Pontifícia Universidade Católica do Rio Grande do Sul
Faculdade de Biociências
PPG- Biologia Celular e Molecular

Aspectos do Metabolismo Energético e da Reprodução de *Hyalella castroi* González, Bond-Buckup & Araujo (Crustacea, Amphipoda, Dogielinotidae) Mantidos em Cultivo Experimental sob Diferentes Dietas

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Dissertação de Mestrado
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DEDICATÓRIA

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Resumo

Foi comparado o efeito de diferentes dietas no metabolismo energético, níveis de lipoperoxidação e atividade da enzima Na^+/K^+ATPase de *Hyalella castroi* assim como sob aspectos reprodutivos. Este crustáceo vive em ambiente límnico no planalto do Rio Grande do Sul, Brasil. Os animais foram coletados durante os meses de outono de 2006 em São José dos Ausentes. Em laboratório, os animais foram mantidos separados por sexo em aquários sob condições controladas e alimentados *ad libitum* por 21 dias com diferentes dietas, sendo estas isocalóricas. No final do período experimental, os animais foram imediatamente congelados para determinação dos diferentes parâmetros bioquímicos. Parte dos animais foram mantidos nas mesmas condições já citadas, porém em aquários que permitiam o contato entre machos e fêmeas. Para análise de alguns parâmetros reprodutivos (número de pareamentos, número de fêmeas ovígeras e número de juvenis eclodidos). A análise estatística revelou diferença significativa na composição bioquímica ente os sexos e as dietas ao longo do cultivo experimental. As dietas foram capazes de alterar o padrão bioquímico dos animais trazidos de campo e determinaram um alto percentual de sobrevivência ao longo do período de cultivo; contudo, não foram adequadas para permitirem o pleno sucesso reprodutivo principalmente em relação ao número de fêmeas ovígeras, a fertilidade e a qualidade dos ovos. Além disso, ambos os sexos mostraram respostas metabólicas e reprodutivas melhores quando alimentados com a dieta 1, a qual possui maior teor de carboidratos (43.19g/100g) e menor de proteínas (30.88g/100g).

REVISÃO DA LITERATURA

1. Introdução

Diferentes adaptações ao meio ambiente têm sido caracterizadas em todos os níveis de organização biológica nos mais diversos organismos. Estas adaptações, tanto estruturais como funcionais, permitiram aos seres vivos à colonização de diferentes habitats. Ao longo dos últimos anos, as adaptações bioquímicas e metabólicas ao ambiente têm sido bastante estudadas em moluscos intertidais, peixes, tartarugas aquáticas e em alguns mamíferos, contudo poucos trabalhos têm abordado a influência de parâmetros ambientais, tais como a hipóxia, a anoxia, a temperatura, o fotoperíodo, a disponibilidade e qualidade de alimento sobre as adaptações do metabolismo intermediário em crustáceos. Tanto pelo número de espécies existentes, como pela diversidade de habitats nos quais vivem, os crustáceos estão entre os animais com o maior êxito durante sua história evolutiva. Esta diversidade é resultado de seus padrões de vida e de estratégias reprodutivas (Sastry, 1983).

Grande parte dos Malacostraca é representada pelos Peracarida que juntamente com os Decapoda são a maioria dos crustáceos, correspondendo a 30% do total. Cerca de 12000 espécies pertencem à classe Peracarida e representam sete ordens, das quais a Ordem Amphipoda é o grupo mais representativo dos ecossistemas aquáticos, aproximadamente 6000 espécies, sendo caracterizado por apresentarem o corpo lateralmente comprimido, olhos compostos e sésseis, e os primeiro e segundo pares de pereópodos chamados de gnatópodos, são geralmente maiores e subquelados, servindo para agarrar (Ruppert e Barnes, 2005).

O táxon Amphipoda é bastante diversificado, incluindo Gammaridea, Hiperiidea, Caprellidea e Ingofiellidea (Ruppert e Barnes, 2005). Os Gammaridea formam um grupo muito grande, predominando espécies marinhas, distribuídas em 69 famílias. Existem ainda representantes na água doce e uma única família (Talitridae) que reúne espécies terrestres. No Rio Grande do Sul, entre ambientes límnicos e marinhos são encontrados representantes de oito famílias de Gammaridea, das quais se destacam Corophidae, Stenothoidae, Hyalidae, Ischyriceridae, Gammaridae, Talitridae, Dogielinotidae (Bento e Buckup, 1999) e Dogielinotidae (González ***et al.***,2005).

O gênero *Hyalella*, pertencente à família Dogielinotidae, é encontrado em uma série de habitats de água doce, como reservatórios permanentes, lagos, tanques e riachos estando muitas vezes aderidas à vegetação, nadando nas colunas d'água ou em buracos cavados no sedimento,

sendo importantes membros da fauna bentônica (Kruschwitz, 1978; Wellborn, 1995; Grosso e Peralta, 1999). No Rio Grande do Sul, há registros de ocorrência de seis espécies deste gênero, sendo uma delas a *Hyalella castroi* González ***et al.***, (2007) encontrada no município de São José dos Ausentes, na localidade do Vale das Trutas (González ***et al.***, 2005.).

O dimorfismo sexual das espécies de *Hyalella* é caracterizado pela presença do segundo par de gnatópodos alargados nos machos. O segundo par de gnatópodos dos machos são usados para o manuseio das fêmeas durante o comportamento de cópula e os primeiros pares pequenos são utilizados para carregá-las. As fêmeas maduras de espécies de Amphipoda são facilmente identificadas pelos seus ovários desenvolvidos, os quais são externamente visíveis, pela presença de um marsúpio e pela presença de ovos dentro do marsúpio (Kruschwitz, 1978).

Alguns aspectos dos crustáceos, principalmente as estratégias reprodutivas, podem ser importantes para a interpretação de dados sobre estudos de bioindicação e para o desenvolvimento de estudos ecotoxicológicos, assim como para programas de conservação. Não somente os aspectos reprodutivos, mas também outras respostas comportamentais dos crustáceos, como as mudanças na alimentação, na locomoção ou no comportamento de pré-cópula podem providenciar respostas preditivas com respeito à bioindicação de toxicidade em determinado ambiente (Rinderhagen ***et al.***, 2000).

A reprodução é um período crítico no ciclo de vida dos animais e está intimamente relacionada com a capacidade reprodutiva, definida como uma porção das energias corporais direcionadas para esse propósito. Em crustáceos, a fecundidade é caracterizada como o número total de ovos no ovário, marsúpio ou externamente presos aos pleópodos das fêmeas (Somers, 1991). Kinne (1961), no entanto, considera ovos, como todas as fases entre a liberação dos ovócitos e todos os estágios de desenvolvimento subseqüentes anteriores a formação do olho no embrião. A fecundidade de uma espécie pode ser relacionada ao tamanho ou peso do animal (Ogawa e Rocha, 1976; Du Preez e McLachlan, 1984; Powell, 1992), a fatores ambientais (Jensen, 1958), a variações latitudinais (Jones e Simons, 1983), a taxa de sobrevivência das larvas e/ou juvenis (Branco ***et al.***, 1992) e, possivelmente, as reservas metabólicas ou energéticas do animal.

A biologia e a ecologia das espécies de Amphipoda do Rio Grande do Sul, ainda são muito pouco conhecidas. Informações sobre a biologia de anfípodos são restritas a *Hyalella azteca,* espécie que ocorre na América do Norte e México. Muitas espécies de Amphipoda, por outro lado, por serem na sua maioria organismos bentônicos, são muito utilizados em testes de

toxicidade e bioensaios para avaliação da qualidade do sedimento dos ecossistemas aquáticos. Este sedimento serve ao mesmo tempo, como depósito e fonte de matéria orgânica e inorgânica, pelo fato de sua camada superficial ser mais permanente que a coluna de água, servindo, portanto, como melhor testemunho das atividades ocorridas recentemente na bacia hidrográfica (Wetzel, 1983; Burton, 1991). Estudos recentes demonstraram que a espécie *Corophium volutator* (Crustacea, Amphipoda), também típica do sedimento está sendo usada na expressão da toxicidade do sedimento em ambientes límnicos (Gerhardt ***et al.***, 2005). Contudo, o número de espécies padronizadas em testes de toxicidade permanece limitado e na sua maioria são utilizados organismos alóctones, especialmente a espécie *Hyalella azteca*, contrastando com a riqueza taxonômica da maioria dos ecossistemas naturais de nosso País (Brendonck e Persoone, 1993).

O estudo do metabolismo intermediário em crustáceos tem demonstrado grande variabilidade inter e intra-espécies, o que torna difícil a determinação de um perfil metabólico padrão. Desconsiderando as diferenças entre os métodos bioquímicos empregados pelos diversos autores esta variabilidade pode ser atribuída a fatores múltiplos, tais como seu habitat (terrestre, marinho, estuarino ou de água doce), estágio do ciclo de muda, maturidade sexual (especialmente em fêmeas), estado alimentar, dieta oferecida e sazonalidade, visto que estes fatores determinam um padrão diferencial de resposta metabólica (Oliveira ***et al.***, 2003).

Dados da literatura sobre o metabolismo de carboidratos em crustáceos confirmam a presença das vias de glicogênese, de glicogenólise e de glicólise em diferentes tecidos (Meenaski e Scheer, 1968; Chang e O'Connor, 1983). As brânquias, os hemócitos, o músculo e o hepatopâncreas têm sido propostos como sítios para ocorrência da via gliconeogênica (Johnston e Davies, 1973; Thabrew ***et al.***, 1971; Oliveira e Da Silva, 1997).

Os principais tecidos de reserva de glicogênio em crustáceos são os músculos, o hepatopâncreas, as brânquias e os hemócitos, porém o local de armazenamento deste polissacarídeo vária de acordo com a espécie (Parvathy, 1971; Johnston e Davies, 1972; Herreid e Full, 1988). O glicogênio armazenado é utilizado nos processos de muda, hipóxia e/ou anoxia, osmorregulação, crescimento, diferentes estágios de reprodução e durante períodos de jejum (Chang e O'Connor, 1983; Kucharski e Da Silva, 1991a; Kucharski e Da Silva, 1991b; Oliveira ***et al.***, 2001 e 2004).

Segundo Chang e O'Connor (1983) a glicose é o principal monossacarídeo presente na hemolinfa de crustáceos, tendo seis destinos principais: a síntese de mucopolissacarídeos, a síntese de quitina, a síntese de ribose e nicotinamida adenina dinucleotídeo fosfato reduzido

(NADPH), a formação de piruvato e a síntese de glicogênio (Hochachka ***et al.***, 1970; Herreid e Full, 1988).

Em crustáceos as concentrações de lipídios são bastante elevadas, apesar de não existir um tecido adiposo diferenciado, os principais locais de armazenamento de lipídios são o músculo e o hepatopâncreas (O'Connor e Gilbert, 1968; Chang e O'Connor, 1983; Herreid e Full, 1988; Kucharski e Da Silva, 1991 a; Oliveira ***et al.***, 2006). Herreid e Full (1988) verificaram que os níveis de lipídios no hepatopâncreas excediam em dez vezes os níveis de glicogênio. Nos crustáceos, as sínteses de ácidos graxos, de diacilglicerol e de triacilglicerol são semelhantes àquela dos mamíferos. Diversos estudos têm demonstrado que durante períodos de grande demanda energética, como a muda e a gametogênese, ocorre uma marcante mobilização de lipídios, principalmente aqueles presentes no hepatopâncreas (Kucharski e Da Silva, 1991b; Rosa e Nunes, 2003a; Oliveira ***et al.,*** 2006).

