysids cannibalizing their young, since young mysids will have less space to hide and avoid contact with adults. Mysids will cannibalize their young if insufficient food is provided (Reitsema & Neff, 1980; Lussier *et al.*, 1988). Accurate feeding concentrations are harder to maintain for higher densities. Underfeeding, causing subsequent cannibalism and overfeeding, resulting in poor water quality, can occur more frequently. In addition, population density affects reproduction; a population that is too dense will stop reproducing and a high proportion of adults and females with empty brood sacs will be present (Lussier *et al.*, 1988).

Flow-through cultures are normally maintained at 15 adults.l^{-1} and static systems are generally maintained at 10 adults.l^{-1} (Lussier *et al.*, 1988). Reproduction consistently occurred at culture densities up to 50 mysids.l^{-1} in our previous experiments with a static water system (Chapter 3) and up to 100 mysids.l^{-1} in the experiments reported here. Ward (1984, 1991) culturing *M. bahia* used a concentration of 2.3 mysids.l^{-1} and Reitsema & Neff (1980) used 6.1 mysids.l^{-1} in recirculating water systems (with *M. almyra*).

In the static culture system described herein, mysids reproduced continuously at densities up to 100 mysids.l^{-1} of sea water; this was the mysid density that promoted the best production. Even at densities of 200 mysids.l^{-1} reproduction was observed, although not as high as at 100 mysids.l^{-1}. This decrease might be related to crowding in the static system causing a coincident increase in cannibalism or deterioration of water quality. Reitsema & Neff (1980) culturing *M. almyra* in a recirculating water system (57 l glass aquarium stocked with broodstock and a 19 l

glass aquarium where hatchlings were collected) obtained up to 400 hatchlings.d^{-1}; average productions were 150-200 hatchlings.d^{-1} using a density of 6.1 mysids.l^{-1} at 25°C. Using the static system up to 500 mysids.d^{-1} with mean production of 273 hatchlings.d^{-1} at 26°C with densities of 100 mysids.l^{-1} were obtained. At 22°C and densities of 50 mysids.l^{-1} up to 600 hatchlings.d^{-1}, with mean production of 232 hatchlings.d^{-1} were obtained. Individual hatchling production by each spawning mysid (between 0.43 and 0.57 hatchlings.spawning mysid^{-1}.d^{-1}) obtained by Reitsema & Neff (1980) was higher than in the current static system (highest production of 0.23 hatchlings.spawning mysid^{-1}.d^{-1}). Nevertheless, considerably higher spawning mysid densities were used in the static culture system, obtaining higher daily production rates (hatchlings.d^{-1}).

The most important factor in culture management in a recirculating or static water system is the build up of biological wastes (Ward, 1991). In the current experiments, water quality appears to be the most limiting factor on reproduction rather than the high mysid densities and associated increase in cannibalism. The deterioration in water quality was caused by high concentrations of prey (*Artemia* nauplii) necessary to feed dense populations of mysids. According to Miller *et al.*, (1990), the toxicity of un-ionized ammonia increased as pH decreased for the related mysid *M. bahia*. Ward (1991) reported that reproduction in *M. bahia* occurred for 24 weeks even when pH steadily decreased from 8.0 to 7.5. Lussier *et al.*, (1988) indicated that pH should be maintained above 7.8 and that below 7.4 reproduction ceased for *M. bahia*. In order to maintain higher mysid concentrations in the static system (over 100 mysids.l^{-1}), more frequent water changes would be necessary to maintain good water quality. More frequent water changes would also reduce the time that hatchlings would spend together with the adults, reducing cannibalism and increasing their chances of survival.

Good aeration and partial water exchanges in the static system were essential in helping to control pH drops caused by oxidation of ammonia by nitrifying bacteria (Ward, 1991). Between the experiments evaluating mysid densities and those evaluating temperature, some modifications were made to the static tank systems. Partial water exchanges of about 30% every week in the biological filtration tank were instituted and gentle aeration in the culture and hatchling trays was begun. These modifications may be responsible for the improved water quality and consequently

resulted in increased production. Production at the 50 mysids.l^{-1} and 26°C (133 ± 69 hatchlings.d^{-1}, almost doubled, (225 ± 80 hatchlings.d^{-1}), after gentle aeration and partial weekly water exchanges in the biological filtration tank were instituted on a weekly basis. Even at lower temperatures, production at 50 mysids.l^{-1} almost doubled after these modifications were made. After the introduction of aeration, ammonia-nitrogen and nitrite-nitrogen concentrations in the trays at the end of 12 h periods were generally lower than before although not statistically significant ($p>0.05$). The drop of pH during the 12 h static period was also slowed; pH never dropped below 7.6 compared to previous experiments (Chapters 2 and 3) when pH dropped below 7.4 at similar mysid densities and temperatures.

Decreasing the age of first reproduction is an effective way of increasing total reproductive output (Lewontin, 1965). Time of appearance of marsupium (indicative of sexual maturity) and release of first brood is directly related to temperature (McKenney, 1996). By increasing temperature, the age of first reproduction and intervals between reproduction periods should decrease, but mysid metabolism would increase correspondly. At higher temperatures, mysids should produce more young and their life cycle should be shorter. However, more food has to be provided to the adult mysids to meet their increased metabolism. The increased metabolism and subsequent increased nitrogen excretion may contribute to poor water quality when compared to culturing mysids at lower temperatures.

Reitsema & Neff (1980) obtained better production with *M. bahia* at high temperatures (25-30°C). In the static water system experiments the highest production was obtained at 22°C instead of 26°C. We expected higher production at 26°C since mysids released their first brood in 13 days at 26°C compared to 21 days at 22°C. Also, time between reproduction periods was probably shorter at 26°C due to the mysids increased metabolism. Nevertheless, survival of spawning mysids in our culture trays at 26°C was lower (69%) than survival of spawning mysids at 22°C (82%) and 18°C (80%). The improved survival of mysids cultured at lower temperatures appears to have a greater effect on production than the higher production rates by mysids cultured at 26°C. Reitsema & Neff (1980) also reported higher long-term survival of adults cultured at lower temperatures (25°C compared to 30°C). Reitsema & Neff (1980) reported that at temperatures below 20°C reproduction on *M. almyra* almost ceased. At 18°C, mysids reproduced continuously in the static system

and production, of 192 ± 66 hatchlings.d^{-1}, was not significantly lower than at 22°C or 26°C at a culture density of 50 mysids.l^{-1}.

Concentration of ammonia-nitrogen and nitrite-nitrogen in the trays at 22°C were usually lower than at 26°C. The pH recorded was also slightly better at 22°C at the end of 12 h static periods. Nevertheless, higher adult survival at lower temperatures, instead of slight improvement in water quality, appears to be the most important factor for obtaining higher production at 22°C.

Reitsema & Neff (1980) reported that *M. almyra* reached sexual maturity between 17-21 days at 28°C. McKenney (1996) reported release of first brood in 15.9 days for *M. bahia* cultured at 28°C. In the static system, *M. almyra* cultured at 26°C released the first brood 13 days after hatching. Leger *et al.*, (1989) reported that *M. bahia* cultured at 23°C reaches sexual maturity in 14 days; similar results were obtained for *M. almyra* cultured at 22°C. Although mysids released their first brood 21 days after hatching, the appearance of marsupium (sexual maturity) was observed at 14 days. In the static system, mysids cultured at 26°C released their first brood 8 days before mysids cultured at 22°C while mysids cultured at 18°C were not sexually mature 29 days after hatching. McKenney (1996) reported that at 19°C *M. bahia* required an additional 10-15 days to mature and an additional 20-25 days to release their first brood in comparison to mysids cultured at 25°C.

In the static system experiments, hatchling *M. almyra* survived better at higher temperatures (26°C) during their first week of life. McKenney (1987) reported optimum temperatures of 28°C for the first week, 23°C for the second week and 20°C for adult mysids. This pattern of declining temperature preference with maturity could explain the better survival observed for mature reproductive mysids when culturing them at lower temperatures and better survival for young at higher temperatures using the static culture system. McKenney (1994) reported better survival for *M. bahia* less than 14 days old at 25°C in comparison to lower temperatures. Growth of mysids was highly influenced by temperature. Mysids grew to 4 mm in 29 days at 18°C; 14 days at 22°C and 7 days at 26°C. Rapid growth and better survival of hatchlings at warmer temperature (26°C) improve the economic practicality of rearing mysids as food for cultured marine species. The 4 mm mysids were one of the goals of this research since

they are the ideal size for feeding to hatchling and juvenile cephalopods cultured in our laboratories (Turk *et al.*, 1986; Lee *et al.*, 1994).

5. THE EFFECTS OF A TOTAL OR PARTIAL REPLACEMENT OF *Artemia* NAUPLII BY ROTIFERS (*Brachionus plicatilis*) ON PRODUCTION, SURVIVAL AND GROWTH OF MYSIDS (*Leptomysis* sp.).

5.1 Introduction

Feeding behavior of *Leptomysis* sp. from the Mediterranean has been described (Wittmann, 1977; 1986; Dauby, 1985; Van Dalfsan, 1986) and is associated with the formation of swarms (Dauby, 1995). During the day, swarms are well formed and mysids spend most of the time in quite stationary groups just above the bottom. At night, swarms break up and mysids swim actively in search of food, close to the bottom (Dauby, 1995). Using *Leptomysis* sp., Mauchline (1969) indicated that more than half of the mysids collected during the day had little or no food in their stomachs. In a similar fashion, Hecq *et al.*, (1981, 1984) showed that enzymatic activity increased during the night, which indicated increased digestion. It appears that *Leptomysis* sp. are mainly omnivorous, as well as cannibalistic (Wittmann, 1978; Dauby, 1985), but not selective feeders (Dauby, 1995).

The brine shrimp (*Artemia* sp.) is widely used as feed in aquaculture because of its availability as dormant cysts (Watanabe, 1987), ease of hatching (Sorgeloos *et al.*, 1983) and potential for packing and enrichment. *Artemia* has been used by several authors to culture mysids (Lussier *et al.*, 1988; Kuhn *et al.*, 1991). Nevertheless, the fluctuating price of *Artemia* cysts is a major factor affecting the costs of mysid production. Therefore, a cheap food must be found to produce mysids in large numbers at a stable, predictable cost (Chapters 3 and 4). Rotifers (*Brachionis plicatilis*) are cheaper to produce than *Artemia* nauplii. In Tenerife, the production costs of one rotifer (using baker's yeast and phytoplankton) were 0.5% of the costs associated with the production costs of one rotifer. Since the effects of feeding rotifers to mysids is not well known, the effects of a partial and a total replacement of *Artemia* nauplii with rotifers were tested in a flow-through culture system.

5.2 Material and methods

Mysids were collected by diving around the island of Tenerife (Canary Islands, Spain) (Figure 20) at depths between 10 and 20 meters using 250 μm mesh size plankton nets. Mysids were identified as *Leptomysis* sp. Identification of the

species was not possible. According to Dauby (1995), the different populations of *Leptomysis* sp. hardly mix. Therefore it is likely that mysids used in out experiments were from the same species.

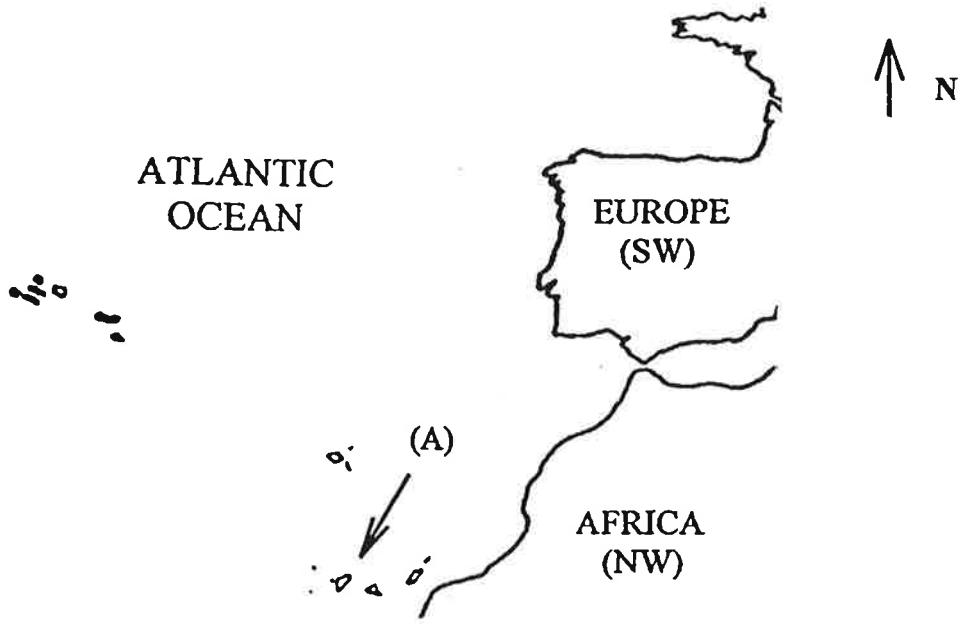

Figure 20 – Tenerife Island (A), where mysids were collected; research was conducted at the culture plant of the "Centro Oceanográfico de Canarias" in Tenerife.

Mysids used in this study were collected at depths between 10 and 20 m, but they were more abundant at shallower depths. Mysid swarms were small, and swam close to the sand and rocks, usually in the shade which probably provides more protection against predators. This type of biotope is similar for the one described by Dauby (1995) for *L. lingvura*. Nevertheless we were not able to confirm if mysids used in this study belonged to this species.

After acclimation periods between one and two days, unsexed adults in good condition were separated to be used in the experiments. The effects of *Artemia* nauplii replacement by rotifers on mysid production, survival and growth were measured using three diets in flow-through systems. The three diets used were: 1) 33% *Artemia* nauplii + 66% rotifers, 2) 100% *Artemia* nauplii , and 3) 100% rotifers. Artemia cysts AF 480, previously enriched (W3-HUFA>10 $mg.g^{-1}$ dry weight) came from INVE AQUACULTURE – ARTEMIA SYSTEMS. Nauplii mean size was 480 µm. Cysts were hatched for 24 h and immediately fed to the mysids. Rotifers (*B. plicatilis*, type L, size between 200-240µm) were cultured and enriched with phytoplankton

(*Tetraselmys suesica*, at concentrations of 30,000 cells.rotifer^{-1} for 6 h) and baker's yeast (L'hirondelle, France, at concentrations between 0.7-1 g.million rotifers^{-1}). Mass culture of rotifers was done in tanks with water volume of 6,500 l. Mean daily concentration was 170 rotifers.ml^{-1} (daily production of 1,100 million rotifers); daily harvesting was between 10-15% of the total.

Nauplii and rotifer were fed *ad libitum* since mysids are cannibalistic and will eat their young if not enough food is provided (Reitsema & Neff, 1980). Prey concentrations were determined on a daily basis before feeding them to the mysids. Since a flow-through was being used, water quality was not controlled. Temperature and salinity were recorded on a daily basis. Two types of culture tanks were used (Figure 21).

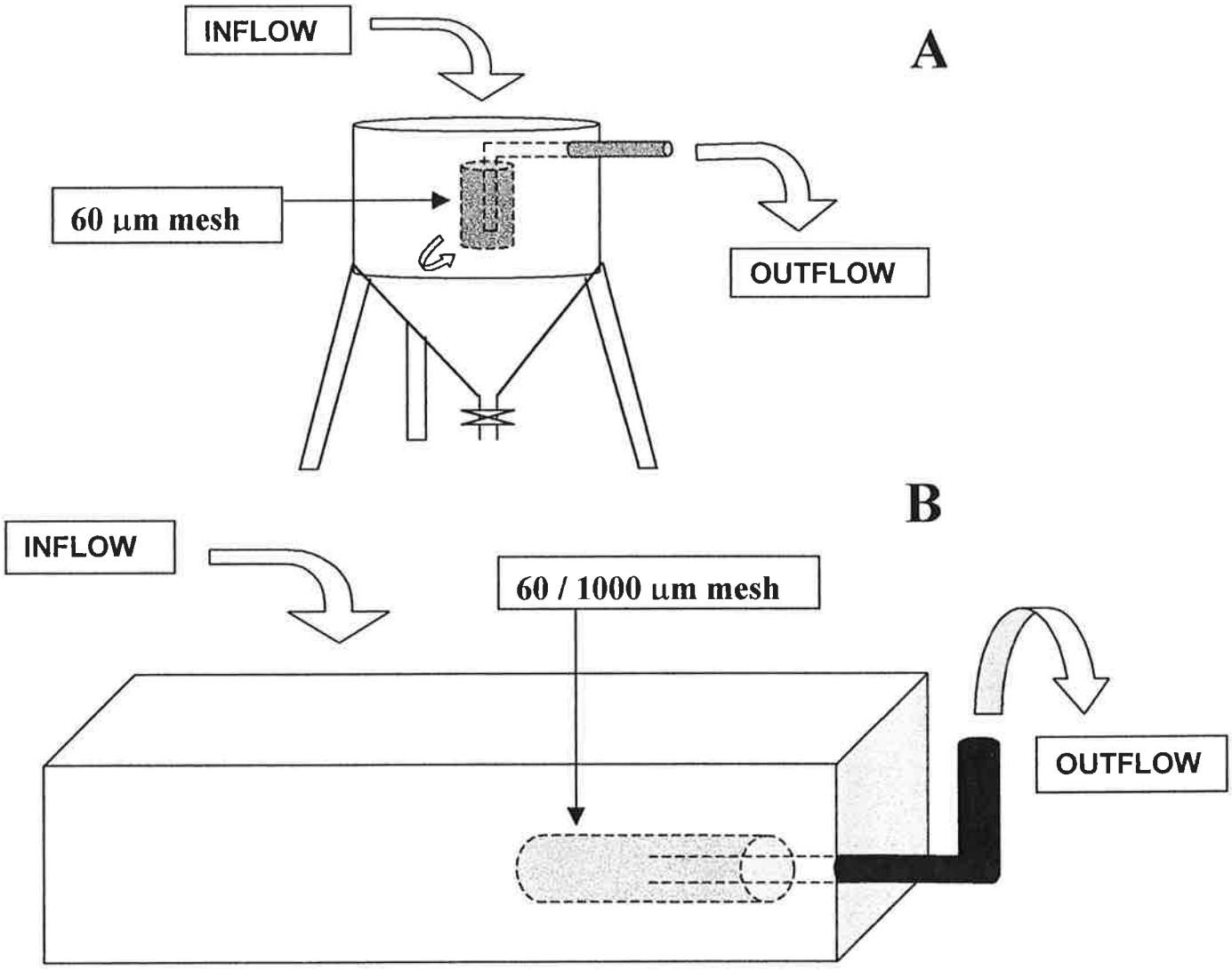

Figure 21 - The diagrams represent the flow-through systems used in Tenerife to culture mysids (Leptomysis sp.). It was composed of 12 conical tanks and 2 rectangular trays. Water volume was 12 l per tank or tray. The conical tanks (A) were used to test mysid growth and survival. The rectangular trays (B) were used in the experiments evaluating production.

The first system was composed of two rectangular plastic trays filled with 12 l of seawater each, with water depth was 8 cm. These trays were used to compare mysid production. The second system was composed of 12 conical tanks (30 cm diameter; 25 cm water depth). These tanks were used to test growth, survival and other biological aspects of the mysids life cycle such as age of the appearance of the marsupium or release of 1st brood, in days.

In all experiments, a continuous water flow of 0.2 l.minute^{-1} (12 l.h^{-1}) promoted a complete water turnover in each culture tank and tray every hour. Prey concentration for each day was: 100 *Artemia* nauplii + 200 rotifers.mysid $^{-1}$ for diet 1, 200 *Artemia* nauplii.mysid^{-1} for diet 2 and 400 rotifers.mysid^{-1} for diet 3, divided into two feeding periods (12:00 h and 24:00 h).

5.2.1 Production experiments

In each of these trays a total of 600 mysids were placed (mysid densities of 50 mysids.l^{-1}). Mysids in each tray were fed one of the three diets. Hatchlings were collected and counted once a day, before the 12:00 h feeding period. Each experiment lasted 20 days. After 20 days, the remaining adult mysids were counted (to determine survival of adults fed each diet during 20 days) and the necessary numbers of adult mysids were added to each tray to equal the numbers at the start of the experiment (600 mysids). Three replicates were made when testing each diet. Differences in production of the offspring grown on the three diets were compared statistically with unpaired student t-test, significance at $p<0.05$ (Zar, 1984). Mysid production was expressed in numbers of hatchlings.d^{-1}.

5.2.2 Survival experiments

Hatchling survival was determined by placing 200 hatchlings in a conical tank. After 20 days the remaining mysids were collected and counted. Adult survival was determined at the end of 20 day periods during the production experiments and in separate trial similar to the ones used to test hatchling survival. Adult and hatchling survival was expressed as a percentage, by dividing the mysids at the end of each experiment by the initial density and multiplying by 100. For each diet tested, a total of five replicates were made. Non-parametric Wilcox matched pairs tests, significance at $p<0.05$ (Zar, 1984) were used to determine differences in survival.

5.2.3 Growth experiments

For each diet being tested, a total of 300 hatchlings were placed in one of the conical tanks. Every three days, 10 mysids were randomly collected from each tank and measured using a dissecting microscope with an ocular micrometer. Total length (mm) was measured from the tip of the rostrum to the tip of the telson. Careful observation of mysids was also preformed to determine the presence of marsupium and number of eggs in the marsupium. When gravid females were observed, a careful observation of the tanks was made on a daily basis to determine when hatchlings were present. A total of three replicates per diet were made. Unpaired student t-test, significance at $p<0.05$ (Zar, 1984) were used to determine statistical differences in growth of mysids fed each diet.

5.3 Results

5.3.1 Production experiments

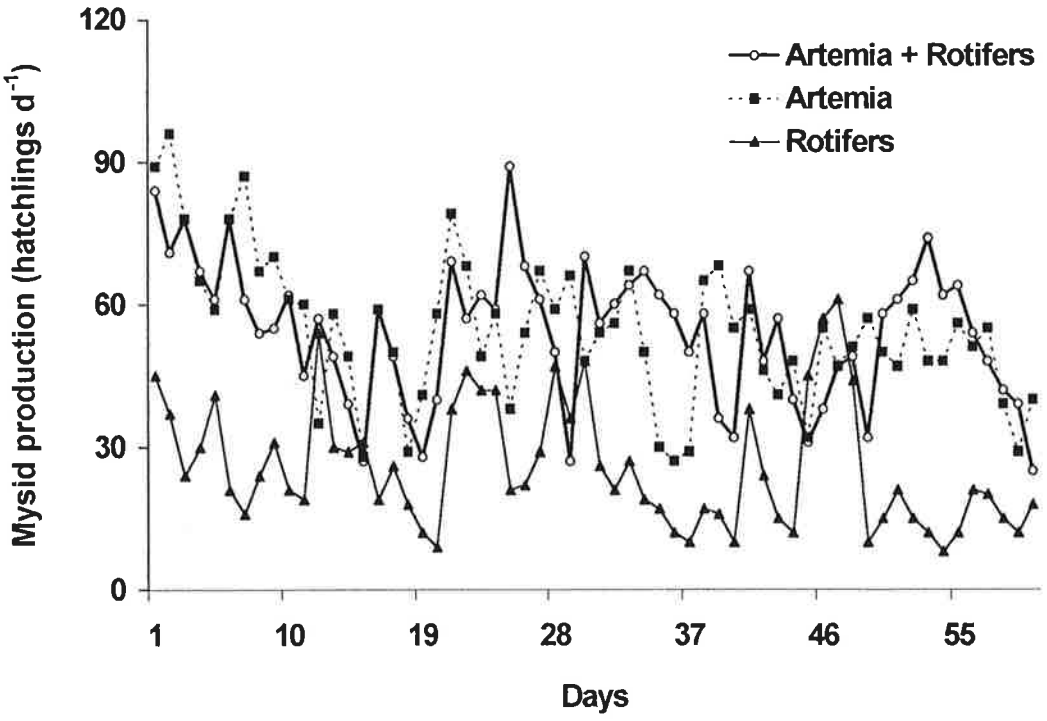

Figure 22 – Daily hatchling production by three populations of 600 mysids. Each population was fed one of three diets: 1) *Artemia* nauplii + rotifers, 2) 100% *Artemia* nauplii and 3) 100% rotifers for 60 days.

Daily production from mysids fed the three diets is shown in Figure 22. Mysids fed diets 1, 2 and 3 produced 57 ± 14 hatchlings.d^{-1}, 55 ± 15 hatchlings.d^{-1} and 21 ± 13 hatchlings.d^{-1}, respectively. This corresponds to 0.1, 0.09 and 0.04 hatchlings produced by each adult mysid fed diets 1, 2 and 3, respectively. Production was not significantly different (p=0.6707; T=0.4283; N=60) between diets 1 and 2, but was significantly different between diets 1 and 3 (p=0.0000 T=10.2608; N=60) and diets 2 and 3 (p=0.0000; T=11.3836; N=60).

5.3.2 Survival experiments

Survival of both hatching and adult mysids fed the three diets after 20 days is shown in Figure 23.

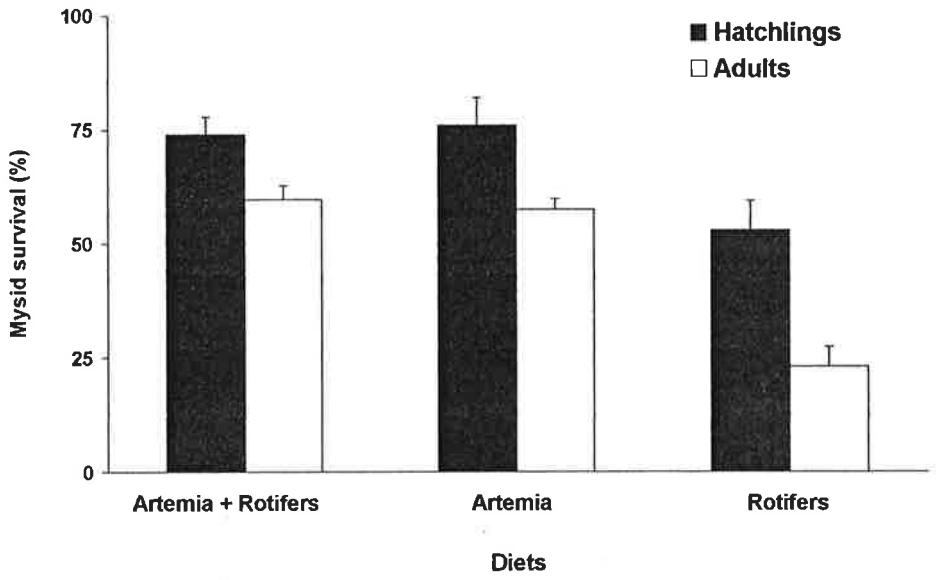

Figure 23 – Survival of hatchlings and adult mysids fed three diets: 1)*Artemia* nauplii + rotifers, 2) 100% *Artemia* nauplii and 3) 100% rotifers. For each diet tested and for both hatchlings and adults, five replicates were made. Bars indicate standard deviations.

Survival of hatchlings fed diets 1, 2 and 3 was 74 ± 4%, 76 ± 6% and 53 ± 7%, respectively. Hatchling survival was not significantly different between diets 1 and 2, (p=0.7874; T=6.5; Z=0.2697; N=5) but was significantly different between diets 1 and 3 (p=0.0431; T=0.0000; Z=2.0226; N=5) and between diets 2 and 3 (p=0.0431; T=0.0000; Z=2.0226; N=5). Survival of spawning aduts fed diets 1, 2 and 3 was 60 ±

3%, 58 ± 2% and 23 ± 4%, respectively. As for the hatchlings, adult mysid survival was not significantly different between diets 1 and 2 (p=0.6858; T=6.0; Z=0.4045; N=5), but was significantly different between diets 1 and 3 (p=0.0431; T=0.0000; Z= 2.0226; N=5) and between diets 2 and 3 (p=0.0431; T=0.0000; Z= 2.0226; N=5). Survival was higher (p<0.05) for hatchlings when compared to adults fed each of the three diets being tested.

5.3.3 Growth experiments

Growth of mysids fed the three diets is shown in Figure 24.

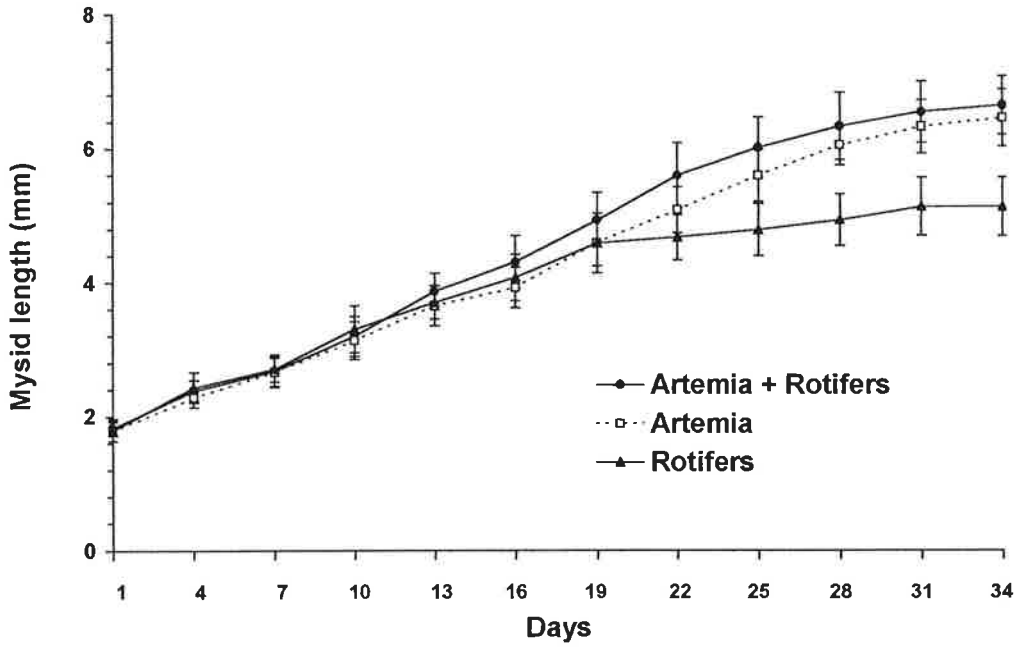

Figure 24 – Growth (mm) of mysids fed three diets: 1) *Artemia* nauplii + rotifers, 2) 100% *Artemia* nauplii and 3) 100% rotifers for 34 days. Every 3 days, 10 mysids were collected from each tank and measured. Three replicates were made for each diet. Bars indicate standard deviations.

During 34 days mysids fed diets 1, 2 and 3 grew up to 6.7 ± 0.4 mm, 6.6 ± 0.4 mm and 5.2 ± 0.4 mm, respectively. The analysis of figure 24 and Table IV (statistical table, Appendix) show that size of hatchlings released by females fed the three diets (1.8 mm) was not significantly different (p>0.05). Growth of mysids fed *Artemia* nauplii+rotifers and mysids fed *Artemia* nauplii exclusively was similar for the first 10 days. After that, mysids fed the mixed diet grew significantly larger (p<0.05) until day 31. At day 34, the age of release of 1st brood by females fed both diets, size was

similar again ($P>0.05$). Comparison between growth of mysids fed rotifers exclusively and the mixed diet of rotifers+*Artemia* nauplii show that growth was similar ($p>0.05$) for the first 10 days. After that, mysids fed the mixed diet grew significantly larger ($p<0.05$) than mysids fed rotifers alone. Growth of mysids fed *Artemia* nauplii or rotifers, exclusively, showed a similar pattern (although different for days 4 and 10) until day 22 of the experiment. From that day on, mysids fed rotifers grew significantly less than mysids fed *Artemia* nauplii.

Female mysids fed rotifers+*Artemia* nauplii (diet 1) and *Artemia* nauplii alone (diet 2) had eggs in their marsupium 22 and 25 days after being born, respectively, and released their first brood at the same age (34 days). Female mysids fed rotifers exclusively (diet 3) had eggs in their marsupium 34 days after birth but did not released or produce any hatchlings even at 40 days. Brood sizes were determined by dissecting and counting the hatchlings in the marsupium. They ranged from 2 to 14, but usually not more than 8 were present in the marsupium. During all experiments, salinity was 36 ppt and water temperature was 22 ± 1°C.

5.4 Discussion

Culture management is the most important factor in achieving highly productive mysid cultures (Lussier *et al.*, 1988). During the present study, a flow-through system was used, contrary to previous experiments (Chapters 2, 3 and 4), in which a static water system was used. This allowed us to focus on the mysid culture without concern for the water quality. In static culture systems, biological wastes accumulate, resulting in poor water quality. High concentrations of ammonia, nitrite and nitrate together with low dissolved oxygen increase mysid mortality (Brandt *et al.*, 1993). According to Ward (1991), in recirculating water systems, the current carries the *Artemia* nauplii to the core where they become trapped or through the core, where they are lost. In the current system, a fine mesh of 60 μm was placed around the outflow. This mesh prevented not only mysids, but also rotifers from exiting the system. Light aeration around the mesh and a slow water flow were used in combination to prevent or reduce clogging. This system worked well throughout the experiments. Meshes were cleaned twice a day before feeding periods and no clogging was registered in any tank or tray.

Lussier *et al.* (1988) reported mysid densities of 15 adults.l^{-1} in flow-through

systems. In the present study, mysid densities in the production experiments (50 mysids.l^{-1}) were the same as in previous experiments (Chapters 2, 3 and 4) in static water systems. We believe that if a static water system could maintain 50 mysids.l^{-1}, so should a flow-through system. However, production by *Leptomysis* sp. in the current flow-through system (0.09 hatchlings.adult^{-1}) was significantly lower when compared to production by *M. almyra* (0.23 hatchlings.adult^{-1}) (Chapter 4) under almost identical conditions. With different mysid densities and temperatures, Reitsema & Neff (1980) obtained with *M. almyra*, between 0.43 to 0.57 hatchlings.adult^{-1}. Brood sizes of females in this study ranged from 2 to 14, but usually not more than 8 were present in the marsupium before release. Reitsema & Neff (1980) report larger brood sizes for *M. almyra*, between 3 and 42 with an average of 10. This indicates that the *Leptomysis* sp. used in this study has a lower reproductive potential compared to *M. almyra*, explaining the lower production obtained in the present study compared to production by *M. almyra* obtained by Reitsema & Neff (1980) and during this research (Chapters 2, 3 and 4).