O músculo parece ser a principal fonte de proteínas nos crustáceos e, em decápodos os níveis de aminoácidos livres nos tecidos atingem valores dez vezes superiores àqueles encontrados em vertebrados. Diversos trabalhos sugerem que estes aminoácidos estariam envolvidos nos processos de osmorregulação, estando principalmente ligados ao controle do volume celular (Huggins e Munday, 1968; Gilles, 1982; Chang e O'Connor, 1983). As proteínas são constituintes estruturais, funcionais e energéticos dos tecidos e tem um importante papel na postura, fertilização e desenvolvimento normal dos embriões de crustáceos (Garcia-Guerrero ***et al.***, 2003; Rodriguez-González ***et al.***, 2006). Outros estudos têm demonstrado variações no conteúdo protéico durante o desenvolvimento ovariano de crustáceos, estas variações podem ser resultado do aumento da biosíntese de muitas proteínas, incluindo enzimas, hormônios e lipoproteínas envolvidas com a maturação gonadal (Yehezkel ***et al.,*** 2000; Rosa e Nunes, 2003a e b; Oliveira ***et al.,*** 2006; Dutra ***et al.,*** 2007a e b).

Muitos estudos têm descrito a influencia do jejum no metabolismo de proteínas, gorduras e carboidratos em crustáceos mostrando uma grande variabilidade interespecífica (Marsden ***et al.***, 1973; Vinagre e Da Silva, 1992; Hervant ***et al.***, 1999; Hardy ***et al.***, 2000; Hervant e Renault, 2002; Vinagre e Da Silva, 2002; Oliveira ***et al***., 2004). Apesar das diferenças ente os métodos analíticos utilizados pelos diferentes autores e o período de jejum, múltiplos fatores unidos a peculiaridades biológicas e ecológicas das diferentes espécies provavelmente contribuem para a diversidade observada. Um fator, que parece não ser levado em conta nas pesquisas publicadas

sobre jejum em crustáceos, é o conteúdo de proteínas ou carboidratos da dieta que estes recebem antes do período de deprivação alimentar (Oliveira ***et al.***, 2004).

Variações sazonais determinam um efeito profundo na composição bioquímica dos organismos, com modificações observadas na dinâmica e nos níveis de lipídios totais durante o ciclo reprodutivo principalmente, no tecido gonadal e hepatopancreático tendo sido analisadas em algumas espécies de Brachyura (Pillay e Nair, 1973) e em outros decápodos (Read e Caulton, 1980; Castille e Lawrence, 1989; Rosa e Nunes, 2003a). Trabalhos desenvolvidos, em nosso laboratório, com *Hyalella curvispina*, *Hyalella pleoacuta* e *Hyalella castroi*, anfípodos característicos da região de planície (*Hyalella curvispina*) e de planalto (*Hyalella pleoacuta* e *Hyalella castroi*) do Rio Grande do Sul, nos permitem verificar, até este momento, um perfil de resposta sazonal do metabolismo de carboidratos, de proteínas e de lipídios; sendo que estes resultados parecem estar correlacionados às condições ambientais, a atividade dos animais e ao período reprodutivo. Observou-se, ainda, que machos e fêmeas diferem quanto ao perfil de resposta anual dos níveis de lipoperoxidação nas diferentes espécies; onde a lipoperoxidação parece estar fortemente associada a comportamentos reprodutivos (Dutra ***et al.***, 2007a, b).

Os radicais livres são continuamente produzidos pela fosforilação oxidativa e por outros sistemas biológicos reagindo rapidamente com a maioria das moléculas orgânicas. Estes radicais podem reagir com lipídios de membrana, proteínas, DNA e também glicídios (Meerson ***et al.***, 1981). Quando reagem com os lipídios de membrana, causam a lipoperoxidação destes, através de uma série de reações, com conseqüente formação de malondialdeído e outras substâncias que quando, aquecidas na presença de ácido tiobarbitúrico, formam um composto rosado, medido espectrofotometricamente (Buege e Aust, 1978; Ohkawa ***et al.,*** 1979; Llesuy ***et al.*** 1985; Halliwell e Gutteridge, 1995). Sabe-se da literatura especializada que o aumento do estresse oxidativo, principalmente, em períodos da alta demanda energética, pode aumentar proporcionalmente, a formação de espécies reativas ao oxigênio e com isto, aumentar a ocorrência do dano oxidativo (Viarengo ***et al.,*** 1991; Correia, 2002; Correia ***et al.,*** 2003; Timofeyev ***et al.***, 2006). Portanto, a utilização e a padronização de uma medida de dano oxidativo (TBARS) em crustáceos podem refletir alterações biológicas, como as que ocorrem no período reprodutivo e ou por alterações no meio ambiente.

Díaz-Muñoz ***et al.***, (1985) mostraram no córtex cerebral de ratos que a atividade enzimática da glutationa diminui durante a noite quando o córtex cerebral sofre um aumento na lipoperoxidação por causa do ritmo de atividade motora e alimentar. Já Fanjul-Moles ***et al.***,

(2003) verificaram que a lipoperoxidação no hepatopâncreas do lagostim *Procambarus clarkii* não é determinada desta forma, apesar deste animal ser noturno e o ritmo respiratório produzir radicais no período da noite, coincidindo neste animal com um aumento dos níveis da glutationa oxidase e da atividade de outros protetores.

A enzima Na^+K^+ATPase utiliza a energia derivada da hidrólise do ATP para bombear para fora da células $3Na^+$ transferindo $2K^+$ da parte externa para o citosol (Shepherd, 1994); funcionando assim como um *antiporter*, sendo um importante instrumento para restaurar o gradiente iônico nas células nervosas seguindo períodos de atividade elétrica como impulsos nervosos e potenciais sinápticos (Shepherd, 1994). Esta enzima dimérica existe em diversas isoformas no cérebro e consome grande parte do ATP disponível (Shepherd, 1994; Bertorello ***et al.***, 1991). Em crustáceos a Na^+K^+ATPase exerce um importante papel na manutenção de gradientes iônicos entre o meio interno (animal) e o habitat; sendo por isto fundamental para a sobrevivência de animais osmorreguladores. Contudo, poucos estudos sobre esta enzima são encontrados em crustáceos dulce-aquícolas (Gilles, 1982; Castilho ***et al.***, 2001). Esta enzima de membrana requer fosfolipídios para sua atividade e é altamente vulnerável ao dano oxidativo visto que sob tais circunstâncias observa-se uma inativação que pode envolver o rompimento dos fosfolipídios do microambiente da enzima ou danos diretos à proteína causados por radicais do oxigênio ou por produtos gerados na lipoperoxidação (Fleuranceau-Fleuranceau-Morel ***et al.***, 1999; Lehtosky ***et al.***, 1999).

Sabe-se que fatores abióticos como a temperatura e a dureza da água podem influenciar no cultivo dos organismos (Lewis e Marki, 1981; Persoone ***et al.,*** 1989), entretanto, dentre todas as variáveis a dieta a qual os organismos estão submetidos tem se mostrado como fator determinante no seu desenvolvimento (Kersting e Van der Leeuw, 1976; Lewis e Marki, 1981; Vijverberg, 1989; Lei ***et al.*** 1990; Kawbata e Urabe, 1998; Beatrici, 2000). Beatrici (2000), ao comparar a resposta de *Daphnia similis* a três diferentes dietas constatou que os indivíduos mantidos com uma dieta combinada de alga (*Selenastrum caprcornutum*) com um complemento alimentar a base de artêmia reproduziam significativamente mais do que quando cultivados apenas com algas. Platte (1993) objetivando alcançar uma forma de aumentar a produtividade dos cultivos de *Ceriodaphnia dubia* obteve resultados semelhantes ao testar o complemento alimentar a base de artêmia como uma forma de incrementar a dieta a base de algas dos organismos.

A importância na quantidade e na qualidade do alimento fornecido pode ser avaliada através do número de filhotes produzidos nos cultivos, uma vez que a dieta pode influenciar

diretamente na capacidade reprodutiva dos indivíduos. Herbert (1978) ao estudar o gênero *Daphnia*, constatou que o número de neonatos produzidos por fêmeas ovígeras depende diretamente de sua alimentação. O número de filhotes, juntamente com a sensibilidade de um organismo a uma substância de referência e o teor de lipídios acumulados são critérios que podem ser adotados para a avaliação da qualidade do cultivo de organismos utilizados em ensaios ecotoxicológicos (Zagatto, 1988).

Em um caranguejo estuarino muito estudado, *Chasmagnathus granulata*, diferentes dietas alteram significativamente as concentrações de glicose, glicogênio e lipídios nos tecidos e na hemolinfa deste crustáceo sendo seus níveis correlacionados positivamente com a concentração de carboidratos da dieta (Kucharski e Da Silva, 1991a). Hernandez-Vergara ***et al.,*** (2003) avaliaram o efeito de diferentes concentrações de lipídios em dietas artificiais oferecidas para o parastacídeo *Cherax quadricarinatus*, e concluíram que os machos investem suas reservas metabólicas para o crescimento, enquanto que as fêmeas, com um alto índice hepatossomático, investem no desenvolvimento gonadal e vitelogênese.

Dutra ***et al.*** (2007c) estudando o lagostim de água doce, *Parastacus brasiliensis*, verificaram que independente da dieta oferecida (rica em carboidratos ou rica em proteínas) mantidos em condições controladas de laboratório (temperatura e fotoperíodo) por 15 dias as marcas metabólicas trazidas de campo pelos animais não são perdidas estando os níveis dos diferentes metabólitos relacionados principalmente ao período reprodutivo. Padrão semelhante foi encontrado por Ferreira ***et al.*** (2005) com as mesmas dietas, porém estudando o anomura de água doce *Aegla platensis.*

2. Justificativa

Os crustáceos são freqüentemente utilizados como bioindicadores e biomonitores em vários sistemas aquáticos, alguns aspectos deste grupo como as estratégias reprodutivas e as respostas comportamentais (mudanças na alimentação, na locomoção ou no comportamento de pré-cópula) podem ser importantes para a interpretação de dados sobre estudos da bioindicação e para o desenvolvimento de estudos ecotoxicológicos (Rinderhagen ***et. al.,*** 2000). Alguns estudos desenvolvidos com crustáceos, especialmente anfípodos, têm demonstrado a importância da determinação de parâmetros bioquímicos como biomarcas em estudos de ecotoxicologia e monitoramento ambiental (Brendonck & Persoone, 1993; Hebel ***et al.***, 1997; Dutra ***et al.***, 2007d).

Tendo em vista a crescente preocupação com as alterações provocadas no ambiente aquático resultante das diversas atividades humanas cresce também, a utilização de

bioindicadores e de testes de toxicidade ou bioensaios para avaliação destes impactos. Vários organismos são utilizados nestes testes, como algas, microcrustáceos, poliquetos, oligoquetos, larvas de insetos e peixes (Plate 1993). Entre os critérios para a seleção destes organismos, destaca-se um amplo conhecimento da distribuição da espécie, localização dentro da estrutura trófica, conhecimento da biologia, hábitos nutricionais e fisiologia, manutenção e cultivo em laboratório (Environmetal Protection Agency-EPA, 1989).

Em Amphipoda, a rápida adaptação às condições de laboratório, com eventos reprodutivos em um mesmo período, desenvolvimento embrionário rápido, elevadas densidades sob as quais são encontrados em seu habitat, a fácil determinação do sexo e o tamanho dos espécimes, facilitam observações de seu ciclo de vida (Krushwitz, 1978; Pennak, 1953; Cooper, 1965; Borowsky, 1991; Duan ***et al.***, 1997). Neste sentido, esta pesquisa visa contribuir para o conhecimento de aspectos do metabolismo energético e da reprodução de *Hyalella castroi* em laboratório, estabelecendo uma dieta adequada para a manutenção desta espécie em cultivo; fornecendo assim, subsídios de cunho bio-ecológico e fisiológico que permitam o uso destes animais autóctones como modelo experimental em estudos futuros de toxicologia e de monitoramento ambiental.

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Aspects of the Energetic Metabolism and Reproduction of the *Hyalella castroi* González, Bond-Buckup & Araujo (Crustacea, Amphipoda, Dogielinotidae) Mantained in Experimental Culture with Different Diets

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Abstract

Was compared the effect of different diets in the energy metabolism (total lipids, cholesterol, proteins and glycogen), in the levels of lipoperoxidation and activity of Na^+/K^+ATPase of *Hyalella castroi*. We also investigated some patterns of the life cycle like survival, formation of reproductive precopulatory mating pairs and number of ovigerous females after 21 days of cultivation with different diets. These crustaceans live in limnetic environments of the plateau (1200m a.s.l) of the state of Rio Grande do Sul, in southern Brazil. The animals were collected in the autumn of 2006 in São José dos Ausentes. In the laboratory, the animals were kept submerged in aquariums, separated by sex, under controlled conditions and were fed *ad libitum*, for 21 days with different diets. At the end of this period, the animals were immediately frozen for determination of biochemical parameters and enzyme. Statistical analysis (ANOVA) revealed significant differences in biochemical composition between the sexes and diets. These diets changed the biochemical patterns of the animals taken from the natural environment, determine a high survival rate, and not improve the reproduction (fecundity and egg quality), these points may be more investigate. In both sexes showed metabolic and reproductive response more adequate when cultivated with diet 1, which was have more carbohydrate (43.19g/100g) and less protein (30.88g/100g) that the diet 2 (carbohydrate = 28.99g/100g and protein = 39-78g/100g).

Introduction

Members of the genus *Hyalella* are common in the Nearctic and Neotropical regions, with 51 described species (González & Watling, 2001). They are found in a variety of freshwater habitats, such as permanent reservoirs, lakes, impoundments, and streams, and often cling to the vegetation, swim in the water, or burrow in the sediment, where they are important members of the benthic fauna (Kruschwitz, 1978; Wellborn, 1995; Grosso & Peralta, 1999). In the Rio Grande do Sul, Brazil, occurred six species of this genus, one this is *Hyalella castroi* González, Bond-Buckup and Araujo (2007) found in the municipally of São José dos Ausentes (1200m a.s.l., in the region of Aparados da Serra), in Vale das Trutas, Rio Grande do Sul (González ***et al.,*** 2005.)

Aquatic organisms exist in a constantly fluctuating habit, with changes in photoperiod, temperature, pH, dissolved organic matter, dissolved oxygen and quality of the food and food supply (Reid and Wood 1976). Organisms must alter of their physiological and biochemical processes in order to cope with these changes.

Carbohydrates are often included in crustacean artificial diets for their protein-sparing effect. By supplying energy to support routine metabolism, a greater quantity of protein is directed towards somatic growth (Shiau and Peng, 1992; Rosas ***et al.***, 2000). However, although glucose is the main sugar circulating in the haemolymph of crustaceans, inclusion of this monosaccharide in the diet of penaeid shrimps was associated with low growth rates, increased mortality and poor protein conversion efficiencies (Abdel-Rahman ***et al.***, 1979; Rosas ***et al.***, 2001; Cuzon ***et al.***, 2001).

In crustaceans, glycogen is stored mainly in the muscles, hepatopancreas, gills, and hemocytes; however, the storage locations vary among different species (Parvathy, 1971; Johnston & Davies, 1972; Herreid & Full, 1988). The stored glycogen is used in the processes of

change, hypoxia and/or anoxia, osmoregulation, growth, different periods during reproduction, and during periods of starvation (Chang & O'Connor, 1983; Kucharski & Da Silva, 1991a; Kucharski & Da Silva, 1991b; Rosa & Nunes, 2003a; Oliveira ***et al.***, 2001 and 2004).

Several studies have evaluated maturation, eye ablation (Sagi ***et al.***, 1997; Wongprasert ***et al.***, 2006), use of hormones (Abdu ***et al.***, 2001), fecundity (King, 1993), and reproductive cycle (Villarreal ***et al.***, 1999; Serrano-Pinto ***et al.***, 2004) of the redclaw crayfish under laboratory conditions. Different diets have been used in these studies. Diet plays an important role in crayfish broodstock condition (Holdich, 2002). Broodstock nutrition is important for reproductive success, because egg and larval production are strongly dependent on the diets offered (Bromage, 1995; Harrison, 1997; García-Ulloa, 2000). Protein is the most critical ingredient in practical diets, because it is expensive and growth responses are affected (Cortés-Jacinto ***et al.***, 2003; Thompson ***et al.***, 2005). According to Harrison (1997), the amount of protein required in broodstock diets for maturation and production of eggs is higher than the level required for growth, because gonad maturation is a process of intense protein synthesis, mainly during vitellogenesis (Abdu ***et al.***, 2000).

The muscle is apparently the main protein-storage location in crustaceans. In decapods, free amino acids in the tissues reach levels ten times higher than those observed in vertebrates. Several studies suggest that these amino acids may participate in osmoregulation, and in the control of cellular volume (Gilles, 1982; Chang and O'Connor, 1983; Schein ***et al.,*** 2004). Other studies have demonstrated a variation in protein content during ovarian development in crustaceans. These variations may result in increased synthesis of several proteins, including enzymes, hormones, and lipoproteins involved in gonad maturation (Yehezkel ***et al.***, 2000; Rosa and Nunes, 2003b).

Lipid concentrations are high in crustaceans, although they have no differentiated adipose tissue but store lipids mainly in muscle tissue and in the hepatopancreas (O'Connor and Gilbert, 1968; Chang and O'Connor, 1983; Herreid and Full, 1988; Kucharski and Da Silva, 1991a; Oliveira ***et al.***, 2006). During periods of high energy demand, such as molting and gametogenesis, large amounts of lipids are mobilized, especially from the hepatopancreas (Kucharski and Da Silva, 1991a; Rosa and Nunes, 2003a; Oliveira ***et al.***, 2006). Herreid and Full (1988) observed that lipid levels in the hepatopancreas were higher than the levels of glycogen.

Malondialdehyde, a breakdown product of lipid endoperoxides, is an expression of lipid peroxidation and has been used with success in aquatic invertebrates as a general indicator of toxicant stress derived from various types of contamination (Zwart ***et al.***, 1999; Livingstone, 2001; Wilhelm Filho ***et al.***, 2001; Timofeyev ***et al.***, 2006). Neuparth ***et al.,*** 2005 described that in *Gammarus locusta* maintained with sediments have high levels of organic matter content present higher levels of lipoperoxidation. Effectively, some authors agree that endogenous variables like nutritional status, age, sex, growth and reproduction influence the peroxidation status of organisms (Viarengo ***et al.,*** 1991; Correia, 2002; Correia ***et al.,*** 2003).

Some studies have reported that the peroxidation of membrane phospholipids induced by reactive oxygen species and/or free radicals leads to alterations in the membrane structure and functions (Halliwell and Gutteridge, 1986; Vercesi ***et al.,*** 1997). These degenerative changes can affect dynamic properties of the membranes such as fluidity and permeability, and consequently the activity of various membrane-associated enzymes (Meccoci ***et al.,*** 1997). Several investigators have reported that lipid peroxidation products disrupt neuronal ion homeostasis by impairing the function of membrane-bound ion-motive ATPases such as Na^+/K^+ATPase (Keller ***et al.,*** 1997; Mark ***et al.,*** 1997).

Dutra ***et al.*** (2007b), working with *H. curvispina* (Shoemaker 1942), suggest that the lipid reserves seem to be an important source of energy used during reproduction, in both males and females; whereas glycogen and proteins may be used during periods of intense activity or intense variation in environmental conditions. This correlation was found too by Chang & O'Connor (1983), Kucharski and Da Silva (1991a), Rosa and Nunes (2003b) and Oliveira ***et al.*** (2006) in their works with other crustaceans. Dutra ***et al.*** (2007b) showed in *H. curvispina* that levels of lipoperoxidation may be related to reproductive behavior, motor and feeding activity, and variation of the photoperiod.

The amphipods species are benthonic organisms, for this they are utilized for toxicity tests and bioassays for evaluation of the water quality of the sediment of the aquatic ecosystem. Recently studies demonstrated that the specie *Corophium volutator* (Crustacea, Amphipoda), tipic of the sediment, is adjusted to expressed of toxicity of the sediment in liminic environments (Gerhardt ***et al.*** 2005). However, the number of the species patronized for the toxicity tests are limited and the major are alloctone organisms, specially the specie *Hyalella azteca*, in contrast with the taxonomic richness of the natural ecosystems in Brazil (Brendonck and Persoone, 1993).

The aim of the present work was to characterize the response of the intermediate metabolism (total lipids, cholesterol, proteins, and glycogen), of the levels of lipoperoxidation (TBARS) and of the activity of Na^+K^+ATPase in *Hyalella castroi* maintained in experimental culture with two different diets. We also investigated some patterns of the life cycle like survival, formation of reproductive couples and number of ovigerous females and juveniles eclosion for patronization of this specie for future use to toxicity tests.

MATERIAL AND METHODS

The animals were cared for in accordance with guidelines such as the Guide for the Care and Use of Laboratory Animal (1996, published by National Academy Press, 2101 Constitution

Ave. NW, Washington, DC 20055, USA) and Brazilian laws. The animal were used with the permission of the Ethic Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (License 06/03423).

Description of the collection place:

The animals were collected between April to June of 2006 (autumn) in a stream in the São José dos Ausentes, RS (28°47'00"S – 49°50'53"W), this place is characterized by little anthropogenic influence. Three periods of collect (April, May and June) permit that were use animals of the same population, but not the same generation, this form we have sure that results are significantly for all population. Animals and macrophytes (*Callitriche rimosa*) were collected by means of fish traps and bottom grabs in same hour of the day.

The animals were transported in cold water (5°C) in insulated containers to the Laboratory of Conservation Physiology of PUCRS (Pontifícia Universidade Católica do Rio Grande do Sul), where they were separated by sex and placed in aerated aquariums for 24 hours without food.

In order to characterize the collect place the following abiotic parameters were measured during months of collect: pH, water temperature and hardness of the water. pH was determined with a portable pHmeter (Quimis/400H), and water temperature with a thermometer of internal scale. The hardness of the water was determined using a classic method of volumetric complexation (Adad, 1982).

Experimental procedure:

After this 24-hour period, the animals were kept submerged in aerated aquariums, in density of 1 animal per liter of water, with an average temperature of 23±1ºC and a photoperiod of 14:10 hours light/dark. The amphipods were divided into two groups, which were fed *ad libitum* in late afternoon, when most of the animals were active, for a period of 21 days. Males

and females stay in the aquariums during 21 days separated with a nylon fabric, but stay in chemical contact, because the water passed by the two parts of aquarium. They were fed one of two diets, the first group (Diet 1) received macrophytes and ration for fishes (ALCOM: fresh shrimp, fish flour, soy protein hydrolizated, corn cream, wheat flour, marine algae flour, dehydrated carrot, leavenings, soy oil, vitamin C, mineral vitaminic supplement, inorganic minerals, additives to pigment and antioxidant BHT), and the second group (Diet 2) received macrophytes and commercial ration for fishes with add spiruline algae (ALCOM: fish flour, soy protein hydrolizated, corn cream, wheat flour, marine algae flour, leavenings, soy oil, vitamin C, mineral vitaminic supplement, inorganic minerals, dehydrated spinach, antioxidant BHT spiruline and prebiotic additive).

The centesimal composition of these rations was determined by ICTA (Instituto de Ciência e Tecnologia dos Alimentos) of the UFRGS (Universidade Federal do Rio Grande do Sul) and showed in Table 1, diet 1 and diet 2 are isocaloric. After 7, 14 and 21 days of experimental culture a group of each diet of each sex was cryoanesthetized, weighed on an electronic balance (± 0.001), and then stored frozen at -80°C until they were used to determine the biochemical parameters.

Reproductive parameters

After of the period of 24 hours, 10 males and 10 females were distributed in each aquarium of the 20 liters in a total of the six aquarium and 60 animals (three aquariums for the diet 1 and three for the diet 2); in this experiment we were permit the physical contact between male and female. The animals were observed every day, during 21 days, and the number of the couples and ovigerous females was quantified (Plaistow, 2003).

Survival and Mortality

The survival and the mortality of the animals during the experimental cultures were registered.