One of the major factors that has prevented the large-scale production of mysids is the price of *Artemia* cysts, that adds significantly to production costs. Food other than *Artemia* (such as artificial diets or phytoplankton) have been tested by Reitsema & Neff (1980) and during the present research (Chapter 3) but proved inadequate. The substitution of *Artemia* with rotifers in the present experiments considerably reduced production costs. Producing rotifers in large quantities was about 99.5% less expensive compared to *Artemia* nauplii. These calculations were based exclusively on the price of *Artemia* cysts compared to baker's yeast and costs related to phytoplankton (*Tetraselmys suecica*) production. Even taking into consideration that the size of the rotifers produced was about one-half of that of an *Artemia* nauplii, a replacement rate of one nauplii by two rotifers used when feeding the mysids resulted in a significant reduction (50%) in production costs. Both *Artemia* cysts incubation and harvesting, and the production and harvesting of rotifers require similar labor.

The complete substitution of *Artemia* nauplii by rotifers resulted in poor results in production and survival of both hatchlings or adult mysids. Production was 21 hatchlings.d^{-1} (0.04 hatchlings.adult^{-1}) for mysids fed 100% rotifers and was significantly lower ($p=0.000$) than the production by mysids fed *Artemia* nauplii (55

hatchlings.d^{-1} or 0.09 hatchlings.$adult^{-1}$). Survival of adult mysids fed 100% rotifers was poor (23%) and significantly lower than mysids fed *Artemia* nauplii (58%). Associated with the poor survival and production of mysids fed 100% rotifers is the fact that only a few dead mysids were found in the culture trays; this is a strong indication that cannibalism (among both hatchlings and adults) could have occurred on a large scale. If cannibalism was not taking place, a lot more dead mysids would be expected to appear when cleaning the culture trays. Survival of hatchlings fed rotifers (53%) was also significantly lower when compared to hatchlings fed *Artemia* nauplii (76%). In addition, mysids fed 100% rotifers were mature (presence of eggs in the marsupium) after 34 days and still had not produced any hatchlings even 40 days after birth, while mysids fed *Artemia* nauplii were mature at 25 days and produced their first brood at 34 days. This strongly indicates that a rotifer diet is inadequate for reproduction. Growth of mysids fed 100% rotifers showed an interesting pattern. Growth was similar initially to mysids fed *Artemia* nauplii for 22 days, or *Artemia* nauplii+rotifers for the first 10 days. After that, growth of mysids fed 100% rotifers slowed significantly in comparison to mysids fed the other diets. According to these results, it appears that a diet composed of rotifers alone would not sustain the culture of *Leptomysis* sp.

Contrary to a total replacement of *Artemia* nauplii by rotifers, a partial replacement of 50% (resulting in a diet composed of 1/3 *Artemia* nauplii + 2/3 rotifers, in numbers) produced good results. In fact, production, survival (hatchlings and adults) and growth was not significantly different from mysids fed *Artemia* nauplii alone. Production by mysids fed the mixed diet, (57 hatchlings.d^{-1} or 0.1 hatchlings.$adult^{-1}$) was even slightly higher when compared to mysids fed *Artemia* nauplii alone. Similarly, survival of adult mysids fed the mixed diet was higher (60%) than that for adult mysids fed *Artemia* nauplii (58%). Mysids fed the mixed diet grew larger than mysids fed *Artemia* nauplii alone from day 13 onwards, but at the end of the experiment size was not significantly different ($p>0.05$). Female mysids fed the mixed diet reached sexual maturity 22 days after birth, compared to 25 days for females fed 100% *Artemia* nauplii. The results obtained during these experiments indicate that although a total replacement of *Artemia* nauplii by rotifers was ineffective, the combined use of rotifers and *Artemia* nauplii was successful and effectively reduced production costs.

In previous experiments, phytoplankton was fed to *M. almyra* (Chapter 3), but poor results were obtained, suggesting that this species is mainly carnivorous, as good results were obtained with *Artemia* nauplii. Nevertheless, species of mysids can feed on phytoplankton (Webb *et al.*, 1987; Dauby, 1995). *Leptomysis* sp. feed on algae and diatoms among other food items (Wittmann, 1978; Dauby, 1985). Based on the analysis of amylase activity, *L. lingvura* appears to be more "herbivorous" than "carnivorous" (Hecq, 1981, 1984). Therefore, we believe that *Leptomysis* sp. which can feed on multicellular algae and phytoplankton are promising organisms for the mass culture of mysids, as use of phytoplankton would greatly reduce production costs by shortening the food chain. *Leptomysis* sp. also prey on copepods (Gaudy & Guérin, 1979; Jerlin & Wooldridge, 1994). In our laboratory, the production of rotifers requires enrichment with baker's yeast and phytoplankton. When the baker's yeast is not consumed and sinks to the bottom of the tanks, it provides copepods with plenty of organic matter to live and reproduce. This fact explains the continuous presence of copepods, in large concentrations at times, in the rotifer culture tanks. Therefore, when rotifers were fed to mysids in our experiments, phytoplankton and copepods were also present. The phytoplankton and copepods, in this case, are "contaminants" or "by-products" to the rotifer culture, but since *Leptomysis* sp. can feed on both phytoplankton and copepods, the rotifer diet was probably a very diverse diet. The concentrations of phytoplankton and copepods in the rotifer culture tanks was variable. Therefore, we do not know the exact concentration of these feeds each time we fed rotifers to the mysids. Nevertheless, the rotifer diet by itself was not effective. The addition of *Artemia* nauplii to the diet was the key to the successful culture. The mixed diet (e.g. rotifers, copepods, microalgae and *Artemia* nauplii), having different kinds and sizes of prey, was probably the key to the good results obtained.

Rotifers have been used to culture *Mysidopsis* sp. The use of rotifers alone or in combination with *Artemia* nauplii produced better results on growth of juvenile *M. bahia* than *Artemia* nauplii alone (Lussier *et al.*, 1988). The use of a mixed diet composed of *Artemia* nauplii and rotifers promoted better survival of juvenile *M. bahia* than *Artemia* nauplii alone, although the enrichment of the *Artemia* nauplii increased survival (Kuhn *et al.*, 1991). Results obtained during the current experiments also indicate that rotifers are beneficial for hatchling mysids. The

analysis of growth curves (*c.f.* Figure 24) indicates that rotifers could be used to grow mysid hatchlings for the first two to three weeks of their life cycle, since the growth of mysids fed the 100% rotifer diet was similar to growth of mysids fed *Artemia* nauplii alone or the mixed diet during this period. Although survival of hatchlings fed 100% rotifer (53%) was significantly lower ($p<0.05$) than survival of mysids fed *Artemia* nauplii (76%) or the mixed diet of rotifers+*Artemia* nauplii (74%), the use of rotifers exclusively for the first part of the life cycle would still promote cost savings compared with the mixed diet.

From the results of the current experiments, we suggest that the ideal feeding regime to grow *Leptomysis* sp. in the laboratory at the lowest production cost would be: 1) to first feed mysid hatchlings a rotifer diet for the first two to three weeks and then, 2) just before sexual maturity is attained, a mixed diet of rotifers+*Artemia* nauplii should be fed to assure that mysids would reach sexual maturity and reproduce. This feeding regime would lower the feeding costs during the first two to three weeks by greater than two orders of magnitude (200 times), as well as during the rest of the mysid culture (100 times). If an increased production would be necessary, the mixed diet of rotifers+*Artemia* nauplii should be given to the mysids through the whole life cycle; the use of the mixed diet would increase production costs slightly due to the use of *Artemia* cysts during the first two to three weeks. Nevertheless, the replacement of the *Artemia* nauplii (50% level) by rotifers during the complete culture process would reduce feeding costs two fold.

6. THE EFFECTS OF SEVERAL AMINO ACIDS ON GROWTH, SURVIVAL AND CONDITION OF THE CUTTLEFISH *Sepia officinalis*.

6.1 Introduction

Due to their particular metabolic performance, cephalopods are a unique model for studying animal nutrition (Lee, 1994). Cephalopod nutrition is relatively recent and has been characterized by the difficulties of holding cephalopods for long periods of time. This is particularly true for squids (Lee, 1994). The majority of the nutrition research has been made with octopuses, much easier to obtain and maintain in captivity (O'Dor & Wells, 1987). Nevertheless, recent advances in culture management and a wider selection of species of squid and cuttlefish adapted to laboratory has started to reverse this process (Lee *et al.*, 1991; Lee, 1992; Castro *et al.*, 1993; DiMarco *et al.*, 1993; Castro & Lee, 1994; Lee *et al.*, 1998). The goal of researchers that produce artificial diets is to reduce the levels of protein in the diets, since protein is the most expensive component in a diet. Nevertheless, due to their amino acid metabolism, artificial diets for cephalopods have to include high levels of protein in their composition (Lee, 1994). Cephalopods require amino acids for structural support (collagen), locomotion (actin/myosin), energy (aerobic amino acid catabolism), oxygen transport (haemocyanin), vision (rhopsin and lens), neurotransmitters (glutamate), hormones (cardiac regulation), pigmentation (melanin) and osmoregulation (free amino acids and hemolymph).

Since cephalopods can feed at rates exceeding 20-50% of their wet body weight (Boucher-Rodoni *et al.*, 1987) and grow at rates between 3 and 15% $BW.d^{-1}$, the amino acid requirement for protein synthesis is high (Lee, 1994; Lee *et al.*, 1998). Cephalopod bodies are composed of 75 to 85% protein on a dry weight basis. In contrast to fishes, cephalopods contain 20% more protein, 80% less ash, 50-100% less lipid and 50-100% less carbohydrate (Lee, 1994). Dietary lipids are used poorly (Lee *et al.*, 1998). Since protein is almost exclusively used for energy, cephalopods are extremely efficient in assimilating protein, with apparent protein digestibility greater than 85-90%. To promote growth, this elevated demand for protein necessitates a high protein diet (Lee, 1994).

Diets of known composition could contribute significant knowledge about cephalopod nutritional physiology (O'Dor & Wells, 1987). In the present study, two preliminary experiments were run to test the acceptance of surimi diets enriched with

casein and some amino acids (first experiment) and to determine its effectiveness, by comparing it with starved cuttlefish (second experiment). After these experiments, other experiments were conducted to evaluate the effects of three essential amino acids (methionine, lysine and leucine) on the growth, survival and food consumption of the cuttlefish, *S. officinalis*. These diets were formulated with varying levels of methionine, lysine and leucine, to determine what levels of methionine, lysine and leucine would promote better growth and survival. The lysine diets were also tested on cuttlefish placed in individual chambers in order to obtain individual data on feeding rate, Instantaneous Growth Rate (IGR) and food conversion.

6.2 Materials and methods

Experiments were conducted at the NRCC in Galveston (*c.f.* Figure 3). For the first experiment testing the acceptability of three surimi diets, 24 cuttlefish (359.8 ± 65.2 g wet weight; mean ± standard deviation; SD) were distributed equally into 6 tanks (each tank was 500 l in volume, 1.6 m diameter) connected to a common filtration system. This tank system (Figure 25) was similar to others used for the culture of cephalopods (Yang *et al.*, 1989). For the second experiment testing another three surimi diets and a control group of unfed cuttlefish, 72 cuttlefish (405 ± 52.1 g ww) were distributed equally into 12 circular tanks. Similar methods to the ones used in the preliminary experiments were used for the experiments testing the effects of amino acids in the surimi diets. In the methionine experiment, a total of 120 laboratory reared cuttlefish (164.0 ± 31.7 g ww) were distributed equally into the same 12 tanks used previously. Following this experiment, a total of 96 cuttlefish (321.8 ± 57.9 g ww) were distributed equally into the same 12 tanks to test lysine. For the leucine experiment, 120 cuttlefish (104.3 ± 30.6 g ww) by the 12 tanks as for the methionine experiment. Before each experiment, the cuttlefish were weighed and distributed randomly so that there were no differences ($p<0.05$) among the means or variances in wet body weight of the cuttlefish in each tank. For the experiment testing lysine fed to cuttlefish in isolation, 16 cuttlefish (451.5 ± 103.7 g ww) were distributed into individual chambers and one of each of the four diets (treatments) was fed to four randomly chosen cuttlefish.

Environmental conditions were standardized for all experiments. Salinity was maintained at 35 ± 2 ppt and temperature was stable at 23 ± 1.5°C.

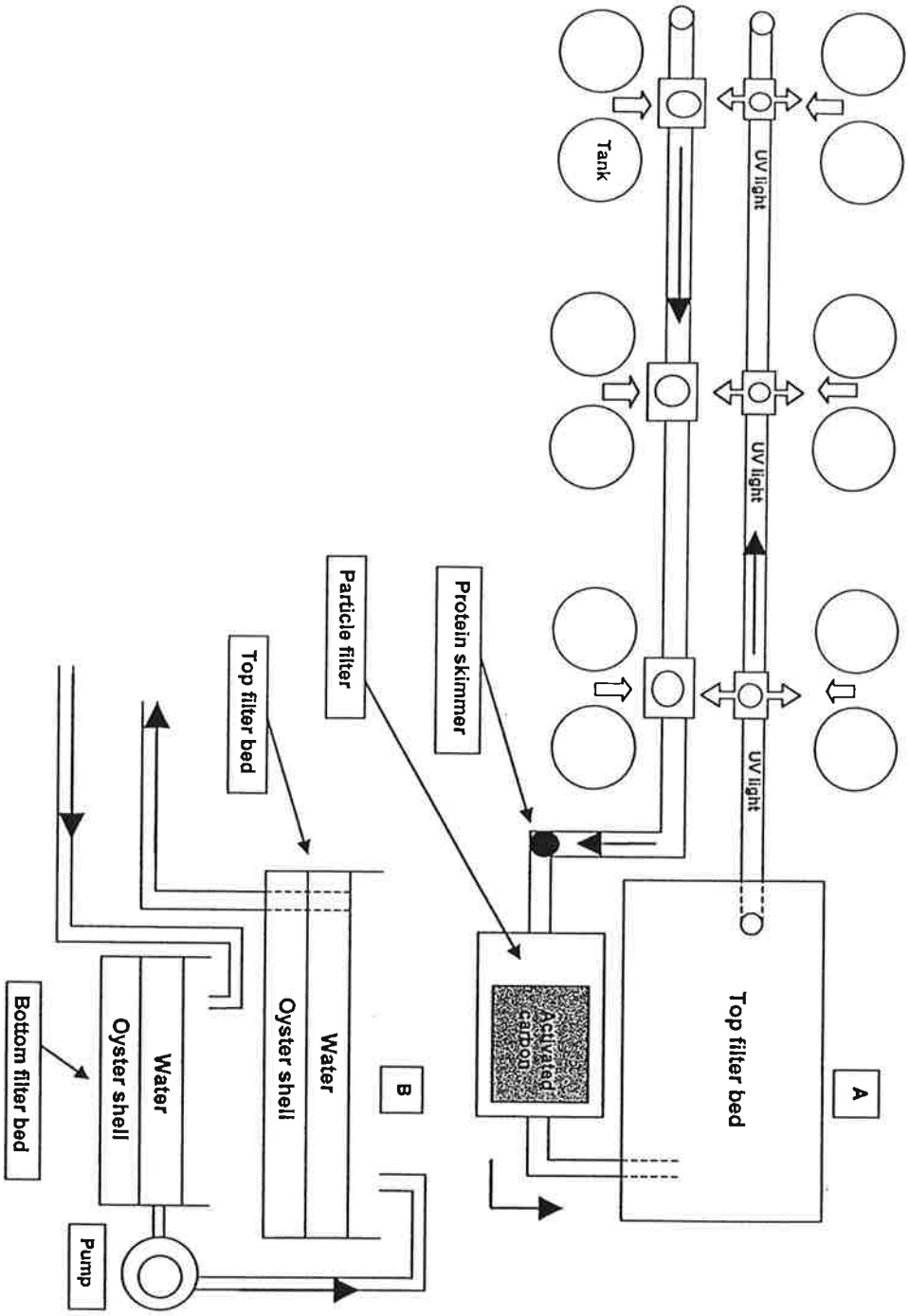

Figure 25 – The diagram represents a recirculating system used to test the effects of surimi diets fed to cuttlefish. The first diagram (A) shows a top view of the top filter bed, the small tank where particle filtration and the activated carbon were placed, (placed next to the lower filter bed) and the 12 circular tanks where the growth experiments took place. The bottom diagram (B) shows a lateral view of the two filter beds. Large arrows show the direction of the water flow. Water entered the culture tanks from the top filter bed after passing through ultraviolet sterelizers. Water coming from the outflow of the culture tanks was collected in a common drain pipe, next to the one used for the inflow. It would then pass through a protein skimmer, particle filter and activated carbon before entering the bottom filter bed. After passing through the oyster shell, water was pumped to the top filter bed, flowed by gravity through the oyster shell and finally flowed by gravity to re-enter the culture trays.