Biochemical Analyses

Metabolites

Metabolic determination for *H. castroi* was done in total homogenates of three pools of twelve males and twelve females each. One pool was used for determination of glycogen and proteins, the second pool for quantification of lipids and cholesterol, and the third pool for quantification of lipoperoxidation levels. Metabolic parameters were determined in quintuplicate by used spectrophotometric methods.

a. Glycogen was extracted from tissues following the method described by Van Handel 1965, and glycogen levels in the animals were determined as glucose equivalent, after acidic hydrolysis (HCl) and neutralization (Na_2CO_3), following the method of Geary ***et al.*** 1981. Glucose was quantified using a Biodiagnostic kit (glucose-oxidase). Results are presented as in mmol/g of animal.

b. Proteins were quantified as described by Lowry ***et al.*** 1951, with bovine albumin (Sigma Co.) as the standard. Results are expressed mg/ml of homogenate.

c. Lipids were extracted from tissue homogenized with an Omni Mixer Homogenizer in a 2:1 (v/v) chloroform-methanol solution, according to Folch ***et al.*** 1957. Total lipids in this homogenate were determined by the sulfophosphovanillin method (Meyer and Walter 1980). This method consists of oxidizing cellular lipids to small fragments after chemical digestion with hot concentrated sulfuric acid. After the addition of a solution of vanillin and phosphoric acid, a red complex is formed witch is measured with spectrophometer (530nm). The levels of total cholesterol were measured by the reactions of cholesterol esterase, cholesterol oxidase and peroxidase enzymes (Labtest Kit/Liquiform). Results are expressed as mg/g of animals.

d. Lipoperoxidation levels were quantified by the method of Buege and Aust (1978) by measuring reactive substances to Thiobarbituric Acid (TBA-RS), using the extraction method of Llesuy ***et al.*** (1985). Results are expressed in nmol of TBARS/mg of protein.

Activity of Na^+/K^+-ATPase

The membrane was extracted from five animals, according to Barnes (1993). The pool were homogenized (10% W/V) in cold buffer Tris (40mM) and phenylmethylsulfonyl fluoride (1 mM; from Sigma, St. Louis, MO) with pH adjusted to 7.40. The homogenate was centrifuged at 10000X*g* at 4°C, and the supernatant was collected and centrifuged at 40.000X*g* (4°C). The pellet was resuspended in the same buffer and centrifuged again at 40.000X*g* (4°C). This last supernatant was then used as the source of Na^+/K^+ATPase. Na^+/K^+ATPase activity was measured according to the method described by Esmann (1988) adjustment by Dutra ***et al.*** (2007d). Incubation medium A contained ATP (5 mM; from Sigma), NaCl (60 mM), KCl (10 mM) and MgCl (40mM), with the pH adjusted to 7.40. In the incubation medium B, KCl was replaced by ouabain (1 mM; from Sigma). Aliquots of homogenate were incubated at 30°C in both mediums A and B, for 30 min with the equivalent of 10 mg of the proteins. The enzymatic reaction was stopped by addition of 10% trichloroacetic acid. The inorganic phosphate released was determined using the method of Chan (1986), in a spectrophotometer at 630 nm. Any difference in phosphate concentration between medium A and B was attributed to Na^+/K^+ATPase activity. All determinations were done in quadruplicate. Results are expressed in μmol of the P*i*.mg of $protein^{-1}.min^{-1}$.

Statistical Analysis

The results are expressed as mean ± standard error. For statistical analysis of the different periods of experimental culture, a one-way ANOVA test was used, followed by a Bonferroni test. For comparisons between different diets and sexes, a two-way ANOVA was used. The

comparisons of experimental culture with dates of the natural environmental and the number of ovigerous females between different diets were did with Student's T test. All the metabolic parameters were homogeneous (Levene test), and were normally distributed (Kolmogorov-Smirnov test). The significance level adopted was 5%. All the tests were done with the program Statistical Package for the Social Sciences (SPSS- 11.5) for Windows.

Results

Abiotic Conditions Analyses

The environmental and experimental culture abiotic factors are present in Table 2. When compared the temperature of the environmental with the experimental culture occurred a significant difference, because the temperature of the collected local was lowest in relation of the experimental culture temperature. The pH and hardness of water were constant in both situations, in environment pH was 7.09 ± 0.21 and in experimental culture 7.00 ± 0.40, already the hardness of the water in environment was 1.12 ± 0.52 ppm of $CaCO_3$ and in experimental culture 0.98 ± 0.46 ppm of $CaCO_3$.

Reproductive parameters

The number of the couples (paired formed) and the ovigerous females feeding with diet 1 and diet 2 are present in Table 3. The amphipods feeding with diet 1 paired 22 % more than the animal maintained with the diet 2. The females feeding with diet 1 showed a higher indice of ovigerous females than the females maintained with the diet 2, however in both cultures, the maximum period of the females showed eggs was 4 days, after this period not was observed juvenis eclosion in aquariums. Although, the number of ovigerous females was low in relation the number of the couples formed in both diets.

Survival rate

The survival of the males and females feeding with diet 1 and with diet 2 are present in Table 4. We verified that during period of experimental culture males and females feeding with diet 1 showed survival rate 11.20% and 13.40%, respectively, higher than the animal feeding with diet 2. However, there was no significant difference between the sexes feeding with the same diet in none of type of diet. The survival rate in diet 1 to vary to 94.5%, until 98.20% and in diet 2 the rate to vary of 83.64% until 89.10%, in both sexes considering the three experiments.

Metabolic parameters

Glycogen:

Figure 1A shows the glycogen concentration in males and females in the natural environmental and cultivation with diet 1. Males of *H. castroi* cultivated by 7 days present a glycogen levels higher (1.3 times) than the animals collected in natural environment, this levels continued increasing until 14 days of culture, and after 21 days this polysaccharide was lower than 14 days and environment. Already, in females was observed an increase in glycogen in 7 days of experiment, and this polysaccharide remain high until finish of the experiment (21 days). There was a significant difference in the behavior of glycogen levels between males and females feeding with diet 1 ($p<0.05$).

Glycogen content in males and females of *H. castroi* in the natural environmental and maintained with diet 2 showed in the Figure 1B. Males feeding with diet 2 showed a peak (1.7 times) of glycogen in 7 days of experiment, and these levels decreased gradually until 21 days of cultivation. In females was observed in 7 days a decrease of 20% in the content of glycogen, after 14 days of culture this polysaccharide increase (30%), and in the finish experiment the glycogen levels returned to the values of 7 days. There was a significant difference in the behavior of glycogen levels between males and females feeding with diet 2 ($p<0.05$).

There was a significant difference in the levels of glycogen during 21 days of cultivation between males submitted to the different diets ($p<0.05$); the same pattern was found to females feeding with different diets ($p<0.05$).

Proteins:

Protein concentrations in males and females in the environment and feeding with diet 1 are showed in Figure 2A. Males showed after seven days of cultivation an increase of 2 times in levels of proteins, in 14 days was found a decrease of 40% in this values, and in the end of the experiment (21 days) the content of proteins returned the levels of 7 days. Already, in females the proteins were higher after 7 days of cultivation, although gradually decreased until 21 days reaching levels lower than the environment and 7 days of culture. There was a significant difference between total protein content in females and males maintained with the diet 1($p<0.05$).

The levels of total protein in males and females in the environment and feeding with diet 2 are showed in Figure 2B. Males feeding with the diet 2 by 7 days showed values of total proteins 2.2 times highest than the males of environment; these levels remained constant until 14 days and decrease after 21 days of experiment returned the values of the animals in natural environment. In females, the levels of total proteins after 7 days of the diet 2 were similar to the amphipods collected in natural environment, although these levels decreased significantly during the time of culture (14 and 21 days). There was a significant difference between total proteins content between males and females maintained with the diet 2 ($p<0.05$).

When was compared different diets we observed a significant difference of the behavior of the total proteins during 21 days of the experiment, the same response was observed in females ($p<0.05$).

Total Lipids:

The concentrations of total lipids in males and females in the environment and feeding with diet 1 are shown in Figure 3A. Levels of this metabolite in males were lowest ($p<0.05$) after seven days of culture and maintained stable until 14 days of experiment, after 21 days of feeding this levels showed a decrease of approximately 2.2 times. Females present values of lipids lowest than the animals of environment after 7 days, although after 14 days this levels was increase, and these animals shows a new decreasing in their lipidic reserve in the end of the experiment (21 days).There was a significant difference between total lipid content of males and females maintained in laboratory feeding with diet 1.

Total lipids content in males and females of *H. castroi* in the environmental and maintained with diet 2 showed in the Figure 3B. The males collected in natural environmental present a total lipids levels approximately 2.6 times highest ($p<0.05$) than the animals fed with the diet 2 by seven days, while the females maintained by the same period no shows a significantly difference in this metabolite ($p>0.05$). We observed that in males the level was decreasing gradually until minimum values in 21 days of culture. Already, the females shows a peak after 14 days of experiment, their levels are 3.7 times highest in relation the females cultivate by 7 days, the levels of total lipids decreasing (2.2 times) in the end of the experimental period (21 days), although these levels were highest. There was a significant difference in the behavior of total lipids levels between males and females feeding with diet 2 ($p<0.05$). There was no significant difference in the levels of total lipids in males submitted to the different diets ($p>0.05$); but the females showed a significant difference between the group that received diet 1 and diet 2 ($p<0.05$).

Cholesterol:

The concentrations of cholesterol in males and females in the environment and feeding with diet 1 are shown in Figure 4A. The males and females of the environment present a content

of total cholesterol 12.7 and 3.7 times, respectively, higher than the animals maintained in laboratory with diet 1 by 7 days. When the levels of this metabolite are compared between the experimental periods, we observed that in males the level was highest after 14 days of experiment, but with 21 days of feeding they shows a turn back to the levels showed after seven days of culture. The same response was observed in females during the experiment cultivation. There was no significant difference between total cholesterol content of males and females maintained in laboratory feeding with diet 1 ($p>0.05$).

The cholesterol content in males and females of *H. castroi* in the environmental and maintained with diet 2 showed in the Figure 4B. The males and females collected in natural environmental present a cholesterol levels approximately 13.3 and 1.8 times highest than the animals fed with the diet 2 by seven days ($p<0.05$). When the levels of this metabolite are compared during the periods of cultivation we verified that males no shows significant difference between the levels of cholesterol ($p>0.05$). Already, in females after 14 days in experimental culture was observed levels 1.8 times highest than of seven days, and cholesterol decreasing (7.0 times) in the end of the period (21 days). There was a significant difference in the behavior of cholesterol levels between males and females feeding with diet 2 ($p<0.05$).

There was no significant difference in the levels of cholesterol in males submitted to the different diets ($p>0.05$); but the females showed a significant difference between the group that received diet 1 and diet 2 ($p<0.05$).

Levels of Lipoperoxidation

Figure 5A shows the levels of lipoperoxidation in males and females in the environmental and maintained with diet 1. Males and females feeding to 7 days were present values of lipoperoxidation lower that animals of the natural environment. After 14 days of experiment the levels of lipoperoxidation decrease in males and increase in females. Already, in the 21 days of

culture this response was inverted, when in males the levels increased and in females the levels decreased. There was a significant difference in the behavior of lipoperoxidation levels between males and females feeding with diet 1 ($p<0.05$).

The levels of lipoperoxidation in males and females of *H. castroi* in the environmental and maintained with diet 2 showed in the Figure 5B. The males and females collected in natural environmental present a lipoperoxidation levels approximately 5.5 and 2.3 times higher, respectively, than the animals fed with the diet 2 by seven days. When the levels of TBARS are compared between the experimental periods, we observed after 14 days, that in males and females the level was increase 2.4 and 2.0 times, respectively, and increase again after 21 days in both sexes. There was no significant difference in the levels of lipoperoxidation between males and females submitted to the diet 2 in the different times of culture ($p>0.05$). There was a significant difference in the levels of lipoperoxidation in males submitted to the different diets ($p<0.05$); the same pattern showed by males was found when was made the comparison between females feeding with diet 1 and diet 2 ($p<0.05$).

Na^+/K^+ ATPase activity

Na^+/K^+ATPase activity in males and females in the environment and feeding with diet 1 was showed in Figure 6A. The males after 7 days of diet 1 present value of Na^+/K^+ATPase activity lowest (1.7 times) that the animals collected in natural environment, in the 14 days the levels showed an increase and returned the initial values in 21 days of culture. Already in females was observed an increase after seven days of culture with diet 1, the values were similar in 14 days, and this activity increased in the finish of the experiment (21 days).

Figure 6B shows the levels of activity of Na^+/K^+ATPase in males and females in the environment and feeding with diet 2. The males collected in the environment showed values of activity of Na^+/K^+ATPase 1.3 times highest than the males feeding with the diet 2 by 7 days, after

14 days of experimental culture was observed an increase of the 2.4 times in this activity, in 21 days the Na^+/K^+ATPase remained elevated in relation of 7 days. Already, in females were verified an increase of 8.1 times in seven days of culture, the levels showed a gradually decrease until 21 days of experiment, however this activity was higher in relation 7 days and environment. There was a significant difference between levels of activity of Na^+/K^+ATPase of males and females maintained with the diet 2 ($p<0.05$). There was a significant difference of the Na^+/K^+ATPase activities values between males maintained with different diets; the same pattern showed by males was found when was made the comparison between females feeding with diet 1 and diet 2 ($p<0.05$).

Discussion

Integral to the development of a diet for any species included the identification of their requirements of protein, lipid and energy. Protein is required to provide the fundamental units amino acids for growth, while dietary lipid provides both essential fatty acids and other some forms of the energy needed for the metabolic processes of growth (D'Abramo ***et al.***, 1997). Further energy can also be derived from the metabolism of protein and some dietary carbohydrates. Most studies on nutrition of freshwater crustaceans are focused on proteins (Cortés-Jacinto ***et al.*** 2003, 2004; Thompson ***et al.***. 2004) and scarce information is available on carbohydrates and lipids (Hernandez-Vergara ***et al.*** 2003). These two nutrients have important roles not only as energy sources but also in development and reproduction of crustaceans. The accumulation of energy reserves in species of crustaceans dependent upon unstable food resources has been reported by several authors (Lee ***et al.***, 1971; Griffiths, 1977; Oliveira ***et al.***, 2003; Rosa and Nunes, 2003b).

In this work diets (1 or 2) determined different responses to glycogen, total proteins, lipoperoxidation levels and Na^+/K^+ATPase activity in both sexes these amphipods, and total lipids and cholesterol levels only in females. The levels of glycogen and total lipids verified in these amphipods, in both diets were similar to levels observed in other crustaceans feeding diet rich in proteins (Kucharski and Da Silva, 1991; Ferreira ***et al.***, 2005). The results may suggest that amphipods present a major content of proteins in natural diet.

Dutra ***et al.*** (2007c) reported that *H. castroi* explore the sediment predominantly, where it finds more organic matter of animal origin, in which it can burrow, these activities require different levels of energy consumption, as well as allowing consumption of organic substances, although her feeding habits is unknown. For *H. azteca*, Hargrave (1970) reported that it is an omnivorous deposit feeder, primarily feeding on algae and bacteria associated with the sediments and aquatic macrophytes. It has been recorded feeding on dead animal and plant matter (Cooper 1965). Byrén ***et al.*** (2002) showed in two species of the amphipods *Monoporeia affinis* and *Pontoporeia femorata* that the settled phytoplankton and detrital organic matter are considered their main found source but bacteria, meiofauna and temporary meiofauna are also included in the diet. Casset ***et al.*** (2001), studying *Hyalella curvispina* in a river in Argentina, suggest that this amphipod is herbivorous, feeding mainly on the phytobenthos and occasionally on sediment.

In the present work we verified that animals that received diet 1 that have less protein (30.88%) and more carbohydrate (43.19%) than diet 2 (protein 39.78% and carbohydrate 28.99%) present a higher rate of survival and number of couples. Although, we was observed in both diets a lower number of ovigerous females and fertility (number of juveniles liberated by females). In present work was not verified juveniles liberated by ovigerous females. These facts can be related with a significant decrease in proteins reserves verified in females in both diets and/or quantity of the proteins in diets (1 and 2) and /or higher temperature of the water in

aquariums. Castiglioni and Buckup (submitted) studying the reproductive strategies in *H. castroi* in laboratory conditions (19°C and 12/12hours light/dark) showed a fecundity of the 7 to 42 eggs and a fertility of the 16 to 36 juveniles per females. In this study developed to Castiglioni and Bond-Buckup (2007) the animals were feed with macrophyte more fish food with 43% of the protein.

The importance of the quantity and quality of the food provided can be evaluated through the offspring produced in the cultures, because the diet can directly influence the reproductive capacity of individuals. Herbert (1978), studying the genus *Daphnia*, observed that the number of neonates produced by ovigerous females depends directly on the food ingestion. The number of juveniles, together with the sensitivity of the organism to some reference substance and the course of accumulation of lipids, can be adopted as criteria to evaluate the quality of the cultures of organisms used in ecotoxicological bioassays (Zagatto, 1988).

According Goimier ***et al.*** (2006) studying *Litopenaeus setiferus* verified that in diet that have protein in excess could produce a stress provoking loss of immune control, melanization and loss of sperm quality change reproductive success. Other studies have been demonstrated that protein in excess (45% of protein) provokes a reduction in growth rate in several shrimp species (García ***et al.***, 1998; Sheen and Huang, 1998; Kureshy and Davis, 2002; Pascual ***et al.***, 2004). A difference in protein catabolism and the stress provoked by changes in internal ammonia concentration was related to the reduction in growth rate of shrimp fed protein in excess (Rosas ***et al.***, 1995, 1996, 2001; Taboada ***et al.***, 1998).

In general, the lowest levels of glycogen in females and males feeding with diet 2 in relation the animals feeding with diet 1 can be explain by lower percentage of carbohydrates contained in this diet (diet 2). Where diet 1 present 43.19g/100g of carbohydrates and diet 2

present 28.99g/100g. Studies developed with other crustaceans, like *C. granulata* (Kucharski and Da Silva, 1991), *A. platensis* (Ferreira ***et al.***, 2005) and *P. brasiliensis* (Dutra ***et al.***, 2007c) shows that increase of the quantity of carbohydrate in diet change the homeostasis of glycogen when authors compared with the animals that received a high-protein diet, determining an increase in levels of this polysaccharide.

In crustaceans as in other animals, the regular functioning of organs such as the brain and muscles requires a constant supply of blood-borne glucose. Haemolymph glucose levels fluctuate in vivo depending on a range of endogenous and exogenous influences including diurnal rhythms (Hall and Van Ham, 1998), moult stage (Telford, 1968b; Chang and O'Connor, 1983), nutritional status (Meenakshi and Scheer, 1961), temperature, captivity, handling, and air exposure (Telford, 1968a; Santos and Keller, 1993a; Speed ***et al.***, 2001). Such variations reflect changes in the balance between anabolic (gluconeogenesis, glycogenesis) and catabolic (glycogenolysis, glycolysis) processes (Hall and Van Ham, 1998), and rates of uptake from the gastrointestinal tract (Verri ***et al.***, 2001).

In this work despite of the animals were given access to food, was observed that males and females feed with diet 2 and males feed with diet 1 not maintained theirs glycogen and lipids reserves during cultivation (21 days). Although, the males and females were maintained in aquariums separated with a nylon tissue, but stay in chemical contact because the water passed by two parts of aquarium, the ovaries of the females matured. According Castiglioni and Buckup (submitted) the winter are the reproductive peak of this species and autumn are season that animal prepared for reproduction (synthesis of the gametes and vittelin, and reproductive behavior); this last season (autumn) was a period that the animals were collected.

Plaistow ***et al.*** (2003) observed that lipid and glycogen reserves of paired males of the *Gammarus pulex* (Linnaeus 1758) are both significantly higher than those of unpaired males, and

indicated that such a cost is more likely to result from pair formation than from the cost of carrying the female, as had previously been assumed. Males benefit from precopulatory mate guarding by maximizing their chances of fertilizing females' eggs once they become receptive. However, the optimal time a male spends guarding each female will depend upon the costs associated with precopulatory mate guarding. Finally, precopulatory mate guarding may be energetically costly (Robinson and Doyle 1985; Elwood and Dick 1990; Sparkes ***et al.*** 1996; Jormalainen ***et al.*** 2001). Robinson and Doyle (1985) showed that in *Gammarus lawrencianus* the feeding rates of males were reduced while they were in precopula, but female feeding rates were unaffected. However, Sparkes ***et al.***. (1996) showed that in the isopod *Lirceus fontinalis* (Rafinesque-Schmaltz 1820), precopulatory mate guarding was associated with only a short-term (<36 h) reduction in glycogen reserves, and there was no reduction when pairs were given access to food.

In this work, male feeding with diet 1 and diet 2 maintained their proteins reserves similar to natural environment after 21 days of the cultivation. Although, female feeding with both diets showed a decrease in these reserves, however females that received diet 1 showed higher decrease in protein levels. A major cost of energy to reproduction activity, principally, synthesis of vittelin in females can be explained the lower quantity of protein, especially in diet 1.

Protein is an essential for tissue growth and maintenance, is an expensive component of formulated diets (Cortés-Jacinto ***et al.***, 2003; Thompson ***et al.***, 2005). When insufficient energy is available in a diet from non-protein sources, protein may be catabolized to meet the energy requirements at the cost of nutrient supply somatic growth (Capuzzo and Lancaster, 1979; Sedgwick, 1979). The most efficient diets contain sufficient non-protein energy sources (lipid and carbohydrate) that are metabolized preferentially to protein to meet general energy requirements, leaving an organism to direct the maximum level of available dietary protein into

growth (Sedgwick, 1979; Bautista, 1986). Barclay ***et al.*** (1983), working with *Penaeus esculentus*, showed that during the period of starvation the abdominal muscle makes the largest contribution of protein to energy metabolism, where small changes in this tissue are sufficient to make a substantial contribution to the overall animal maintenance.

The levels of proteins present in diet 1 (30.88g/100g) and diet 2 (39.78g/100g) may be determine the low number of ovigerous females and low fertility. Rodríguez-González ***et al.*** (2006) showed that a dietary protein level of about 30% maximizes the size of the spawning population of *Cherax quadricarinatus*. The authors suggests that such protein level is optimal to meet nutritional requirements for reproductive females, while other studies determining optimal dietary protein requirement for spawning female redclaw, *Procambarus clarkii* (27% crude protein) (Cortés-Jacinto ***et al.***, 2004). The different protein levels used in their experiment yielded a fecundity value (8.5–9.2 eggs/g) similar to that reported by Yeh and Rouse (1994) (7.8–10 egg/g female). Broodstock nutrition is important for reproductive success, because egg and larval production are strongly dependent on the diets offered (Bromage, 1995; Harrison, 1997; García-Ulloa, 2000; Rodríguez-González, 2001). According to Harrison (1997), the amount of protein required in broodstock diets for maturation and production of eggs is higher than the level required for grow out, because gonad maturation is a process of intense protein synthesis, mainly during vitellogenesis (Harrison, 1990; Abdu ***et al.***, 2000; García-Guerrero ***et al.***, 2003).

In the present work the levels of total lipids and cholesterol of the animals feeding with the diet 1 showed a tendency of decrease in the beginning of the experiment and maintained stable until the end of the culture in males and females; already in both sexes feeding with diet 2 the response is biphasic, in the first moment this reserves decrease, but with the continuation of culture was happen a increase in levels of these metabolites.

Dutra ***et al.*** (2007a) described that in *H. castroi*, that proteins, lipids and cholesterol were depleted during precopula and copula (winter) because *H. castroi*, like other crustaceans, produce large eggs. Egg size is related to maternal investment, mainly the lipid metabolism (Rosa and Nunes 2003 a and b). Lipids are the main source of energy throughout embryonic development, and the amount of lipids is generally correlated with the size of eggs and the time interval between spawning and hatching (Petersen and Anger 1997; Rainuzzo ***et al.*** 1997). Cholesterol is a vital component of cell membranes and is the precursor of bile acids, steroids, and molting hormones. It is reported to be an essential nutrient for growth and survival of all crustacean species (Abdel-Rahman ***et al.***, 1979).