Because of the extensive use of protein for their highly aerobic metabolism, cephalopods produce ammonia rapidly. Water filtration and consequent improvement in water quality is one of the most important factors that imporved the success of maintenance of cephalopods in recirculating seawater tanks (Lee *et al.*, 1998). Since nitrate-nitrogen concentrations above 80 $mg.l^{-1}$ cause cuttlefish to become highly agitated and easily startled (Forsythe *et al.*, 1991), ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen were kept below 0.1 $mg.l^{-1}$, 0.1 $mg.l^{-1}$ and 50 $mg.l^{-1}$, respectively, by making water changes when necessary. The pH was maintained between 7.8 and 8.1 through addition of sodium bicarbonate. The pH is an important factor to the successful culture of cephalopods as their oxygen transfer physiology is very sensitive to even small changes in pH (Portner *et al.*, 1996). Low light intensities (2.8 lux) were maintained 24 $h.d^{-1}$.

The methionine experiment tested four diets with different levels of methionine (0.95, 1.58, 3.43 and 11.76 g of methionine.kg^{-1} ww); the lysine experiment tested four diets with different levels of lysine (0.72, 2.31, 6.54 and 10.49 g of lysine.kg^{-1} ww); the leucine experiment tested four diets with different levels of leucine (1.25, 3.07, 8.42 and 12.02 g of leucine.kg^{-1} ww). All diets were formulated using surimi as a base (Castro *et al.*, 1993). The composition of the first two surimi diets, used to test the acceptability of the prepared diets by the cuttlefish is shown in Table V (Appendix). The composition of the methionine, lysine and leucine diets is shown in Tables VI, VII and VIII, respectively (Appendix). The surimi diets were prepared according to the procedure shown in Table XI (Appendix). During the week prior to the start of the experiments, the cuttlefish were slowly transitioned from thawed frozen shrimp to thawed catfish fillets. This procedure was done to ensure better acceptance of the surimi by the cuttlefish, as small pieces of catfish resemble surimi. For all experiments, one of each of the three or four diets (treatments) was fed to cuttlefish in three randomly chosen tanks.

The first experiment and the experiment testing leucine lasted 15 days. The second experiment and the experiments testing methionine and lysine lasted 30 days. Each cuttlefish was weighed on day 1, 15 and 30. The individual rearing experiment lasted 21 days and cuttlefish were weighed on days 1, 14 and 21. Initial ration levels were set at 5% $BW.d^{-1}$ allowing for a slight excess of food. Later, these rations were adjusted based on ingestion and mortality rates. The daily amount of food given to each tank was presented in two rations: 50% at 09:00h and 50% at 16:00h. The food was

presented for an hour and then uneaten food was removed, weighed and multiplied by a correction factor to account for water absorption and leaching.

Data were used to calculate: 1) Instantaneous Growth Rate (IGR) (% Body Weight.d^{-1}) = ((ln w2 - ln w1)/t)*100, where w2 and w1 are the final and initial wet weights of the cuttlefish, ln the natural logarithm and t the number of days of the time period; 2) Feeding Rate (FR) (% BW.d^{-1}) = (FI/Average w(t))*100, where FI is the food ingested in a period of time (t), w(t) is the average wet weight of the cuttlefish during the period of time (t) in days; and 3) Food Conventions (FC) (%) = FI/(w2-w1). Mean weight gained was determined by subtracting the mean weight at the start from the mean weight at the end of each experiment. A non parametric Kruskal-Wallis ANOVA test (Zar, 1984) determined significant differences in both weight and IGR's of cuttlefish fed the different diets.

At the end of the methionine and lysine experiments, two cuttlefish were sacrificed per tank (6 cuttlefish per diet). Samples were taken from the mantle, digestive gland and blood of each cuttlefish to determine and compare the amino acid composition (except glutamine and tryptophan) of these tissues. All samples were frozen before being analyzed by high-performance liquid chromatography (Lehninger *et al.*, 1993). The total amino acid composition of the mantle and digestive gland tissue was determined along with the free amino acid composition of the blood. Additionally, the amino acid composition of these tissues was compared with the amino acid composition from mantle, digestive gland and blood of shrimp-fed cuttlefish and cuttlefish unfed for 28 d (McCliment *et al.*, submitted). Amino acids in Tables and Figures were abreviated as followed; alanine (ALA), arginine (ARG), aspartic acid (ASP), cystine (CYS), glutamate (GLU), glycine (GLY), histidine (HIS), isoleucine (ISO), leucine (LEU), lysine (LYS), methionine (MET), phenylalanine (PHE), proline (PRO), serine (SER), therionine (THR), tyrosine (TYR) and valine (VAL). A non-parametric Kruskal-Wallis ANOVA test (Zar, 1984) determined significant differences between the amino acid composition of cuttlefish fed the artificial diets and both shrimp-fed and unfed cuttlefish.

For the first two preliminary experiments and the methionine experiment, all cuttlefish were tagged individually. A small numbered tag was fixed to the upper anterior mantle of each cuttlefish (FTF-69 Fingerling tag; Floy tag, Seattle, WA). During these three experiments a high mortality rate (63%, 34% and 40%, respectively) was associated with the tagging procedure (e.g. handling and perforation of the

cuttlefish mantle). Furthermore, almost 50% of the tagged animals ripped off their tags, making it impossible to identify them. For these two reasons cuttlefish were not tagged during the lysine and leucine experiments and all values were determined as means of the cuttlefish in each diet treatment.

6.3 Results

6.3.1 Preliminary experiments

Table X (Appendix) shows the Mean IGR's at the end of the two experiments. From the 24 cuttlefish used at the start of the experiment, only 9 survived (37%) the 15 days of the experiment. Three cuttlefish fed diet 1 survived and had IGR's of 0.28, 0.72 and 0.47% $BW.d^{-1}$. Feeding rates were 0.48, 0.65 and 1.48% $BW.d^{-1}$ and food conversions were 58.4, 111.5 and 31.7, respectively. Only 2 cuttlefish fed diet 2 survived at the end of the experiment and had IGR's of 1.10 and 0.03% $BW.d^{-1}$, respectively. Feeding rates were 0.55 and 1.14% $BW.d^{-1}$ and food conversions were 2.43 and 240.0, respectively. Four cuttlefish fed diet 3 survived. One of them had a negative IGR (lost weight) of –0.02% $BW.d^{-1}$ and a feeding rate of 1.94% $BW.d^{-1}$. The other three cuttlefish fed this diet had IGR's of 1.07, 0.60 and 0.23% $BW.d^{-1}$. Feeding rates were 3.0, 1.7 and 4.1% $BW.d^{-1}$ and food conversions were 2.8, 2.8 and 14.5, respectively.

For the second experiment, 36 of the 72 cuttlefish survived the 30 days of the experiment (50%). Survival was 56%, 44%, 44% and 56% for cuttlefish fed diets 1, 2 and 3 and starved cuttlefish, respectively. The majority of the cuttlefish that survived had ripped off their tags, so individual data was very scarce and IGR's were calculated as a mean for cuttlefish fed each diet. Mean IGR's (MIGR's) were negative for all diets and starved cuttlefish, both during the first and second half of the experiment. MIGR's for the first 15 days of the experiment were-0.26, -0.24, -0.34 and –0.30% $BW.d^{-1}$ for diets 1, 2, 3 and starved cuttlefish, respectively. At the end of the experiment, MIGR's were –0.24, -0.26, -0.43 and –0.34% $BW.d^{-1}$, for diets 1, 2, 3 and starved cuttlefish, respectively. During this second experiment, cannibalism on dead or dying cuttlefish was observed in the tanks were the three diets were being fed, but no cannibalism was observed in the tanks were cuttlefish were maintained without being fed.

6.3.2 Methionine experiment

6.3.2.1 Feeding, food conversion, growth and mortality rates

Feeding rates varied between diets and with the exception of the diet with the highest methionine level (diet 4), rates increased during the second half of the experiment. Feeding rate for diet 1, with the lowest methionine level (0.71% $BW.d^{-1}$), was lower ($p<0.05$) than for the other three diets. Feeding rates for diets 2, 3 and 4 were 2.27%, 2.09% and 1.94% $BW.d^{-1}$, respectively, and were not different ($p>0.05$) from each other. During the first half of the experiment (days 1-15) food conversion was higher for all diets. During the second half of the experiment (days 16-30) all but diet 4 produced negative food conversions. Diet 4 produced the best food conversion rate (12.01) during the entire experiment; food conversions for the remaining diets were 14.9 for diet 1, 60.7 for diet 2 and 18 for diet 3. There were no differences in food conversions between all diets, denoting the high variance between tanks and diets..

Figure 26 shows the mean instantaneous growth rate (MIGR) during the first and second halves of the experiment (days 1-15 and 16-30, respectively) and for the total length of the experiment (days 1-30).

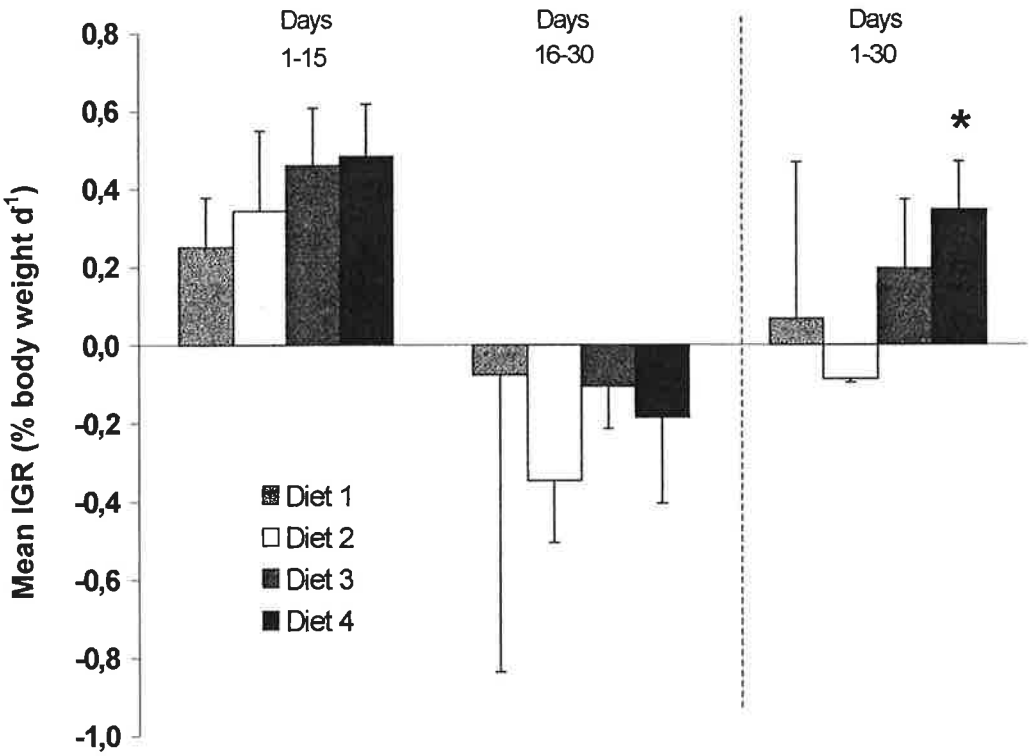

Figure 26 – Mean Instantaneous Growth rates (MIGR's) of cuttlefish for the first and second halves of the experiment testing the amino acid methionine. Bars indicate standard deviations. The "*" sign indicates that the value was significantly different ($p<0.05$) when compared to the other ones obtained during that period of time.

During the first half of the experiment, MIGR for cuttlefish fed the four diets varied between 0.25% $BW.d^{-1}$ and 0.48% $BW.d^{-1}$. Cuttlefish fed diets with higher methionine levels had higher MIGR's. During the second half of the experiment none of the diets promoted growth; all MIGR's were negative, indicating that the cuttlefish lost weight. The MIGR's for the complete experiment were 0.07 ± 0.40% $BW.d^{-1}$ for diet 1, -0.09 ± 0.01 $BW.d^{-1}$ for diet 2, 0.2 ± 0.18% $BW.d^{-1}$ for diet 3 and 0.35 ± 0.12% $BW.d^{-1}$ for diet 4; the diet with the highest methionine level (diet 4) promoted growth ($p<0.05$). The highest individual growth rate (IGR) for the entire experiment was by a cuttlefish fed diet 3 (1.08% $BW.d^{-1}$). A cuttlefish fed diet 4 had an IGR of 1.54% $BW.d^{-1}$ during a 15 days period.

The mean increase in body weight was 4.3 ± 8.5 g for diet 1, -4.3 ± 10.3 g for diet 2, 10.2 ± 7.9 g for diet 3; the highest increase occurred on diet 4 where each cuttlefish grew an average of 17.7 ± 9.8 g (Figure 27).

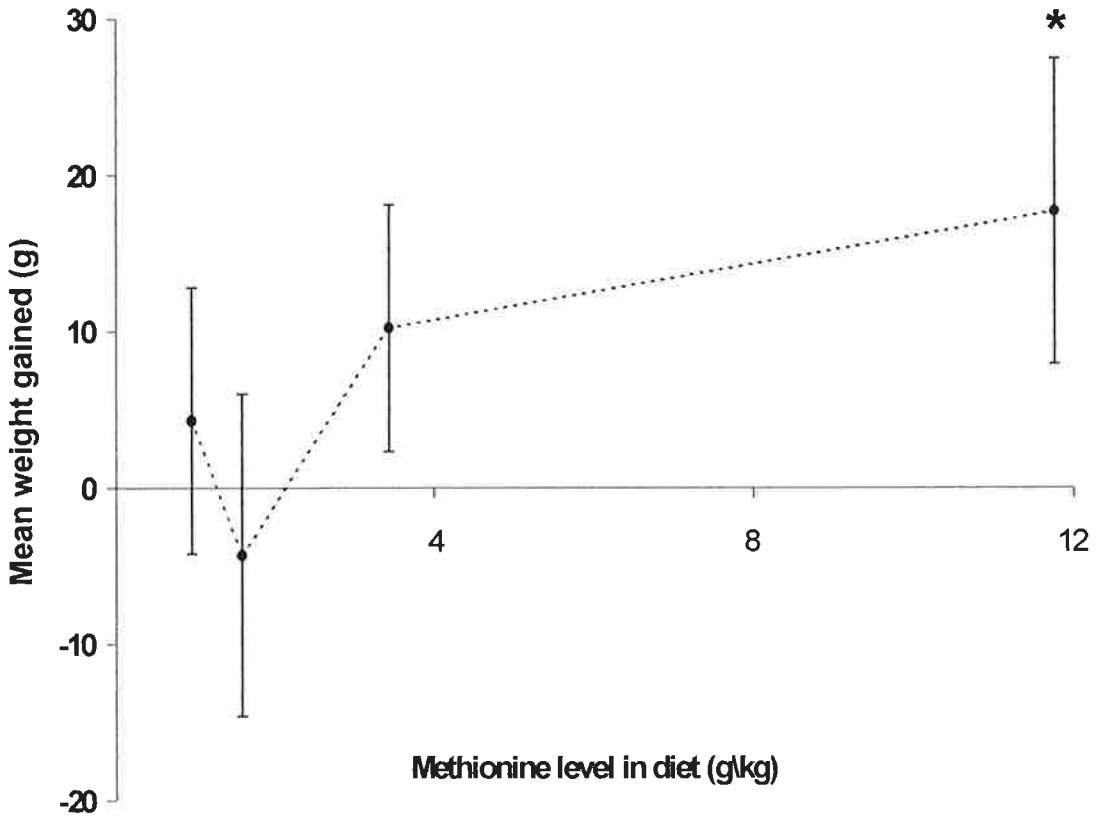

Figure 27 – Mean increase in body weight (g) of cuttlefish fed four diets testing the amino acid methionine. Bars indicate standard deviations. The "*" sign indicates that that value was significantly different ($p<0.05$).

There was a difference in weight ($p<0.05$) for cuttlefish fed diet 4 at the end of the experiment, while weights of cuttlefish fed diets 1, 2 and 3 were not different ($p>0.05$). Results from the experiments testing methionine indicated that only the diets with the highest levels of methionine (11.76 g methionine.kg^{-1}) produced growth ($p<0.05$).

Mortality rates (8%) were low during the first part of the experiment but during the last 15 d of the experiment there was a considerable increase in mortality (32.5%); total mortality was high (39.17%). Mortality for cuttlefish fed diet 1 was the lowest (26.7%). Highest mortality (53.3%) was for cuttlefish fed diet 4.

6.3.2.2 Comparison between the amino acid composition of the diets

The amino acid composition of the four diets was analyzed and compared (Table XI, Appendix). Overall, the amino acid composition of diet 1 was different ($p<0.05$) from the composition of diet 4 (with highest amino acid concentrations in diet 4); the composition of diets 2 and 3 was not different ($p>0.05$). The only individual amino acids that had different ($p<0.05$) concentrations for the four diets were cystine and methionine; concentrations of these two amino acids increased ($p<0.05$) from diet 1 to diet 4.

6.3.2.3 Composition of cuttlefish tissues

For the cuttlefish fed the four methionine diets, Table XII (Appendix) shows the total amino acid composition of mantle tissue ($mg.l^{-1}$), digestive gland tissue ($mg.l^{-1}$), and the free amino acid composition of the blood ($\mu g.l^{-1}$).

6.3.3 Lysine experiment

6.3.3.1 Feeding, food conversion, growth and mortality rates

Feeding rates between diets were not different ($p>0.05$), varying between 2.67% (diet 2) and 2.79% $BW.d^{-1}$ (diet 4). Feeding rate for diet 1 and 3 were 2.69% and 2.77% $BW.d^{-1}$, respectively.

During the first 15 days of the experiment food conversion was higher, although not significant ($p<0.05$) for cuttlefish fed diets 2, 3 and 4. During the second half of this experiment, there were no differences ($p<0.05$) between food conversions from cuttlefish fed all diets. Cuttlefish fed diet 4 produced the best food overall food conversion (10.7). The remaining food conversion rates were –40.8 for diet 1, 26.7 for

diet 2 and 80.9 for diet 3. There were no differences in food conversions ($p>0.05$) between all diets at the end of the experiment, denoting the high variability between tanks and diets.

Figure 28 shows the mean instantaneous growth rate (MIGR) for the first and second halves of this experiment (days 1-15 and 16-30, respectively) and for the total length of the experiment (days 1-30). MIGR's for diets 2, 3 and 4 were higher in the first half of the experiment, although the decrease in growth rate for diet 4 was small (from $0.33 \pm 0.21\%$ BW.d^{-1} to $0.27 \pm 0.16\%$ BW.d^{-1}) and insignificant ($p<0.05$).

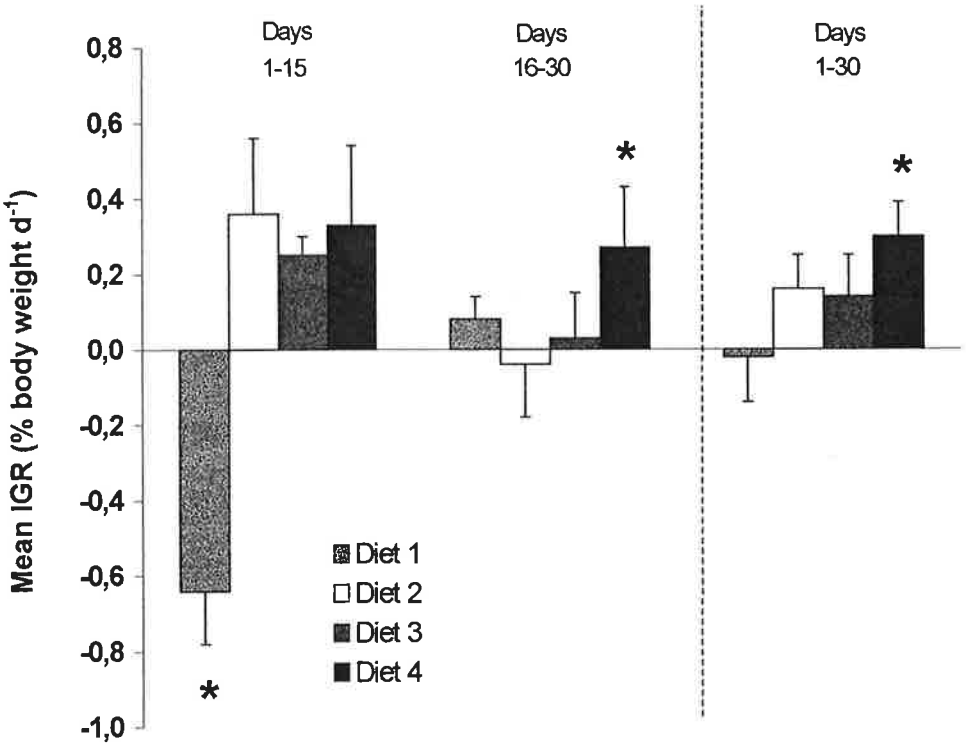

Figure 28 - Mean Instantaneous Growth rates (MIGR's) of cuttlefish for the first and second halves of the experiment testing the amino acid lysine. Bars indicate standard deviations. The "*" sign indicates that the value was significantly different ($p<0.05$) when compared to the other ones obtained during that period of time.

Cuttlefish fed diet 1 had a negative growth rate during the first half of the experiment ($-0.64 \pm 0.14\%$ BW.d^{-1}) but had a positive growth rate during the second half ($0.08 \pm 0.06\%$ BW.d^{-1}). MIGR's for the entire experiment were $-0.02 \pm 0.12\%$, $0.16 \pm 0.09\%$, $0.14 \pm 0.11\%$ and $0.30 \pm 0.09\%$ BW.d^{-1} for cuttlefish fed diets 1, 2, 3 and 4, respectively. There was no positive association between amino acid concentration and MIGR for the first 15 days of the experiment. However, for the entire experiment,

diet 4, with higher concentrations of lysine (10.49 $g.kg^{-1}$ ww) promoted higher ($p<0.05$) growth rates.

Cuttlefish fed diet 1 lost weight (an average of 2.4 ± 12.3 g per cuttlefish). Cuttlefish fed diet 2 grew an average of 17.7 ± 10.9 g, and those fed diet 3 grew an average of 16.5 ± 12.1 g. Diet 4, with the highest content of lysine (10.49 g $lysine.kg^{-1}$ ww) promoted an average gain in weight of 29.8 ± 11.8 g per cuttlefish (Figure 29). There were no variations in weights ($p>0.05$) for cuttlefish fed diets 1, 2 and 3. In contrast, cuttlefish fed diet 4 weighed more ($p<0.05$) at the end of the experiment.

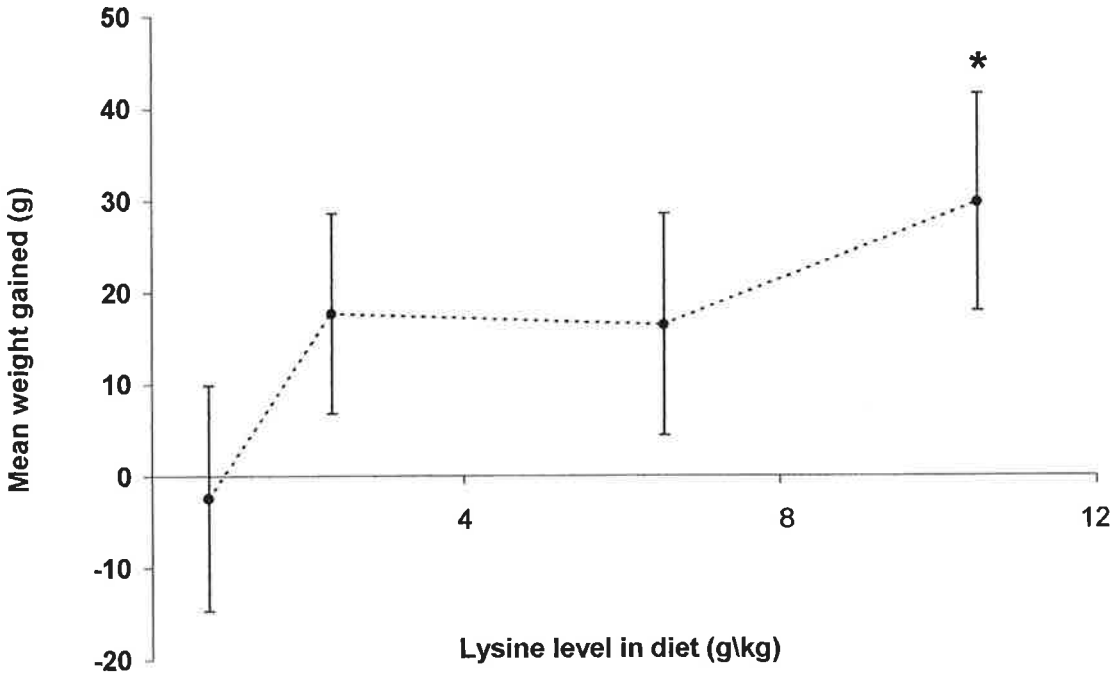

Figure 29 – Mean increase in body weight (g) of cuttlefish fed four diets testing the amino acid lysine. Bars indicate standard deviations. The "*" sign indicates that that value was significantly different ($p<0.05$).

Mortality was lower (18.75%) than for the methionine experiment. Mortality rates were 12.5% for cuttlefish fed the low lysine diet and 20.8% for cuttlefish fed the remaining 3 diets.

6.3.3.2 Composition of cuttlefish tissues

For the cuttlefish fed the four lysine diets, Table XIII (Appendix) shows the total amino acid composition of mantle tissue ($mg.l^{-1}$), digestive gland tissue ($mg.l^{-1}$), and the free amino acid composition of the blood ($\mu g.l^{-1}$).

6.3.4 Comparison between surimi-fed, shrimp-fed and unfed cuttlefish

Table XIV (Appendix) compares cuttlefish fed the methionine diets with both shrimp-fed and unfed cuttlefish. Table XV (Appendix) presents the same comparisons for cuttlefish fed the lysine diets. The "-" or "+" indicates that the concentration of that amino acid was lower or higher ($p<0.05$), respectively, when compared with the concentration in shrimp-fed and unfed cuttlefish. When compared with shrimp-fed cuttlefish, no amino acid concentration was higher ($p>0.05$) in the cuttlefish fed the surimi diets. Cuttlefish fed the diet with the highest methionine level (diet 4) had similar overall amino acid levels in their tissues ($p>0.05$) when compared to shrimp-fed cuttlefish. However, the aromatic amino acids (tyrosine, phenylalanine and histidine) in the blood as well as proline and arginine (used for energy) in the mantle, were lower ($p<0.05$). As well, for cuttlefish fed diet 2, the majority of the amino acids in the tissues (especially the digestive gland) had lower ($p<0.05$) concentrations. Proline and arginine were also lower ($p<0.05$) in the mantle of cuttlefish fed all four surimi diets. Cuttlefish fed diets 1 and 3 were in an intermediate condition.

When comparing cuttlefish fed the methionine diets to unfed cuttlefish, the aromatic amino acids (tyrosine, phenylalanine and histidine) were lower ($p<0.05$) in the mantle of cuttlefish fed diet 2, and lower ($p<0.05$) in the blood of cuttlefish fed diet 4. In contrast, the branch-chained amino acids (valine, leucine and isoleucine) were higher ($p<0.05$) in the blood of cuttlefish fed diets 2 and 4. Alanine (except for cuttlefish fed diet 3) and proline were also present in higher ($p<0.05$) concentrations in the blood of cuttlefish fed all methionine diets. Compared to shrimp-fed cuttlefish, cuttlefish fed the diets with lowest (diet 1) and the highest (diet 4) lysine levels had overall lower ($p<0.05$) amino acid concentrations, especially in the digestive gland. The aromatic amino acids (tyrosine, phenylalanine and histidine) were also lower ($p<0.05$) in the blood of cuttlefish fed diets 2 and 4, and in the digestive gland of cuttlefish fed diet 2. Cystine was lower ($p<0.05$) in the digestive gland of cuttlefish fed all lysine diets, and serine was lower ($p<0.05$) in all the three tissues of cuttlefish fed diet 1. Cuttlefish fed the intermediate lysine diets (diets 2 and 3) had the closest amino acid composition when compared with shrimp-fed cuttlefish.

When comparing cuttlefish fed the lysine diets with unfed cuttlefish, proline was higher in the blood of cuttlefish fed all four lysine diets, in the digestive gland of cuttlefish fed all but diet 3 and in the mantle of cuttlefish fed diet 1 (all $p<0.05$). The

basic amino acids (arginine and lysine) were also higher ($p<0.05$) in the digestive gland of cuttlefish fed diet 2. The branch-chained amino acids (valine, leucine and isoleucine) were higher ($p<0.05$) in the blood of cuttlefish fed diets 1, 3 and 4 and in the digestive gland of cuttlefish fed diet 2. In contrast, serine was lower in all three tissues of cuttlefish fed diet 1. Cystine was lower in digestive gland of cuttlefish fed diets 1 and 4, and lower in the blood of cuttlefish fed diets 1 and 2. Histidine was also lower in the blood of cuttlefish fed diet 1 (all $p<0.05$).

6.3.5 Individually reared cuttlefish

Feeding rates, IGR's and food conversion for surviving cuttlefish from the individually reared experiment are presented in Table XVI (Appendix). Feeding rates varied between 1.0 and 3.9% $BW.d^{-1}$. Cuttlefish fed diet 4, with the highest lysine level (10.49 g $lysine.kg^{-1}$ ww) had the highest IGR's at the end of the experiment (0.48, 0.36 and 0.26% $BW.d^{-1}$ for the three surviving cuttlefish). All cuttlefish fed diet 1, with the lowest lysine level (0.72 g $lysine.kg^{-1}$ ww) had negative IGR's (-0.37, -0.35 and -0.18% BW d^{-1}). The highest food conversions were from cuttlefish fed diet 4 (between 6.14 and 6.74), and only cuttlefish fed diet 4 produced growth ($p<0.01$). Due to the slower water circulation in these tanks, ammonia-nitrogen was higher than recommended, and this high concentration likely caused the death of 5 of the 16 cuttlefish.

6.3.6 Leucine experiment

6.3.6.1 Feeding, food conversion, growth and mortality rates

The experiment tested leucine was initially planned for 30 days, as the previous ones testing other amino acids. However, high mortality occurred during the experiment, due to bacterial infection. After 15 days, 51.7% of the cuttlefish had died (73.3%, 53.3%, 43.3% and 40.0% for diets 1, 2, 3 and 4, respectively). Therefore, the experiment was interrupted at that time. Results obtained are shown in Table XVII (Appendix). Feeding rates were 0.24%, 1.72%, 1.81% and 1.59% $BW.d^{-1}$ for diets 1, 2, 3 and 4, respectively. Food conversions were 0.91, 4.72, 20.04 and 1.43 for cuttlefish fed diets 1, 2, 3 and 4, respectively.

MIGR for cuttlefish fed diet 4 (1.12% $BW.d^{-1}$) was among the higherst registered for all experiments. Nevertheless, this high value was not obtained by eating the surimi diets. The smaller cuttlefish fed these diets died; therefore, the average was done with the remaining cuttlefish (the bigger ones) which gave high growth rates,

which did not correspond to real growth rates. MIGR for diets 1, 2 and 3 were 0.26%, 0.37% and 0.09% $BW.d^{-1}$, respectively. Also, bigger cuttlefish were cannibalizing on the smaller ones, or the ones in worse condition, wihich contributed to the high food conversions (1.43) for cuttlefish fed this diet.

6.4 Discussion

Prepared diets for cephalopods are being tested to lower the costs of mariculture (Lee *et al.*, 1991; 1998). Among the Cephalopoda, high growth rates are a result of high rates of protein synthesis and highly efficient retention of synthesized protein. For example, in *Octopus vulgaris*, the proportion of synthesized protein retained for growth can be higher than 90% (Houlihan *et al.*, 1990). Cephalopod water content is approximately 80% with protein (16.6%) being the next most abundant component (Iwasaki & Harada, 1985). Carbohydrates are approximately 1% (Vlieg, 1984) and lipids are usually less than 2%; neither are candidates for energy production (Hochachka *et al.*, 1975). Thus, protein is the major source of energy in cephalopods (Lee, 1994). The rapid growth rate of cephalopods requires highly efficient ingestion, digestion and assimilation of protein. This reliance on amino acid metabolism makes it essential for prepared diets to contain balanced levels of essential amino acids in order to promote growth. In contrast to the protein-to-energy (P/E) ratio that is utilized as an indicator of the nutritional value of terrestrial vertebrate diets, amino acid ratios are the best measure of the nutritional value of cephalopod diets (Lee, 1994). Therefore, the addition of known levels of amino acids, particularly the essential amino acids, is of paramount importance in the determination of well balanced diets for cephalopods.

A diet low in energy may be sufficient for meeting metabolic needs, but no growth or subnormal growth can result. The surimi diets tested in the present experiments produced modest growth. During early trials cuttlefish did not accept commercial surimi (Lee *et al.*, 1991), but more recently, Castro *el al.*, (1993) reported that cuttlefish readily accepted prepared surimi diets (fish based) when compared to classical pelleted diets (shrimp based). No growth was obtained during those experiments due to the low nutritional value of the diets. It was postulated that the lack of growth was due to the low nutritional value of the diet, and that this could be improved with supplementation. Castro & Lee, (1994) tested growth on plain surimi diets and surimi diets with casein supplementation. Only the supplemented diet promoted significant growth.

In the present experiments, only the diets with the highest levels of methionine and lysine supplementation promoted significant growth ($p<0.05$). Even the remaining high protein diets with lower levels of methionine and lysine did not promote growth ($p>0.05$). A positive association was found between growth and the level of the amino acids in the diets, since diets with higher levels of methionine and lysine promoted higher growth rates. The highest growth rate in the present experiments (0.48% $BW.d^{-1}$) for a cuttlefish fed the diet with highest lysine level in the individual trial was approximately 7 times lower than growth rates recorded during normal laboratory maintenance of this species (3.2% - 4% $BW.d^{-1}$; obtained when feeding thawed shrimp by Forsythe *et al.*, 1994). However, similar IGR's for surimi diets (between 0.4% and 0.8% $BW.d^{-1}$) were found in previous experiments (Castro *et al.*, 1993; Castro & Lee, 1994).