The two diets determined different response in levels of the Na^+/K^+ATPase activities in males and females and after 7 days of experimental culture already were possibly to observe change in the environmental pattern for this enzyme In both diets, the females always showed values of the Na^+/K^+ATPase activity higher that males, this fate can be related with more intense degradation of the glycogen and total lipids. The animals feeding with diet 2 were present levels of activity of Na^+/K^+ATPase higher in relation that animals feeding with diet 1 during the period of the study (21 days).

The fact of the levels of Na^+/K^+ATPase activity was lower in animals feeding with diet 1 maybe can explain by the higher levels of lipoperoxidation. Some works reported that the peroxidation of membrane phospholipids induced by reactive oxygen species and/or free radicals leads to alterations in the membrane structure and functions (Halliwell and Gutteridge, 1986; Vercesi ***et al.*** 1997). These degenerative changes can affect dynamic properties of the membranes such as fluidity and permeability and consequently the activity of various membrane-associated enzymes (Meccoci ***et al.*** 1997). Several investigators have reported that lipid peroxidation

products disrupt neuronal ion homeostasis by impairing the function of membrane-bound ion-motive ATPases such as Na^+/K^+ATPase (Keller ***et al.*** 1997; Mark ***et al.*** 1997).

Dutra ***et al.*** (2007b) showed in females of *H. castroi* a peak of lipoperoxidation in autumn. This season, antecede the peak of reproduction that occurred in winter (Araujo ***et al.*** 2005), the males showed too a peak of lipoperoxidation, during the period of precopulation and copulation (autumn) because they consume more energy for precopulatory mate guarding and carrying females during this period. However, these animals not pared in laboratory, but females showed mature ovaries, we can be suggesting that they maintained the preparation for the reproductive period.

In conclusion, our results showed that these diets changed the biochemical patterns of the animals taken from the natural environment and but not can be improve the reproduction (fertility and egg quality), these points may be more investigate. In both sexes showed metabolic response more adequate when cultivated with diet 1, which was have more carbohydrate, less protein and major quantity of protein of animal origin.

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Captions to Tables:

Table 1: Centesimal composition of the diets 1 and diet 2.

Table 2: Water temperature (°C), pH, and Hardness of water (ppm of $CaCO_3$) in natural environmental and experimental conditions. The results are expressed as mean ± standard error of the mean.

Table 3: Number of the couples and ovigerous females of the *Hyalella castroi* maintained in experimental culture with different diets.

Table 4: Indices of the survival of the males and females of the *Hyalella castroi* maintained in experimental culture with different diets.

Captions to Figures:

Figure 1: Concentration of glycogen of *Hyalella castroi* in environment and maintained in experimental culture. (A) Males and females feeding with diet 1; (B) Males and females feeding with diet 2. Columns represent the mean, and bars represent the standard error of the mean. Results are expressed in mmol/g. The same letter represents a significant difference between the days of culture or environment. # a significant difference between sexes maintained with the same diets. **&** represents significant difference between males feeding with different diets. * represents significant difference between females feeding with different diets.

Figure 2: Concentration of total proteins of *Hyalella castroi* in environment and maintained in experimental culture. (A) Males and females feeding with diet 1; (B) Males and females feeding with diet 2.Columns represent the mean, and bars represent the standard error of the mean. Results are expressed in mg/ml. The same letter represents a significant difference between the days of culture or environment. **#** a significant difference between sexes maintained with the same diets. **&** represents significant difference between males feeding with different diets. ***** represents significant difference between females feeding with different diets.

Figure 3: Concentration of total lipids of *Hyalella castroi* in environment and maintained in experimental culture. (A) Males and females feeding with diet 1; (B) Males and females feeding with diet 2. Columns represent the mean, and bars represent the standard error of the mean. Results are expressed in mg/g. The same letter represents a significant difference between the days of culture or environment. **#** a significant difference between sexes maintained with the same diets. ***** represents significant difference between females feeding with different diets.

Figure 4: Concentration of cholesterol of *Hyalella castroi* in environment and maintained in experimental culture. (A) Males and females feeding with diet 1; (B) Males and females feeding with diet 2. Columns represent the mean, and bars represent the standard error of the mean. Results are expressed in mg/g. The same letter represents a significant difference between the days of culture or environment. **#** a significant difference between sexes maintained with the same diets. ***** represents significant difference between females feeding with different diets.

Figure 5: Levels of lipoperoxidation of *Hyalella castroi* in environment and maintained in experimental culture. (A) Males and females feeding with diet 1; (B) Males and females feeding with diet 2. Columns represent the mean, and bars represent the standard error of the mean. Results are expressed in nmol of TBARS/mg of protein. The same letter represents a significant difference between the days of culture or environment. **#** a significant difference between sexes maintained with the same diets. **&** represents significant difference between males feeding with different diets. ***** represents significant difference between females feeding with different diets.

Figure 6: Activity of Na^+/K^+ATPase of *Hyalella castroi* in environment and maintained in experimental culture. (A) Males and females feeding with diet 1; (B) Males and females feeding with diet 2. Columns represent the mean, and bars represent the standard error of the mean. Results are expressed in μmol of Pi/min. mg of protein. The same letter represents a significant difference between the days of culture or environment. **#** a significant difference between sexes maintained with the same diets. **&** represents significant difference between males feeding with different diets. ***** represents significant difference between females feeding with different diets.

Table 1

Compound	Diet 1 Macrophyte and Ration 1	Diet 2 Macrophyte and Ration 2
Water content (g/100g)	5.30	7.26
Ashes (g/100g)	11.02	14.15
Protein (g/100g)	30.88	39.78
Fat (g/100g)	6.19	4.99
Fiber (g/100g)	3.59	4.83
Carbohydrates (g/100g)	43.19	28.99
Total Caloric Value (Kcal/100g)	351.59	319.99

Table 2

Abiotic Factors	Environmental	Experimental Culture
Temperature	16.40± 0.35	23.00 ± 1.00
pH	7.09 ± 0.21	7.00 ± 1.00
Hardness of Water	1.12 ± 0.52	0.98 ± 0.65

Table 3

Days	Couples Diet 1	Couples Diet 2	Ovigerous Females Diet 1	Ovigerous Females Diet 2
1	8	10	0	0
2	10	9	0	0
3	10	9	0	0
4	2	2	0	1
5	2	2	0	1
6	3	2	0	1
7	3	2	0	1
8	2	0	2	0
9	2	1	2	0
10	2	1	2	0
11	2	1	1	0
12	3	1	0	0
13	2	1	0	0
14	2	3	0	0
15	4	3	0	0
16	5	3	0	0
17	3	4	0	0
18	3	1	0	0
19	3	2	0	0
20	3	3	0	0
21	3	3	0	0
Total	77	63	2	1

Table 4

	1º Culture	2º Culture	3º Culture	Mean
Males –Diet 1	98.20%	94.55%	96.36%	96.37%
Females –Diet 1	98.20%	96.36%	98.20%	97.59%
Males –Diet 2	83.64%	89.10%	85.45%	86.06%
Females –Diet 2	87.27%	85.45%	85.45%	86.06%

Figure 1

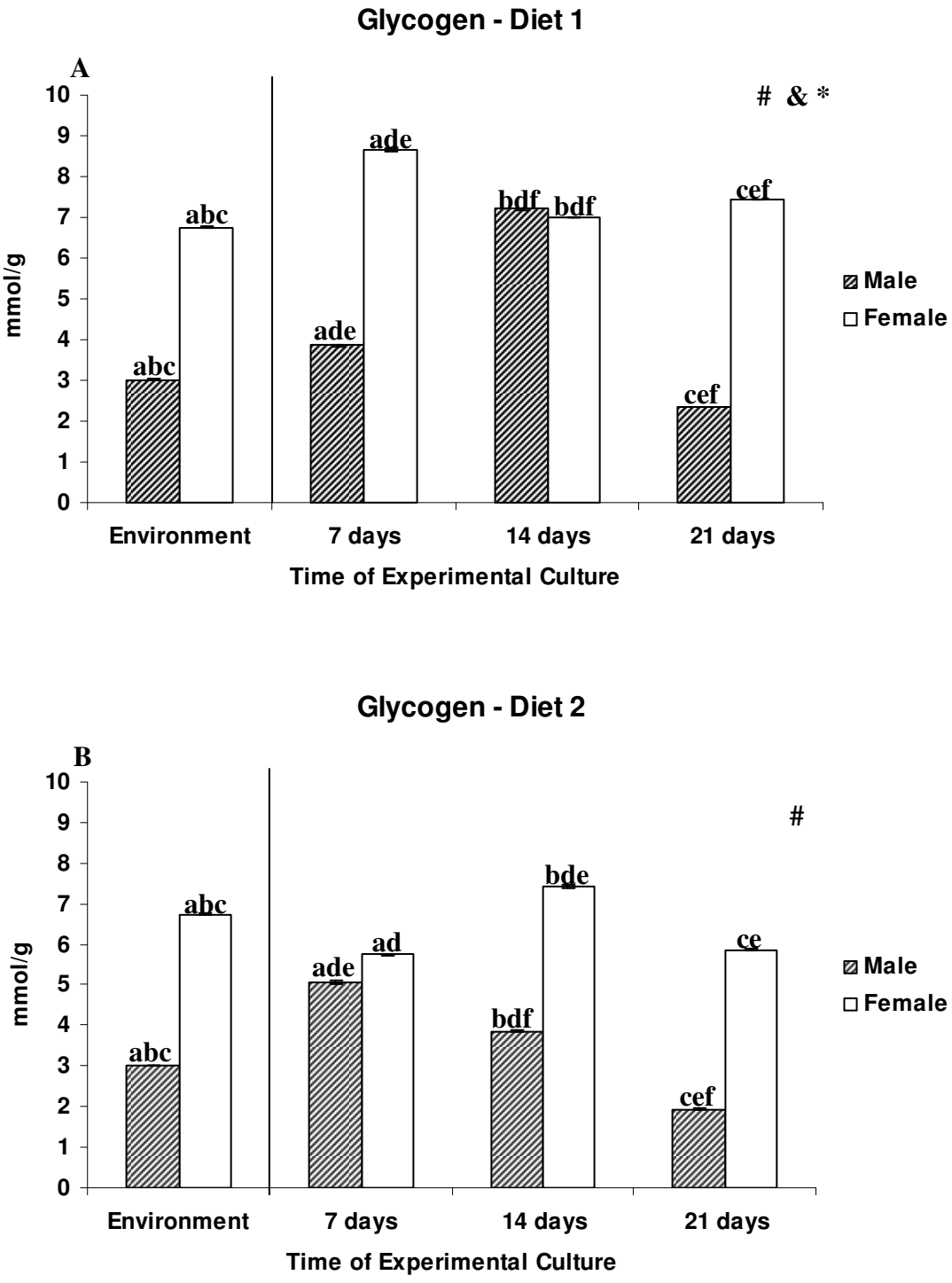

Glycogen - Diet 1
A
& *
mmol/g
abc
abc
ade
ade
bdf
bdf
cef
cef
Male
Female
Environment
7 days
14 days
21 days
Time of Experimental Culture
Glycogen - Diet 2
B
#
mmol/g
abc
abc
ade
ad
bdf
bde
cef
ce
Male
Female
Environment
7 days
14 days
21 days
Time of Experimental Culture

Figure 2

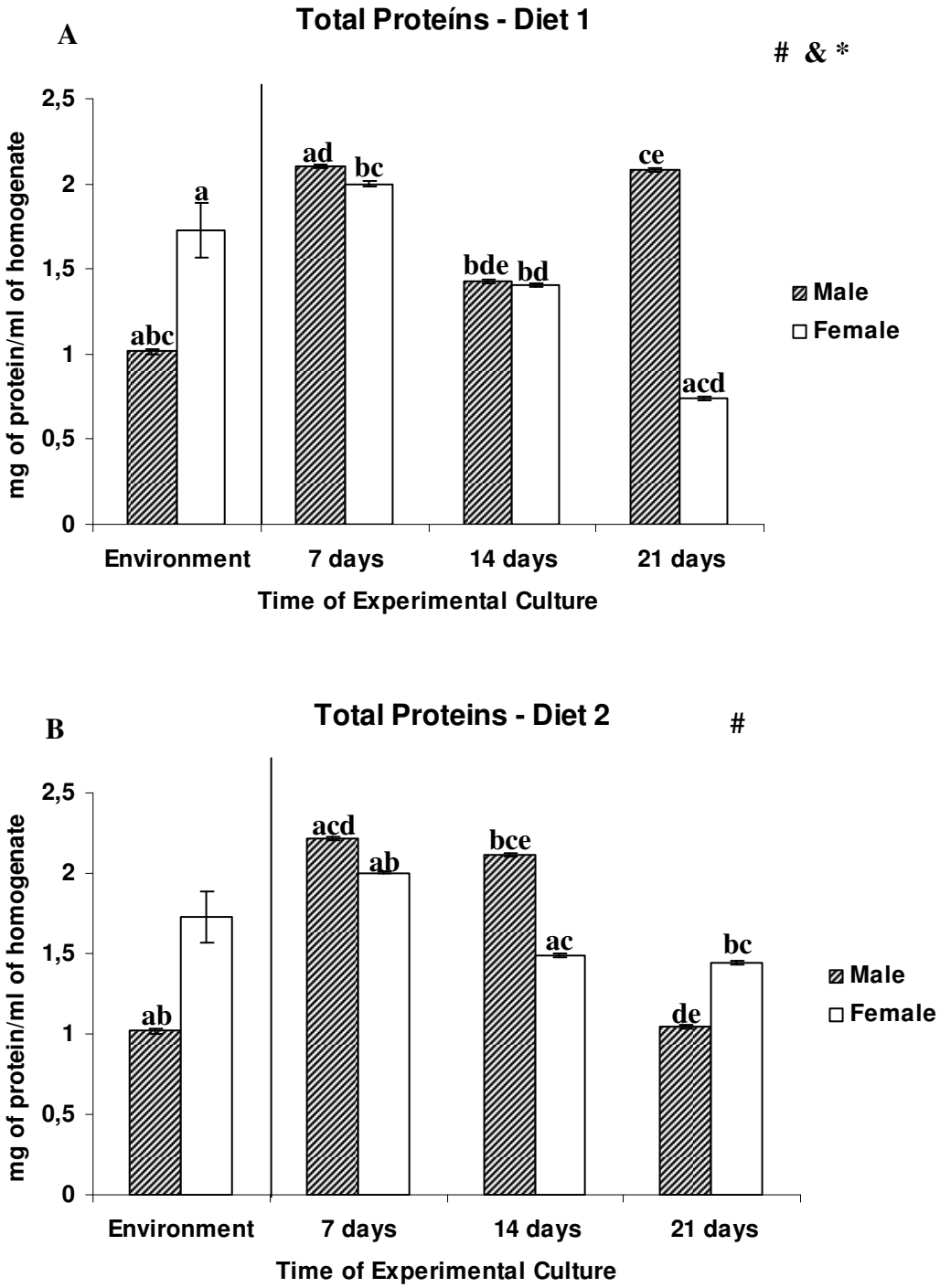

A
Total Proteíns - Diet 1
& *
mg of protein/ml of homogenate
2,5
2
1,5
1
0,5
0
abc
a
ad
bc
bde
bd
ce
acd
Male
Female
Environment
7 days
14 days
21 days
Time of Experimental Culture
B
Total Proteins - Diet 2
#
mg of protein/ml of homogenate
ab
acd
ab
bce
ac
de
bc
Male
Female
Environment
7 days
14 days
21 days
Time of Experimental Culture

Figure 3

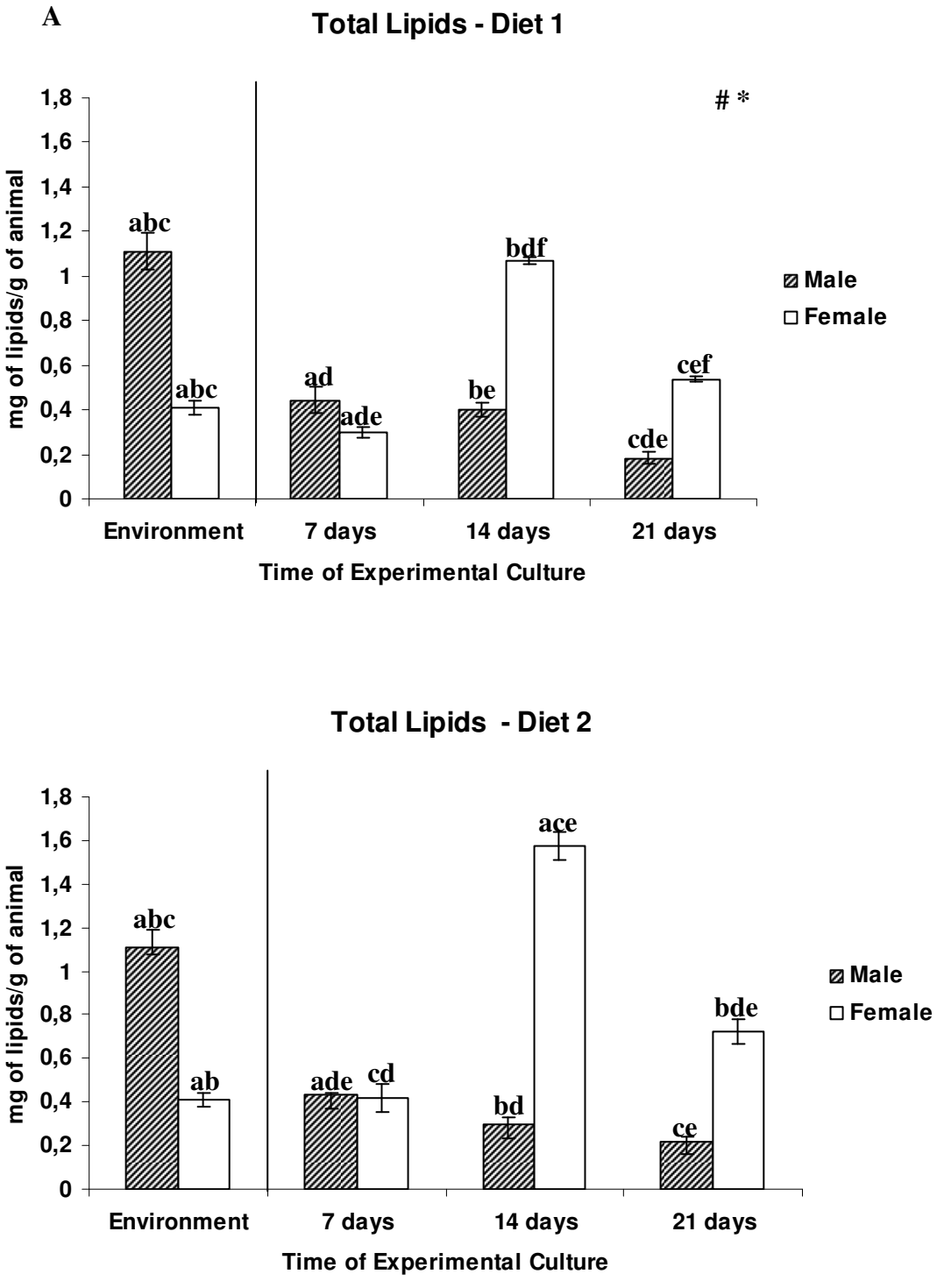
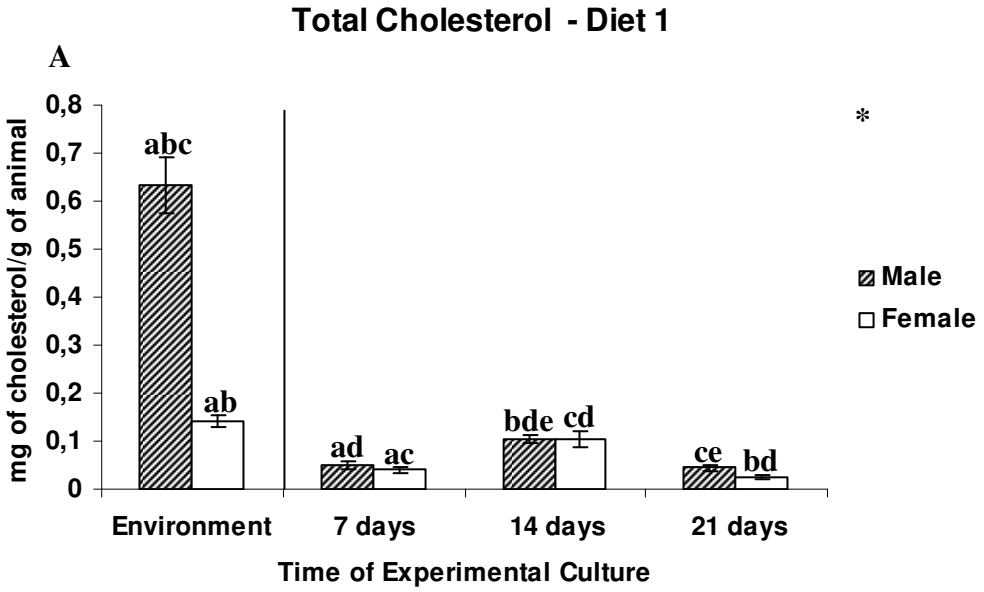

A
Total Lipids - Diet 1
*
mg of lipids/g of animal
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
abc
abc
ad
ade
be
bdf
cde
cef
Male
Female
Environment
7 days
14 days
21 days
Time of Experimental Culture
Total Lipids - Diet 2
mg of lipids/g of animal
abc
ab
ade
cd
bd
ace
ce
bde
Male
Female
Environment
7 days
14 days
21 days
Time of Experimental Culture

Figure 4


Total Cholesterol - Diet 1
A
mg of cholesterol/g of animal
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
abc
ab
ad
ac
bde
cd
ce
bd
*
Male
Female
Environment
7 days
14 days
21 days
Time of Experimental Culture

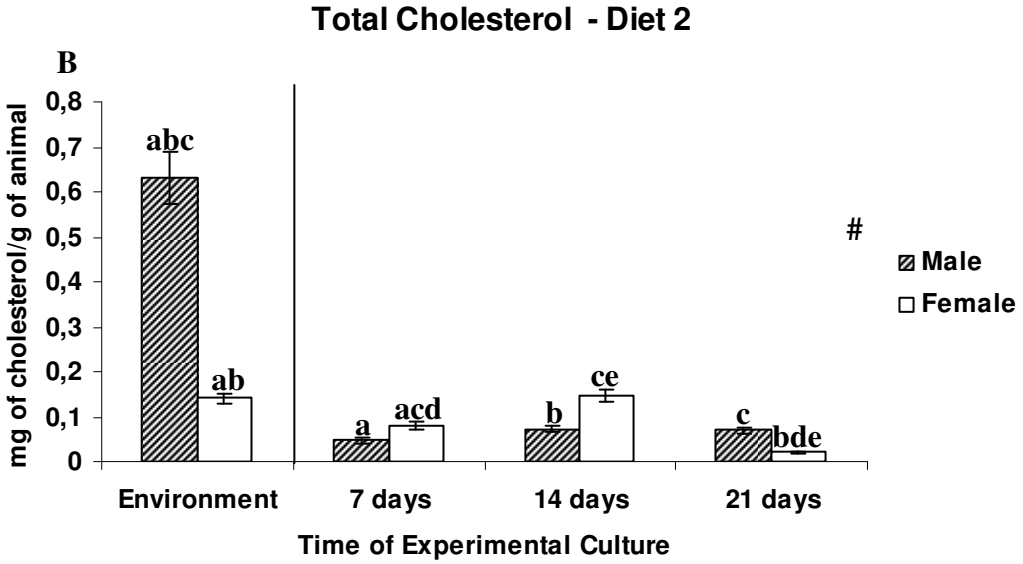
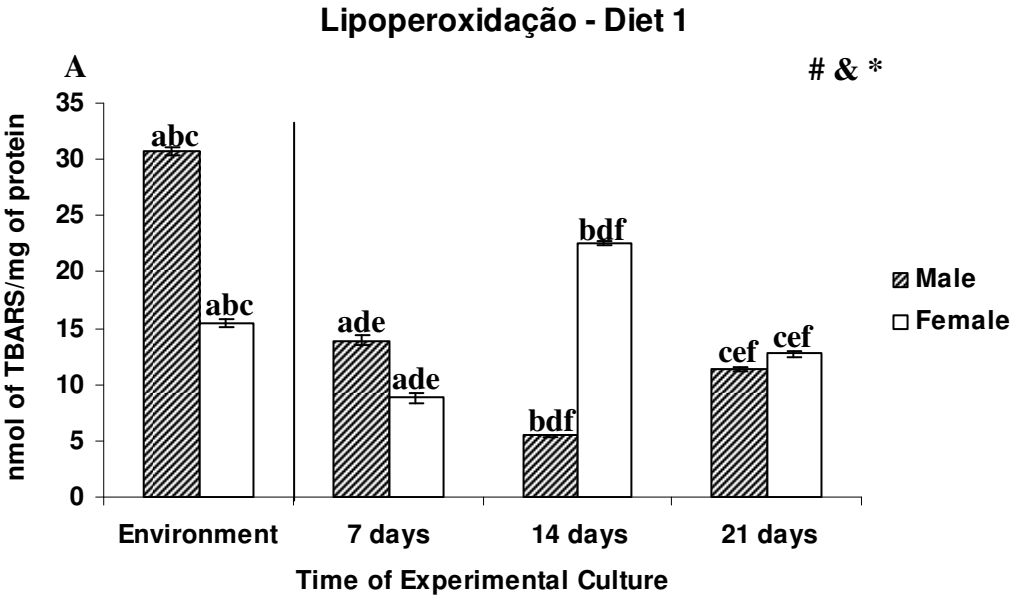