Feeding rates on surimi diets during the present experiments (< 4% $BW.d^{-1}$) were considerably lower than feeding rates on the normal laboratory maintenance diet of thawed shrimp (6-8% BW d^{-1}), determined in previous experiments (Castro *et al.*, 1993). Feeding rates during the present experiments were also lower than rates during transition periods when cuttlefish were fed thawed catfish fillets (up to 9% $BW.d^{-1}$; Table XVIII, Appendix). Castro *et al.*, (1993) reported a feeding rate between 8% and 9% $BW.d^{-1}$ on surimi diets. Forsythe *et al.*, (1994) found a feeding rate on thawed shrimp up to 12% $BW.d^{-1}$ in cuttlefish of 500 g in wet weight, and from 5% to 7% $BW.d^{-1}$ for larger cuttlefish.

During the experiment testing lysine, feeding rates were higher than the rates during the testing of methionine, and this may be due to the condition of the cuttlefish. Cuttlefish were not tagged for the lysine experiments. Thus, the handling and stress incurred during the lysine experiment was considerably reduced. Tagging may also have contributed to high mortality. In the methionine experiment and the individually reared lysine experiment, feeding rates on the low protein diet were lower ($p<0.05$) than for the remaining diets. This situation did not exist during the lysine experiment, where feeding rates were similar ($p>0.05$) among the four diets. There was a decrease ($p<0.05$) in feeding rates in all the diets tested from the first half to the second half of the experiment. Food conversions were also higher for the diets with the highest levels of methionine and lysine. These observations are likely due to negative post-ingestive feedback as well as low nutritional value of the diets. Since cephalopods have high-energy costs for feeding (O'Dor & Wells, 1987) a low energetic diet may inhibit

feeding. This may indicate that although surimi was attractive, it was still nutritionally deficient. By using the current prepared diets, feeding, growth and survival of cephalopods are comparable to fish larvae being weaned from natural to prepared diets (Dabrowski *et al.*, 1978; Lindberg & Doroshov, 1986).

The amino acid concentration in mantle tissue, digestive gland and blood from cuttlefish fed surimi diets were compared with the concentration in cuttlefish fed shrimp or left unfed (McCliment *et al.*, submitted). The overall composition of the mantle of cuttlefish fed the methionine diets showed little variation compared to cuttlefish fed shrimp (*c.f.* Table XIV, Appendix), with the higher variations being the amino acids proline and alanine (lower concentrations for all diets), both utilized for energy as TCA cycle substrates (Ballantyne *et al.*, 1981). For both experiments, amino acid concentrations in the tissues of cuttlefish fed the surimi diets were generally lower ($p<0.05$) than concentrations in cuttlefish fed shrimp (*c.f.* Tables XIV and XV, Appendix). The lower concentrations were found mainly in the mantle of cuttlefish fed diet 2, and accounted for almost all the negative variation in amino acid concentration. However, amino acid concentration in the mantle of cuttlefish fed the other three diets had lower concentrations of proline and alanine ($p<0.05$). In the experiment testing lysine, proline and alanine were the only amino acids with lower mantle concentrations in cuttlefish fed surimi (*c.f.* Table XV, Appendix) compared to cuttlefish fed thawed shrimp. Significantly lower ($p<0.05$) concentrations of proline in the mantle of cuttlefish fed all diets in the methionine experiment, and in diet 1 in the lysine experiment, were obtained. Proline is the amino acid that is most easily oxidized into glutamate (Lehninger *et al.*, 1993) that is then used to produce energy either by deamination or transamination into α-ketoglutarate for the TCA cycle (Ballantyne *et al.*, 1981). Therefore, proline is the first amino acid to be naturally depleted. Additionally, the lower ($p<0.05$) concentration of proline in the blood (*c.f.* Tables XIV and XV, Appendix) is also explained by the fact that proline is catabolized and used as energy.

When comparing unfed cuttlefish with cuttlefish fed the methionine and lysine diets, higher ($p<0.05$) concentrations of proline were found in tissues of cuttlefish fed the diets, especially in the blood (*c.f.* Tables XIV and XV, Appendix). For the methionine experiment, alanine (another amino acid used for energy) was present in higher ($p<0.05$) concentrations in the blood (*c.f.* Table XIV, Appendix). This indicates that cuttlefish fed the diets were in better condition than unfed cuttlefish, since this pool of amino acids used for energy was elevated, especially in the blood. Higher ($p<0.05$)

concentrations of branch-chained amino acids such as valine, leucine and isoleucine were also found in the blood of cuttlefish fed the methionine (*c.f.* Table XIV, Appendix) and lysine (*c.f.* Table XV, Appendix) diets compared to unfed cuttlefish. Higher concentrations ($p<0.05$) of these three amino acids were also found in the digestive gland (*c.f.* Table XV, Appendix). These amino acids are converted into acetyl Co-A and succinyl-CoA, substrates for the TCA cycle, and used as energy, especially in the mantle (Hochachka *et al.*, 1975).

Cephalopods mobilize amino acids from the mantle and digestive gland tissues during starvation (O'Dor & Wells, 1987; Castro *et al.*, 1992). Amino acid concentrations in cuttlefish fed the surimi diets were generally lower when compared with cuttlefish fed shrimp. In contrast, higher ($p<0.05$) concentrations of the amino acids used for energy (proline and alanine and the branch-chained amino acids) were generally present in the digestive gland and/or blood when compared with unfed cuttlefish (*c.f.* Tables XIV and XV, Appendix). These amino acids are catabolized to TCA cycle substrates and their absence from the blood indicates nutritional stress. Alanine is produced when proline is catabolized by transamination to glutamate (Storey *et al.*, 1978), predicting a high energy diet. In the methionine experiment, alanine concentrations in the mantle of cuttlefish fed all diets were lower ($p<0.05$) than the ones found in cuttlefish fed shrimp. Another indicator that the diets were not sufficiently balanced to promote normal growth is that there was a lower ($p<0.05$) concentration of histidine in the blood of cuttlefish fed the surimi diets compared with cuttlefish fed shrimp (*c.f.* Tables XIV and XV, Appendix). Histidine, also a precursor to glutamate, has a more complex catabolic pathway than proline (Lehninger *et al.*, 1993), and is depleted at a slower rate. This is based on the fact that its concentration was not lower ($p>0.05$) in the mantle, the most stable of the tissues analyzed, but was lower ($p<0.05$) in the blood, where transport and catabolism was more rapid.

The unfed cuttlefish are in a state of severe nutritional stress with extremely low levels of the metabolically important amino acids in their blood as well as other tissues. This indicates that our supplemented surimi was not sufficiently balanced to promote normal growth, but was balanced enough to maintain cuttlefish with minimal growth rates. It appears that cuttlefish fed the surimi diets were in an intermediate condition; i.e. not as healthy as cuttlefish fed shrimp, yet not as nutritionally stressed as unfed cuttlefish. In short, metabolically important amino acids were disappearing from the storage tissues but were still present in the blood.

Tables XIV and XV (Appendix), when comparing the relative effects of supplementation between the methionine and lysine diet series, indicate that the cuttlefish fed the methionine diets were in worse nutritional condition than cuttlefish fed the lysine diets. When comparing cuttlefish fed the methionine diets to cuttlefish fed thawed shrimp, greater variations where found compared to cuttlefish fed the lysine diets (*c.f.* Table XV, Appendix), especially in the mantle and digestive gland. Also, lower concentrations of the aromatic amino acids (tyrosine, phenylalanine and histidine), were present in the mantle and blood of cuttlefish fed the methionine diets when compared to unfed cuttlefish (*c.f.* Table XIV, Appendix), while no lower concentrations of these three amino acids were found when comparing the cuttlefish fed the lysine diets and unfed cuttlefish (*c.f.* Table XV, Appendix). Therefore, it is possible that the methionine diets had a more negative nutritional impact on body condition than the lysine diets.

The digestive gland is the most metabolically active organ in cuttlefish, and for both experiments it showed a greater change in amino acid concentration than the other tissues analyzed. In the digestive gland, concentrations of several amino acids, particularly essential amino acids, were lower ($p<0.05$) when compared with cuttlefish fed shrimp (*c.f.* Tables XIV and XV, Appendix). Due to poor nutritional quality of the diets, this may have been caused by a decrease of substrate. Since less protein or amino acids arrive in the digestive gland, less enzymes are needed for catabolic enzyme production. Also, after using their small lipid reserves are depleted (mainly concentrated in the digestive gland), cuttlefish begin to rapidly use protein and amino acids as their main fuel reserve (Boucher-Rodoni *et al.*, 1987). Being that the digestive gland is an extremely well vascularized organ, the breakdown of amino acids occurs here faster than in other tissues like the mantle.

These facts indicate that both methionine and lysine are essential amino acids for the cuttlefish, *S. officinalis*, and possibly for other cephalopods. Thus, ideal prepared diets for cephalopods will have to take into account the type and amount of amino acids used for supplementation. Additionally, some nucleotides (ATP and AMP) and amino acids (proline) have proved to be chemoattractants for cephalopods (Lee, 1992). Besides visual stimuli from the food, the potential effects of chemical stimuli are of great importance (DiMarco *et al.*, 1993). A good diet can be energetic and digestible, but if it is not detected it will not stimulate consumption.

7. CONCLUSIONS

Experiments with mysids in the static water system revealed that *M. almyra* can indeed be cultured in large scale. During the initial experiments (chapter 2), concentrations of up to 50 mysids.l^{-1} were maintained in 20 l trays where the water was exchanged with a biological filtration system every 12 h. The use of large densities enables small systems to produce large quantities of mysids, saving considerable amounts of space and water. Survival of newly hatched mysids in the initial experiments (chapter 2) after two weeks was about 40%. During 14 days, hatchlings were added to the tray twice a day and towards the end of each 14 day trial some of the mysids previously released into the trays grew to be large enough to cannibalize their hatchlings. Survival was improved in the next experiments (chapters 4 and 5) by placing mysids of similar size in each tray or tank. Mysids grew up to 4 mm (an appropriate size to harvest and feed them to cephalopod hatchlings) in only 7 days at temperatures that ranged from 17°C to 31°C., and released their first brood 13 days after being born at temperatures that ranged from 17°C to 31°C. Production could probably be improved by increasing the number of mature females, and simultaneously decreasing the intervals between water exchanges, feeding and hatchling collection. Nevertheless, by using a static water system, close attention has to be paid to water quality, especially the maintenance of pH values above 7.5. Below this value, mortality increased greatly and reproduction stopped.

The only factor that prevented the inexpensive production of *M. almyra* was the inability to find a less expensive diet to grow them. The acceptance of phytoplankton would have been a potential method of reducing the production costs but this species did not grow or reproduce when fed algae. *Artemia* nauplii were indeed a good food item, but its price prevents an economical large-scale mysid culture, vital for cuttlefish culture in large numbers. It is possible that *M. almyra* could be cultured on rotifers, or at least that their use could considerably reduce the costs of mysid culture by reducing the dependance on *Artemia*. Other researchers have used rotifers to grow *Mysidopsis* sp. with similar results when compared to *Artemia* nauplii (in cases even better, especially when feeding hatchlings).

The enrichment of *Artemia* nauplii with HUFA enhanced survival of hatchlings, but not production or growth. Nevertheless, hatchlings have to be grown to an appropriate size (about 4 mm). This growth period is of one week at higher water temperatures (27°C), or two weeks at lower temperatures (22°C). By using HUFA

enriched *Artemia* nauplii, more mysids survived the growth periods making mysid production (costs per mysid produced) similar when using HUFA enriched or *Artemia* nauplii without enrichment. Therefore, the enrichment of *Artemia* nauplii is benefitial. The use of zooplankton (mainly copepods) produced similar results on both production and survival; nevertheless, costs associated to collection still raised feding costs when compared to feeding HUFA enriched *Artemia* nauplii. Therefore, under these conditions, this would not be a good food source to produce mysids in large scale. Phytoplankton and artificial diets had significantly lower production and survival rates compared to the other diets tested. They were not a satisfactory food item to grow *M. almyra*.

We believe that *M. almyra* can be successfully cultured in a static water system at higher densities (up to 100 mysids.l^{-1}) than reported previously. Even higher densities might be possible but it would require more frequent water changes than twice a day to maintain water quality. Reproduction occurred at densities higher densities (200 mysids.l^{-1}), although deteriorating water quality in the static system resulting in poor production at this mysid density (chapter 4). Sustained production was obtained at lower temperatures compared to previous reports (chapter 4). Culturing broodstock at lower temperatures (22°C) should sustain adequate production, reduce production costs (as less food is necessary for mysids), improve water quality and lower the mortality rate of mature adults. Nevertheless, it is necessary to culture hatchlings at higher temperatures (about 26°C) in order to obtain both rapid growth and better survival during the first week of life. A cost effective culture strategy must address the finding that *M. almyra* adults and hatchlings have markedly different optimal temperatures for growth and survival.

The introduction of aeration in the trays and a partial weekly water exchanges for the biological filtration system improved water quality and consequently mysid production (chapter 4). In the static water system used, automation of the production system to set water exchanges at once every 6 h would improve water quality and hence increase production.

The use of rotifers as a replacement of *Artemia* nauplii to reduce mysid production costs (chapter 5) was tested in the Spanish Oceanographic Institute in the Canary Islands (Spain) since phytoplankton and rotifer production are established in their aquaculture plant. Mysids used in this case were *Leptomysis* sp., collected around the island of Tenerife. A flow-through system was used, as aquaculture in this facility is

done in flow-through systems (at the NRCC recirculating systems are used). If the replacement of *Artemia* nauplii by rotifers was successful, a project involving large scale production in this institute in Tenerife could be implemented, and therefore flow-through systems were used here. Unfortunately, the direct comparison between production, survival and growth of mysids used in both locations was not possible since different genera and culture systems were used. There was no possibility of using the same species in both locations because mysids used in each were not found in the other one. As for the culture systems, a standardization between the two places could have been used (static systems could have been used also in Tenerife). However, the priority was to determine if mysid culture could be achieved, in a small scale, using the conditions existing in each research facility so that results could be directly applied in larger systems for the large production of mysids.

The total replacement of *Artemia* nauplii by rotifers was unsuccessful, but a partial replacement (50% less *Artemia* nauplii used) promoted similar results as *Artemia* nauplii alone. When using rotifers, phytoplankton (used to enrich rotifers) and copepods (that grow in the rotifer culture tanks) were also fed to the mysids. Since several species of *Leptomysis* can feed on phytoplankton, its use might have been beneficial, contrary to *M. almyra*, that did not accept or do well with phytoplankton alone. The use of rotifers reduced production costs related to feeding approximately 99%; further studies (higher concentrations of rotifers and less *Artemia* nauplii) should be conducted in order to determine the effect on the culture of mysids and potential to lower production costs.

The successful partial replacement of *Artemia* nauplii by rotifers (chapter 5) represents the most important accomplishment obtained in the experiments. Nevertheless, the lower production by *Leptomysis* sp. compared to *M. almyra* would require larger quantities of adult mysids to produce the same number of hatchling. For *Leptomysis* sp., maturity and release of first brood required a larger period of time (at similar temperatures). Results obtained from research in both places indicated that *M. almyra* has a faster life cycle, and would be more appropriate for large scale production, if it could be successfully cultured with rotifers, as was seen for *Leptomysis* sp.

An attempt for the mass production of mysids is planned to be initiated in the future in Tenerife. Phytoplankton, adult *Artemia* and rotifers will be placed in a large volume, outdoor tank with flowing sea water. The phytoplankton will grow continuously, feeding the *Artemia* which will also continuously reproduce. Copepod populations coming in with the inflow will also grow in the tank, and rotifers will also be added and

reproduce, feeding on the phytoplankton and the baker's yeast that will be supplied. Finally, large numbers of mysids will be added and they will be able to graze on the copepods, phytoplankton and *Artemia* nauplii. If a balance (predator-prey) inside the tank is maintained, mysid production will take place continuously. Mysids will then be collected daily, both to be used as food for young cephalopods, and to reduce their numbers in the tank and maintain the balance in that system. This system, when well balanced, will allow mass mysid production at reduced costs (eventually with only phytoplankton and baker's yeast being added to the tank).

The surimi diets used in the experiments (chapter 6) were not effective for promoting satisfactory growth and survival of cuttlefish. Diet palatability is an important factor as soon as the cephalopod grabs the food. The surimi diets used in these experiments were not attractive enough and therefore feeding rates are considerably lower when compared to natural diets. This contributed to the small growth rates. The possibility of a low protein digestibility of the artificial diets might also be an important factor, contributing to the small growth rates.

From a strictly commercial point of view, the experimental diets (surimi) used did not produce good results. In fact, they promoted growth rates generally lower than 0.5% $BW.d^{-1}$, several times lower than rates obtained when feeding catfish fillets (up to 3.8% $BW.d^{-1}$) (*c.f.* Table XVIII, Appendix) that serve as a base for their preparation, while being considerably more expensive to produce (due to the addition of proteins, amino acids or minerals, and also the intensive labour it requires). The amino acid composition of the high protein diets (diets 2, 3 and 4) used in the methionine experiment were generally higher than the catfish fillets (Table XIX), and therefore should have produced better growth. This indicates that the major problem to solve is the palatability of the diets. Cuttlefish did not find the diets attractive and the feeding rates were considerably lower compared to natural foods. This resulted in poor growth.

Initial experiments with diets supplemented with four amino acids and casein delievered different results. In the first experiment, all diets promoted negative growth, similar to unfed cuttlefish. In the second experiment all diets promoted moderate growth (close to 0.5% $BW.d^{-1}$). Nevertheless, surimi was readily accepted by cuttlefish and low growth rates were attributed to the low nutritional value of the diets. During the next group of experiments, surimi diets were supplemented with different concentrations of some essential amino acids to test their effects on growth, survival and body condition of cuttlefish. The fact that cuttlefish accept artificial diets (and grow) is a significant

step towards the production of a successful artificial diet for cephalopods. Also, these diets of known composition are a powerful tool with which to study cephalopod metabolism, particularly the effects of amino acids.

The fully supplemented methionine and lysine were the only ones that promoted significant growth. This indicates that these amino acids are indeed essential. This strongly indicates that an effective artificial diet will have to be carefully balanced, especially in the levels of essential amino acids, in order to promote growth.

The analysis of body composition indicated also that some amino acids such as the branch-chained and the aromatic, and particularly proline are very important in cuttlefish metabolism and a well balanced diet has to include adequate concentrations of these amino acids. Proline should be considered an essential amino acid in cephalopod nutrition since it was the one that presented the higher variations in the tissues analyzed. Results obtained from the variation in amino acid concentration of the tissues indicates that the mantle is the most stable tissue analyzed.

The amino acid body composition indicated that cuttlefish fed the surimi diets were in intermediate condition. Several essential amino acids (from the aromatic and branch-chained groups) were present (in the blood) in lower concentrations compared to cuttlefish fed shrimp but were present in higher concentration when compared to unfed cuttlefish. This suggests that these metabolically important amino acids were desapearing from storing tissues but were still present in the blood of cuttlefish fed the surimi diets.

The addition of inert substances such as chromium oxide (that are not digested by the cuttlefish and appear in their initial concentrations in the feces) allows the determination of digestibility of proteins or other components of the diet. Digestibility studies have to be done to better understand the quality of these diets and improve them.

The initial objectives proposed for this research were accomplished; results obtained from mysid culture in both Galveston and Tenerife indicate that mysids could be grown at sufficiently low production costs to allow mysid culture at a commercial scale. The reduced cost mysids would allow the culture of several hundreds or thousands of *S. officinalis* hatchlings. Nevertheless, the production of artificial diets that would significantly reduce production costs (associated with live or natural feeds) of *S. officinalis* were not completely satisfactory. This research is still in its early stages and further research is necessary before an adequate artificial diet for cephalopods is attained.

As for future trends on research in concerning mysid culture, several paths can be followed. One would be the use of rotifers (low production costs) and/or the use of existing zooplankton populations in the water when using flow-through systems (such as copepods, present in the rotifer culture tanks) to considerably reduce mysid production costs. Another topic of research would be the identification of a mainly herbivorous mysid species, that could be cultured on phytoplankton alone. Both these two situations would lower production costs to levels that would allow large-scale mysids culture (and consequently some species of cephalopods as *S. officinalis*).

Another area of research that can improve mysid culture is the determination of suitable mysid species for large-scale culture; those are the ones with higher fecundity and reproduction rates (associated to a faster life cycle).

Concentration future research on the use of rotifers, phytoplankton or other cheap type of food, as well as the mysid species that do better in culture are the most important factors for the large-scale production of mysids, since results from this research indicates that the type of culture system, although important, is not as critical as the two previous aspects.

Further research on artificial diets will have to include digestibility and palatability studies. The first ones are vital to understand why some energetic surimi diets such as the fully supplemented methionine diet, with high amino acid concentrations when compared to catfish fillets (*c.f.* Table XIX, Appendix) did not promote acceptable growth. Although feeding rates were lower than for natural diets, cuttlefish accepted the surimi and better growth rates were expected. As for palatability, it is another vital aspect to be improved in order to obtain a satisfactory artificial diet, since artificial diets were clearly less attractive than natural feeds.

Finally, the effects of other amino acids, particularly proline, should be tested. Results obtained from the body composition of cuttlefish tissues analyzed indicate that this amino acid is intensely used by the cuttlefish, particularly when not enough food or low energy diets are provided. Therefore, the determination of the adequate levels of proline in an artificial diet should be of great importance for the production of a suitable artificial diet for cephalopods.

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APPENDIX

Table I - Numbers of adult and juvenile (< 2 mm total length) mysids in the trays on days 1, 15, 30 and 45 of the preliminary trial.

	Tray 1			**Tray 2**		
	Adults	Juveniles	**Total**	Adults	Juveniles	**Total**
Day 1	120	0	**120**	120	0	**120**
Day 15	117	107	**224**	195	87	**282**
Day 30	216	92	**308**	280	108	**388**
Day 45	242	117	**359**	265	167	**432**

Table II - Growth in length of *M. almyra* fed *Artemia* nauplii and *Artemia* nauplii enriched with HUFA (SELCO®) for 15 days. The "a" letter indicates that the value was significantly different ($p < 0.05$).

Trial 1			**Trial 2**		**Trial 3**	
Growth (mm)	**Enriched *Artemia***	***Artemia***	**Enriched *Artemia***	***Artemia***	**Enriched *Artemia***	***Artemia***
Day 1	1.96	1.95	1.70	1.70	1.90	1.90
Day3	2.69	2.51	2.57	2.51	2.40	2.43
Day 5	3.14	3.00	3.54	3.66	3.23	3.17
Day 7	3.63 [a]	3.36 [a]	4.31 [a]	4.77 [a]	4.11 [a]	4.57 [a]
Day 9	3.74 [a]	3.54 [a]	5.26	5.40	5.31	5.43
Day 11	4.63 [a]	4.26 [a]	5.88	6.12	5.86	5.89
Day 13	5.07 [a]	4.71 [a]	6.38	6.21	5.91	5.96
Day 15	6.02 [a]	5.21 [a]	6.23	6.03	6.10	6.05

Table III - Independent one tail Student T-test comparing production between four different mysid densities (mysids.l^{-1}).

Mysid densities	**T**	**DF**	**p**
25-37.5	5.479	58	0.000
25-50	4.757	58	0.000
25-100	11.517	58	0.000
37.5-50	-0.071	58	0.943
37.5-100	7.872	58	0.000
50-100	7.657	58	0.001

Table IV - Statistical comparison of growth from mysids fed three diets during 34 days. Diets were: 1) – *Artemia* nauplii+ rotifers; 2) – *Artemia* nauplii; 3) – Rotifers. Values of probability (p) are followed by the T value. N was 30 for all comparisons. Significant differences ($p<0.05$) are shown in bold, followed by the "*" sign.

Days		1-2	1-3	2-3
1	**p**	0.1435	0.7812	0.1709
	T	1.5036	0.2803	1.4043
4	**p**	**0.0323 ***	0.3138	**0.0110 ***
	T	2.2488	1.0252	2.7173
7	**p**	0.8036	0.6135	0.3256
	T	0.2510	0.5106	1.0000
10	**p**	0.2742	0.1796	**0.0481 ***
	T	1.1145	1.3751	2.0635
13	**p**	**0.0168 ***	**0.0489 ***	0.3883
	T	2.5364	2.0562	0.8759
16	**p**	**0.0009 ***	**0.0114 ***	0.0812
	T	3.7139	2.7022	1.8070
19	**p**	**0.0005 ***	**0.0023 ***	0.9469
	T	3.9420	3.3396	0.0672
22	**p**	**0.0000 ***	**0.0000 ***	**0.0000 ***
	T	5.3963	9.8730	4.8856
25	**p**	**0.0005 ***	**0.0000 ***	**0.0000 ***
	T	3.9336	11.6014	8.1330
28	**p**	**0.0087 ***	**0.0000 ***	**0.0000 ***
	T	2.8201	13.3694	11.6148
31	**p**	**0.0360 ***	**0.0000 ***	**0.0000 ***
	T	2.1990	12.4790	12.8622
34	**p**	0.0870	**0.0000 ***	**0.0000 ***
	T	1.7720	13.6749	12.4240

Table V - Composition of the surimi diets used in the preliminary experiments to test the acceptance of artificial diets (surimi) by the cuttlefish *S. officinalis*.

EXPERIMENT #1	DIET 1 (%)	DIET 2 (%)	DIET 3 (%)
Catfish	85.5	79	49
Casein	6	6	12
Salt	2	2	2
Shrimp meal			10
Corn Starch			5
ARG	0.500	1.269	2.030
PRO	0.500	1.476	2.361
GLU	0.500	0.500	0.700
LEU	0.500	0.968	1.357
ISO		0.502	0.802
VAL		0.432	0.691
THR		0.468	0.748
LYS		0.877	1.403
MET		0.434	0.694
TYR		0.848	1.357
TRP		0.500	1.000
HIS		0.227	0.363
Vitamines	2	2	2
Minerals	2		
Chromic Oxide	0.5	0.5	0.5

EXPERIMENT #2	DIET 1 (%)	DIET 2 (%)	DIET 3 (%)
Catfish	85.5	79.0	85.0
Casein	6	6	
Salt	2	2	2
ARG	0.50	1.27	1.27
PRO	0.50	1.48	1.48
GLU	0.50	0.50	0.50
LEU	0.50	0.97	0.97
ISO		0.50	0.50
VAL		0.43	0.43
THR		0.47	0.47
LYS		0.88	0.88
MET		0.43	0.43
TYR		0.85	0.85
TRP		0.50	0.50
HIS		0.23	0.23
Vitamines	2	2	2
Minerals	2	2	2
Chromic Oxide	0.5	0.5	0.5

Table VI - Composition of the surimi diets fed to cuttlefish (*S. officinalis*) in the experiment testing the amino acid methionine.

Ingredient	DIET 1 (%)	DIET 2 (%)	DIET 3 (%)	DIET 4 (%)
Catfish	51.500	85.025	84.682	79.312
Casein				6
Salt	2	2	2	2
Corn Starch	31.428			
THR	0.63	0.48	0.48	0.41
GLU	1.98	1.40	1.40	1.01
PRO	2.38	2.15	2.15	1.80
VAL	0.47	0.35	0.35	0.22
MET	0	0	0.34	1.18
ISO	0.60	0,.8	0.48	0.36
LEU	1.01	0.68	0.68	0.54
TYR	0.46	0.37	0.37	0.25
LYS	1.06	0.84	0,84	0.67
ARG	1.50	1.23	1.23	1.27
TRP	0.5	0.5	0.5	0.5
Vitamins	2	2	2	2
Minerals	2	2	2	2
Chromic Oxide	0.5	0.5	0.5	0.5

Table VII - Composition of the surimi diets fed to cuttlefish (*S. officinalis*) in the experiment testing the amino acid lysine.

Ingredient	DIET 1 (%)	DIET 2 (%)	DIET 3 (%)	DIET 4 (%)
Catfish	51,50	85,52	85,07	80,49
Casein				6
Salt	2	2	2	2
Corn Starch	16,04			
Cellulose	16,04			
THR	0,63	0,48	0,48	0,41
GLU	1,98	1,40	1,40	1,01
PRO	2,38	2,15	2,15	1,80
VAL	0,47	0,35	0,35	0,22
MET	0,41	0,34	0,34	0,00
ISO	0,60	0,48	0,48	0,36
LEU	1,01	0,68	0,68	0,54
TYR	0,46	0,37	0,37	0,25
LYS	0	0	0,45	0,67
ARG	1,50	1,23	1,23	1,27
TRP	0,5	0,5	0,5	0,5
Vitamins	2	2	2	2
Minerals	2	2	2	2
Chromic Oxide	0,5	0,5	0,5	0,5

Table VIII - Composition of the surimi diets fed to cuttlefish (*Sepia officinalis*) in the experiments testing the amino acid leucine.

Ingredient	DIET 1 (%)	DIET 2 (%)	DIET 3 (%)	DIET 4 (%)
Catfish	51,500	85,180	84,680	80,195
Casein				6
Salt	2	2	2	2
Corn Starch	32,03			
THR	0,63	0,48	0,48	0,409
GLU	1,98	1,40	1,40	1,008
PRO	2,38	2,15	2,15	1,799
VAL	0,47	0,35	0,35	0,215
MET	0,41	0,34	0,34	0,290
ISO	0,60	0,48	0,48	0,362
LEU	0	0,19	0,69	0,540
TYR	0,46	0,37	0,37	0,246
LYS	1,06	0,84	0,84	0,670
ARG	1,50	1,23	1,23	1,266
TRP	0,50	0,50	0,50	0,50
Vitamins	2	2	2	2
Minerals	2	2	2	2
Chromic Oxide	0,5	0,5	0,5	0,5

Table IX - Method used to make the surimi diets used in all experiments. The base of diets was thawed fresh water catfish fillets.

How to make surimi

- **Boil** the **water** that is going to be used with the catfish to make the paste (blend).
- Add Sodium Bicarb. $Na(HCO_3)$ At 0.025%. This is: **(Liters of water used)*0.250= g of $Na(HCO_3)$**.
Example: For 16 liters of water - 16*0.250 = 4 g $Na(HCO_3)$

- **Adjust the pH** of the water (by adding NaOH or Hcl). The pH has to be maintained between **6.6** and **7.4**
- **Cut the catfish fillets** in small portions (cubes of about 3 cm each side).
- **Blend** the catfish with water.
- Pass the paste by a **sieve** into a bucket, after blending, until almost everything is gone by the sieve.
- Weight the remains that have not passed through the sieve (**Waste**).
- **Add salt** (**NaCl**). 1.5 g of NaCl for every kg of paste. (kg Paste - kg waste * 1.5) = g NaCl.
- Let the paste **rest overnight**.
- Carefully **remove supernate** that formed during the night.
- **Centrifuge** the remaining of the paste. Centrifuging takes **15 minutes**, at **3000 r.p.m.**, at **8°C**.
- **Discart supernate** and put the remaining paste in the bucket.
- Let it **rest overnight**.
- Before cooking, **add all the ingredients** to the paste (**amino Acids**, **Proteins**, **Vitamins**, **Minerals**, etc).

- COOKING:
- **Cook for aproximately 45 minutes at 40°C.**
- **Cook for aproximately 45 minutes at 90°C.**

Table X - Mean Instantaneous Growth Rates (% Body Weight.d^{-1}) of cuttlefish (*Sepia officinalis*) fed artificial diets enriched with casein and four amino acids (arginine, proline, glutamine and leucine) during two experiments.

Exp. I		Exp. II
30 days	**Duration**	16 days
72	**n**	24
50%	**Survival**	37%
	Mean IGR	
-0.24	**Diet 1**	0.48
-0.26	**Diet 2**	0.49
-0.44	**Diet 3**	0.57
-0.34	**Starved**	-

Table XI - Comparison between amino acid concentration of the 4 diets used in the experiment testing methionine. The "*" indicates that these concentrations were significantly ($p<0.05$) different between them.

A. acids	ASP	THR	SER	GLU	PRO	GLY	ALA	VAL	CYS
Diet 1	**15.4***	**92.7***	**5.9***	217.4	**347.6***	**9.1***	**9.2***	**75.5***	**1.4***
Diet 2	32.7	77.9	13.3	188.0	303.7	21.4	20.2	60.7	**6.6***
Diet 3	26.2	68.3	**10.7***	166.8	265.9	17.6	16.1	51.9	**29.5***
Diet 4	**38.0***	**48.0***	**24.1***	150.3	**181.6***	**15.5***	**18.5***	**41.8***	**79.1***

A. acids	MET	ISO	LEU	TYR	PHE	LYS	HIS	ARG
Diet 1	**4.1***	**85.4***	**122.4***	28.5	**5.9***	**133.8***	**2.7***	**212.6***
Diet 2	**10.0***	73.6	102.1	44.0	12.5	118.9	5.8	193.0
Diet 3	**43.2***	66.6	94.6	41.1	**10.1***	103.5	**4.6***	167.6
Diet 4	**62.8***	**47.5***	**75.8***	29.9	**22.7***	**74.8***	**11.9***	**106.9***

Table XII - The amino acid composition of the tissue of cuttlefish (n=6) fed methionine diets. Total amino acid composition of the mantle and digestive gland is expressed in $mg.l^{-1}$ and free amino acid composition of the blood as $\mu g.l^{-1}$ (means, in bold; standard deviations below).

	MET	CYS	ARG	LYS	ALA	PRO	ASP	GLU	GLY	SER	THR	VAL	LEU	ISO	TYR	PHE	HIS
DIET 1																	
Mantle	**25.9**	**21.4**	**70.0**	**67.3**	**46.1**	**81.8**	**83.5**	**133.1**	**45.3**	**33.9**	**34.5**	**36.4**	**66.0**	**37.4**	**22.6**	**31.0**	**14.5**
	11.0	7.9	34.9	29.5	20.1	45.0	36.3	52.4	21.1	15.2	15.1	15.6	26.9	15.6	9.8	13.6	5.9
Digestive Gland	**23.8**	**42.0**	**47.4**	**69.6**	**39.3**	**34.6**	**82.8**	**106.9**	**37.6**	**36.0**	**38.0**	**44.5**	**63.0**	**41.4**	**29.1**	**38.6**	**18.8**
	10.4	22.7	18.4	27.5	14.0	16.1	34.0	36.5	15.8	16.1	16.8	19.2	25.2	17.9	13.8	17.6	7.6
Free blood	**11.0**	**18.2**	**4.9**	**10.6**	**18.1**	**24.7**	**2.8**	**5.0**	**10.3**	**9.6**	**8.7**	**13.1**	**27.4**	**12.2**	**28.9**	**11.0**	**9.5**
	4.1	2.8	2.9	4.5	5.3	9.4	2.0	3.7	1.5	2.4	5.7	3.0	14.9	2.7	18.5	10.0	3.0
DIET 2																	
Mantle	**20.8**	**19.1**	**53.4**	**55.2**	**40.2**	**79.7**	**68.6**	**115.2**	**38.5**	**28.3**	**28.9**	**29.5**	**55.5**	**31.2**	**17.9**	**25.2**	**11.8**
	9.2	10.3	24.2	25.2	17.3	41.1	31.7	49.4	16.7	12.8	13.2	12.8	25.2	14.2	8.4	11.4	4.8
Digestive Gland	**18.9**	**32.5**	**39.6**	**56.0**	**36.0**	**32.7**	**74.5**	**100.9**	**34.4**	**32.2**	**33.5**	**39.1**	**56.0**	**36.5**	**22.9**	**32.9**	**16.4**
	5.8	9.7	13.6	25.2	12.3	12.7	26.2	37.3	12.3	12.2	13.3	12.9	18.3	12.3	6.9	11.9	5.3
Free blood	**8.6**	**12.7**	**6.7**	**11.1**	**20.1**	**44.1**	**1.5**	**3.7**	**10.1**	**10.0**	**12.2**	**21.5**	**29.4**	**17.3**	**14.4**	**3.7**	**8.4**
	2.9	3.9	6.2	4.0	5.5	30.1	0.8	2.3	1.9	2.6	6.5	8.0	12.8	6.3	5.5	1.4	1.8
DIET 3																	
Mantle	**28.5**	**22.4**	**75.8**	**75.6**	**52.7**	**114.6**	**90.0**	**152.1**	**47.9**	**37.0**	**30.5**	**40.9**	**73.7**	**41.2**	**25.8**	**34.8**	**15.8**
	8.5	10.1	21.2	22 .0	14.2	25.2	25.0	38.8	15.2	10.8	17.3	11.0	20.2	12.0	8.2	10.5	4.3
Digestive Gland	**20.0**	**31.3**	**40.7**	**59.6**	**35.1**	**36.0**	**73.9**	**94.3**	**38.2**	**33.2**	**34.7**	**40.6**	**55.6**	**36.7**	**25.3**	**35.1**	**16.8**
	7.5	16.1	17.1	23.1	13.6	14.3	29.5	36.5	16.6	14.1	14.6	14.4	21.4	14.6	10.4	14.0	6.1
Free blood	**11.6**	**27.4**	**8.1**	**12.6**	**21.9**	**51.2**	**3.8**	**7.6**	**14.1**	**12.6**	**9.6**	**16.9**	**24.3**	**13.6**	**18.0**	**8.4**	**9.5**
	7.0	19.3	7.2	10.0	10.4	36.8	5.1	10.9	8.5	7.7	8.0	11.3	21.4	10.6	9.8	10.3	2.7
DIET 4																	
Mantle	**28.0**	**21.1**	**63.2**	**69.0**	**44.5**	**97.6**	**85.2**	**135.2**	**44.5**	**34.4**	**35.7**	**39.1**	**67.9**	**38.9**	**23.2**	**32.4**	**14.7**
	15.7	9.8	30.6	30.1	21.8	41.2	38.3	49.2	21.8	15.8	16.8	17.2	26.5	17.2	10.1	14.6	6.3
Digestive Gland	**22.1**	**37.1**	**46.5**	**72.0**	**38.9**	**34.8**	**84.0**	**108.9**	**38.2**	**36.3**	**39.6**	**44.5**	**61.9**	**40.9**	**27.5**	**37.4**	**18.2**
	13.5	26.7	33.0	48.6	24.1	19.2	53.7	72.7	21.7	21.9	24.7	26.6	37.5	24.9	15.7	21.8	10.0
Free blood	**9.0**	**45.6**	**9.2**	**13.0**	**21.4**	**39.0**	**1.7**	**3.2**	**13.7**	**12.1**	**12.0**	**22.1**	**28.3**	**16.5**	**13.9**	**4.6**	**9.2**
	5.7	64.4	10.4	4.7	6.9	7.2	1.0	1.9	5.5	3.6	3.8	8.1	9.2	5.5	5.3	2.3	2.7

Table XIII - The amino acid composition of the tissue of cuttlefish (n=6) fed lysine diets. Total amino acid composition of the mantle and digestive gland is expressed in $mg.l^{-1}$ and free amino acid composition of blood as $\mu g.l^{-1}$ (means, in bold; standard deviations below).

	MET	CYS	ARG	LYS	ALA	PRO	ASP	GLU	GLY	SER	THR	VAL	LEU	ISO	TYR	PHE	HIS
DIET 1																	
Mantle	**41.2**	**27.0**	**104.3**	**113.3**	**77.7**	**84.7**	**139.4**	**181.2**	**65.4**	**19.0**	**60.0**	**58.7**	**107.6**	**60.7**	**38.8**	**52.8**	**28.9**
	14.5	11.5	43.6	41.1	26.8	18.1	49.1	96.8	19.9	10.2	21.2	19.8	29.9	20.5	14.4	19.2	9.9
Digestive Gland	**19.3**	**20.9**	**39.3**	**60.7**	**36.8**	**75.5**	**76.7**	**89.9**	**36.4**	**9.4**	**36.5**	**37.2**	**57.4**	**37.1**	**25.9**	**36.3**	**39.3**
	3.1	3.9	7.4	10.1	6.6	17.7	14.0	17.3	7.9	1.8	6.1	7.7	9.4	6.0	3.9	6.2	7.4
Free blood	**9.3**	**11.4**	**7.5**	**13.2**	**23.5**	**30.5**	**4.0**	**9.8**	**14.5**	**1.0**	**12.0**	**17.4**	**23.9**	**15.2**	**16.2**	**7.8**	**7.5**
	4.2	5.9	5.1	6.9	13.5	6.6	2.9	8.1	4.0	0.9	4.1	0.7	7.2	4.4	6.1	4.0	5.1
DIET 2																	
Mantle	**36.8**	**22.9**	**89.5**	**99.3**	**68.7**	**107.0**	**122.7**	**177.3**	**62.8**	**50.1**	**52.3**	**53.8**	**95.6**	**54.7**	**35.7**	**47.7**	**25.8**
	20.5	11.0	53.1	54.2	36.1	65.1	66.5	89.4	34.6	26.9	28.0	26.9	46.7	27.1	17.9	24.6	12.9
Digestive Gland	**33.9**	**32.2**	**68.6**	**104.3**	**62.3**	**60.7**	**124.0**	**146.9**	**62.1**	**56.4**	**40.0**	**65.6**	**95.5**	**62.9**	**43.0**	**59.4**	**33.4**
	9.1	11.8	18.4	30.4	15.1	12.3	33.8	28.5	20.9	16.7	24.5	20.8	24.1	19.7	13.6	20.0	11.6
Free blood	**10.2**	**8.6**	**2.9**	**10.6**	**18.7**	**30.5**	**1.4**	**2.2**	**13.7**	**9.7**	**8.7**	**17.8**	**24.6**	**18.4**	**11.3**	**4.4**	**6.6**
	4.7	2.8	1.3	5.4	6.0	12.2	0.7	0.8	4.3	3.3	2.2	10.0	12.2	5.8	5.6	2.6	3.7
DIET 3																	
Mantle	**48.7**	**30.4**	**131.2**	**128.6**	**98.1**	**195.8**	**164.5**	**229.8**	**78.0**	**64.1**	**70.8**	**70.4**	**123.9**	**71.0**	**46.5**	**64.1**	**34.3**
	21.3	15.1	53.5	60.2	34.3	100.6	71.2	78.0	27.2	33.2	31.2	32.2	30.7	28.4	22.7	27.0	15.3
Digestive Gland	**27.9**	**29.5**	**58.0**	**88.2**	**54.3**	**45.8**	**113.1**	**130.8**	**50.7**	**48.6**	**52.4**	**57.0**	**81.3**	**52.9**	**33.7**	**52.3**	**29.3**
	14.2	16.1	28.9	44.7	23.9	22.9	49.0	54.6	23.9	22.0	24.7	29.6	33.9	26.4	18.3	26.1	14.8
Free blood	**9.7**	**42.6**	**2.8**	**14.2**	**19.5**	**34.3**	**2.0**	**4.4**	**13.4**	**14.5**	**11.9**	**20.8**	**30.3**	**17.3**	**19.6**	**6.2**	**11.6**
	4.6	41.2	1.7	5.5	5.1	8.2	1.7	3.9	4.4	3.6	1.6	7.6	8.5	4.5	5.5	2.5	3.5
DIET 4																	
Mantle	**44.2**	**20.5**	**117.8**	**121.8**	**91.4**	**173.4**	**137.6**	**199.7**	**69.6**	**52.2**	**58.7**	**58.7**	**116.1**	**60.6**	**42.9**	**55.3**	**29.4**
	12.7	11.0	45.9	38.1	36.1	92.5	45.6	60.7	17.2	23.9	19.2	18.8	28.1	19.1	12.7	16.6	8.7
Digestive Gland	**22.1**	**22.3**	**42.7**	**71.9**	**40.4**	**47.0**	**85.9**	**110.9**	**39.1**	**42.0**	**32.2**	**47.3**	**66.7**	**44.7**	**27.6**	**39.0**	**24.0**
	6.0	6.4	13.2	16.3	12.4	10.2	22.4	22.4	15.7	9.7	16.5	10.5	16.3	10.4	9.0	11.6	5.1
Free blood	**6.6**	**36.4**	**4.6**	**14.4**	**16.7**	**42.3**	**2.0**	**5.1**	**12.1**	**11.7**	**11.7**	**27.8**	**39.8**	**23.1**	**12.0**	**4.3**	**7.9**
	3.7	46.2	2.5	8.9	4.3	18.5	1.6	4.6	4.2	4.9	5.7	16.8	24.0	12.7	2.4	1.9	3.0

Table XIV - Comparison of cuttlefish fed the four methionine diets with shrimp-fed and unfed cuttlefish. The - or + indicates that the concentration of the amino acid in that tissue was significantly lower or higher ($p<0.05$) when compared with shrimp-fed or unfed cuttlefish.

Diet vs. shrimp	MET	CYS	ARG	LYS	ALA	PRO	ASP	GLU	GLY	SER	THR	VAL	LEU	ISO	TYR	PHE	HIS
Mantle DIET 1					-	-											
Digestive Gland				-			-	-	-					-			
Free blood		-				-			-	-		-					-
Mantle DIET 2	-		-	-	-	-	-		-	-	-	-	-		-	-	-
Digestive Gland	-	-		-	-		-	-	-	-			-	-	-	-	-
Free blood		-								-					-	-	-
Mantle DIET 3					-	-											
Digestive Gland	-	-		-	-		-	-	-	-		-	-	-	-	-	-
Free blood				-											-		-
Mantle DIET 4					-	-											
Digestive Gland							-										
Free blood				-											-	-	-
Diet vs. unfed	**MET**	**CYS**	**ARG**	**LYS**	**ALA**	**PRO**	**ASP**	**GLU**	**GLY**	**SER**	**THR**	**VAL**	**LEU**	**ISO**	**TYR**	**PHE**	**HIS**
Mantle DIET 1																	
Digestive Gland																	
Free blood		+			+	+			-			+					
Mantle DIET 2	-						-		-	-	-				-	-	-
Digestive Gland																	
Free blood		-			+	+			-			+	+	+		-	
Mantle DIET 3						+											
Digestive Gland																	
Free blood						+											
Mantle DIET 4																	
Digestive Gland																	
Free blood					+	+						+	+	+	-	-	-

Table XV - Comparison of cuttlefish fed the four lysine diets with shrimp-fed and unfed cuttlefish. The - or + indicates that the concentration of the amino acid in that tissue was significantly lower or higher ($p<0.05$) when compared with shrimp-fed or unfed cuttlefish.

Diet vs. shrimp	MET	CYS	ARG	LYS	ALA	PRO	ASP	GLU	GLY	SER	THR	VAL	LEU	ISO	TYR	PHE	HIS
Mantle DIET 1						-				-							
Digestive Gland	-	-		-	-		-	-	-	-		-	-	-	-	-	-
Free blood		-								-					-		-
Mantle DIET 2																	
Digestive Gland		-															
Free blood		-				-				-					-	-	-
Mantle DIET 3																	
Digestive Gland		-															
Free blood						-									-	-	
Mantle DIET 4																	
Digestive Gland	-	-		-	-		-		-			-	-	-		-	
Free blood						-									-	-	-
Diet vs. unfed	**MET**	**CYS**	**ARG**	**LYS**	**ALA**	**PRO**	**ASP**	**GLU**	**GLY**	**SER**	**THR**	**VAL**	**LEU**	**ISO**	**TYR**	**PHE**	**HIS**
Mantle DIET 1						+				-							
Digestive Gland		-				+				-							
Free blood		-				+				-		+	+	+			-
Mantle DIET 2																	
Digestive Gland			+	+	+	+		+				+	+	+			+
Free blood		-				+								+			+
Mantle DIET 3																	
Digestive Gland																	
Free blood						+				+		+	+	+			
Mantle DIET 4																	
Digestive Gland		-				+											
Free blood						+						+	+	+			

Table XVI - Feeding rates, food conversions and Instantaneous Growth Rates for cuttlefish kept in isolated chambers and fed 4 different surimi diets.

Diets	I	I	I	II	II	II	III	III	IV	IV	IV
Feeding rate (%BW.d^{-1})	1.3	1.1	1.0	2.2	3.9	2.1	1.6	1.9	1.7	3.0	2.2
Food Conversion	-3.5	-2.8	-5.4	13.7	-34.3	16.5	28.1	16.0	6.5	6.1	6.2
IGR (%BW.d^{-1})	**-0.37**	**-0.39**	**-0.18**	**0.16**	**-0.11**	**0.13**	**0.06**	**0.12**	**0.26**	**0.48**	**0.36**

Table XVII – Results obtained from the experiment testing the amino acid Leucine. Mean Instantaneous Growth Rates (MIGR) and feeding rates are expressed as %BW.d^{-1}. Mortality is expressed as a percentage.

	MIGR	Feeding Rate	Food Conversions
Diet 1	**0.26**	**0.24**	**0.91**
Diet 2	**0.37**	**1.72**	**4.72**
Diet 3	**0.09**	**1.81**	**20.04**
Diet 4	**1.12**	**1.59**	**1.43**

Table XVIII - Feeding rates, food conversions and Instantaneous Growth Rates for cuttlefish kept in individual chambers and fed thawed catfish.

Tanks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Feeding rate (%BW.d^{-1})	9.0	4.2	6.2	5.2	8.3	3.7	5.8	5.5	7.6	5.6	7.6	5.5	9.4	7.2	3.8	7.2
Food conversions	2.9	3.5	3.1	3.2	3.3	3.9	3.2	2.7	2.7	2.7	2.8	2.6	2.5	2.9	2.5	2.5
IGR (%BW.d^{-1})	**3.14**	**1.20**	**2.01**	**1.65**	**2.54**	**0.94**	**1.77**	**2.04**	**2.88**	**2.12**	**2.71**	**2.10**	**3.83**	**2.52**	**1.53**	**2.88**

Table XIX - Comparison between amino acid composition (mg.g^{-1}) of surimi diets used in the methionine experiment and amino acid composition in thawed catfish fillets.

Amino acids	ASP	THR	SER	GLU	PRO	GLY	ALA	VAL	CYS
Diet1	15.4	92.7	5.9	217.4	347.6	9.1	9.2	75.5	1.4
Diet2	32.7	77.9	13.3	188.0	303.7	21.4	20.2	60.7	6.6
Diet3	26.2	68.3	10.7	166.8	265.9	17.6	16.1	51.9	29.5
Diet4	38.0	48.0	24.1	150.3	181.6	15.5	18.5	41.8	79.1
Catfish	**37.0**	**21.9**	**16.9**	**43.0**	**18.7**	**35.6**	**31.8**	**1.6**	**19.5**

Amino acids	MET	ISO	LEU	TYR	PHE	LYS	HIS	ARG
Diet1	4.1	85.4	122.4	28.5	5.9	133.8	2.7	212.6
Diet2	10.0	73.6	102.1	44.0	12.5	118.9	5.8	193.0
Diet3	43.2	66.6	94.6	41.4	10.4	103.5	4.6	167.6
Diet4	62.8	47.5	75.8	29.9	22.7	74.8	11.9	106.9
Catfish	**8.0**	**14.4**	**27.6**	**8.3**	**11.7**	**6.2**	**27.3**	**15.8**