Total Cholesterol - Diet 2
B
mg of cholesterol/g of animal
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
abc
ab
a
acd
b
ce
c
bde
#
Male
Female
Environment
7 days
14 days
21 days
Time of Experimental Culture

Figure 5


Lipoperoxidação - Diet 1
A
& *
nmol of TBARS/mg of protein
35
30
25
20
15
10
5
0
abc
abc
ade
ade
bdf
bdf
cef
cef
Male
Female
Environment
7 days
14 days
21 days
Time of Experimental Culture

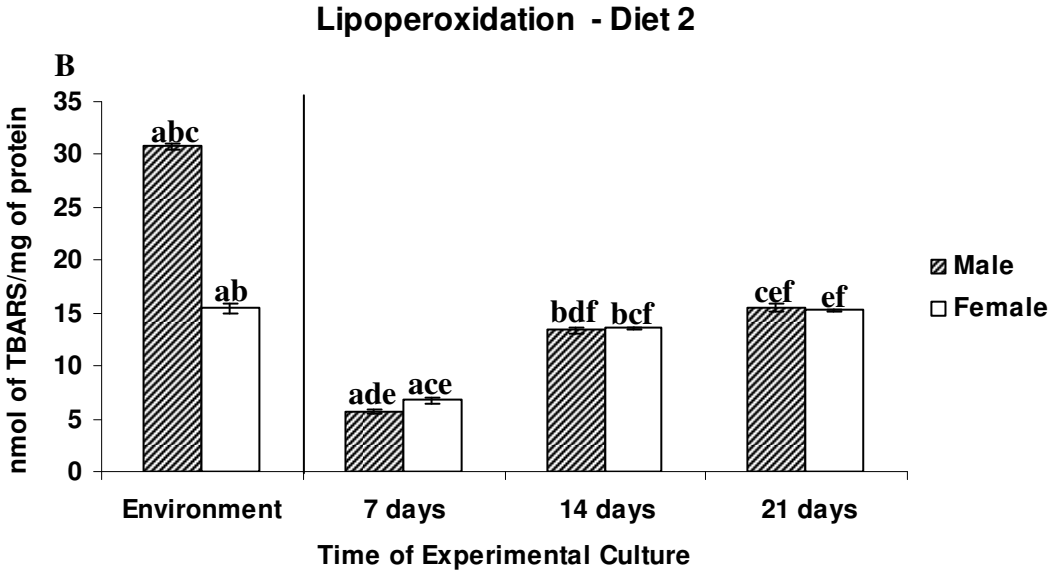
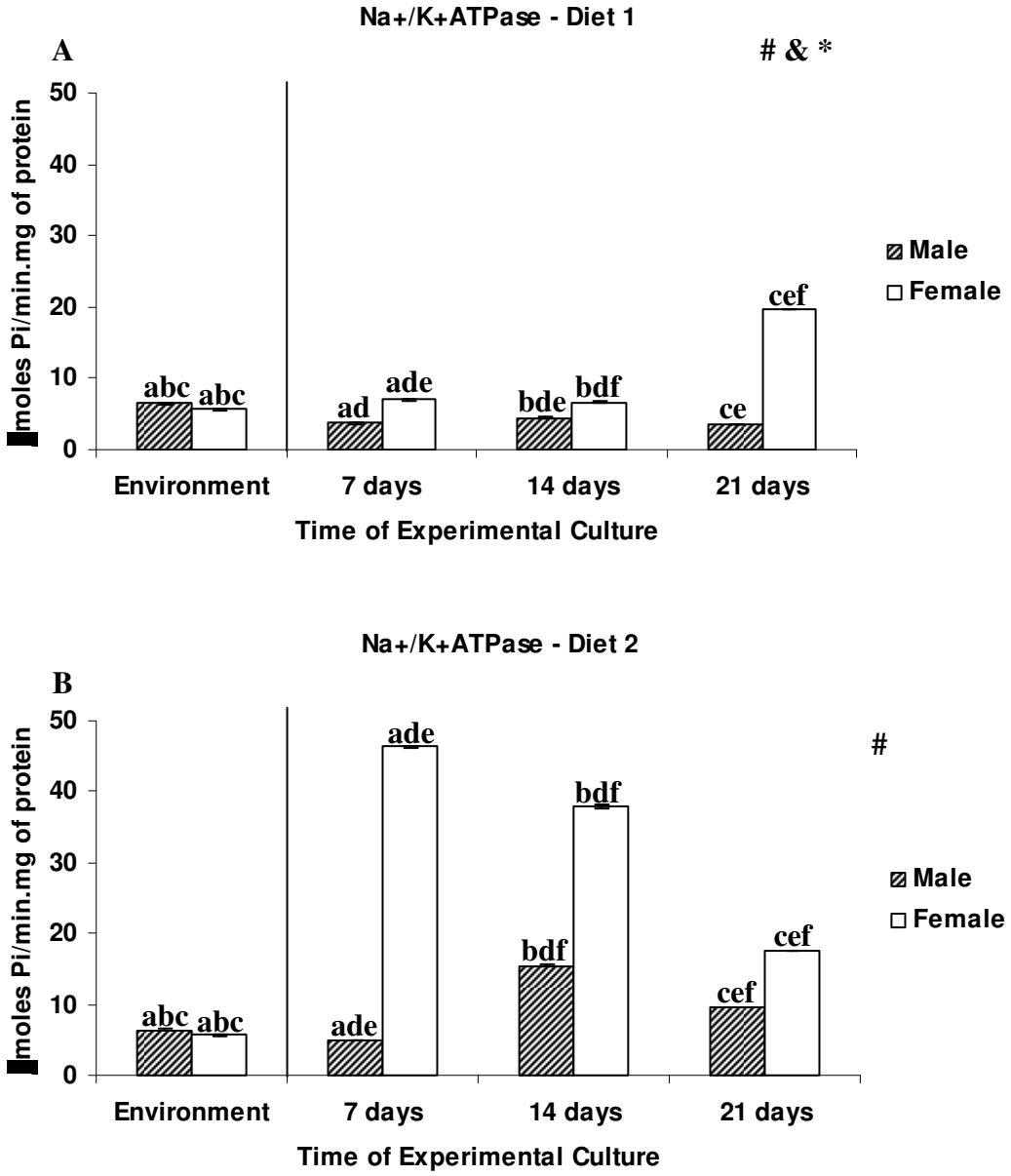

Lipoperoxidation - Diet 2
B
nmol of TBARS/mg of protein
35
30
25
20
15
10
5
0
abc
ab
ade
ace
bdf
bcf
cef
ef
Male
Female
Environment
7 days
14 days
21 days
Time of Experimental Culture

Figure 6


Na+/K+ATPase - Diet 1
A
& *
μmoles Pi/min.mg of protein
50
40
30
20
10
0
abc abc
ad ade
bde bdf
ce cef
Environment
7 days
14 days
21 days
Time of Experimental Culture
Male
Female
Na+/K+ATPase - Diet 2
B
#
μmoles Pi/min.mg of protein
50
40
30
20
10
0
abc abc
ade ade
bdf bdf
cef cef
Environment
7 days
14 days
21 days
Time of Experimental Culture
Male
Female

CONSIDERAÇÕES FINAIS

O presente estudo visou padronizar uma espécie de Hyalella, *Hyalella castroi*, em cultivo experimental em laboratório para sua possível utilização posteriormente em testes de toxicidade. De acordo com o objetivo proposto e os resultados obtidos neste trabalho, podemos concluir que:

- As duas dietas administradas aos anfípodos foram capazes de alterar os padrões metabólicos que estes animais apresentaram em campo.

- Embora, as duas dietas tenham suprido as necessidades calóricas dos animais, nenhum dos grupos conseguiu reproduzir em laboratório (apesar de serem encontrados pares reprodutivos e fêmeas ovígeras).

- Ambos os sexos de *Hyalella castroi* mostraram respostas metabólicas mais adequadas quando cultivadas com a dieta 1, a qual possui uma maior quantidade de carboidratos, menor concentração de proteínas e uma maior quantidade de proteínas de origem animal. Tais resultados aliados aos de campo sugerem que há uma maior quantidade de proteínas em sua dieta natural.

- Ao final deste estudo podemos constatar que a dieta ideal para o cultivo desta espécie ainda não foi definida, devendo novos testes serem arrolados.

ANEXO I

INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS
DEPARTAMENTO DE CIÊNCIAS DOS ALIMENTOS
RELATÓRIO DE ENSAIO

NÚMERO**: 171B/2004**

DATA ENTRADA: 10/10/2005
DATA SAÍDA: 27/10/2005

SOLICITANTE: **Bibiana Kaiser Dutra**
Fone: 9191.1954

AMOSTRA: Macrófita *Callitriche rimosa*

ENSAIOS:

Umidade.....93,84g/100g
Cinzas.....1,26g/100g
Proteina 1,24g/100g
Lípidios 0,28g/100g
Fibra Bruta.....1,34g/100g
Carboidratos 2,04g/100g
VCT 9,52Kcal/100g

Prof. **Adriano Brandelli**
Coordenador Prestação de Serviços

Heloisa H. Chaves Carvalho
Nutricionista
CRN 1484

Referências: Ministério da Agricultura. Laboratório Nacional de Referência Animal. Métodos analíticos para análise e seus ingredientes. Brasília, 1981. V.2: Métodos físicos e químicos.

Official methods of the Association of Official Analytical Chemists. AOAC, 1995. Cap. 4, seção 4.5.0.1.

Portaria n. 108 de 04 e 11 de setembro de 1991, Método n. 04. Diário Oficial da União, Brasília, p. 11813-19819, 17 setembro de 1991. Seção 1.

Normas Analíticas do Instituto Adolfo Lutz. 3 Ed. São Paulo, 1985. V. 1: Métodos Químicos e Físicos para análise de alimentos.

Válido somente para a(s) amostra(s) fornecida(s) pelo interessado.

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Prestação de serviços - telefone: (051) 3316-6248 - Fax: (051) 316-7048
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INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS
DEPARTAMENTO DE CIÊNCIAS DOS ALIMENTOS
RELATÓRIO DE ENSAIO

NÚMERO**: 170B/2004** **DATA ENTRADA: 10/10/2005**
DATA SAÍDA: 27/10/2005

SOLICITANTE: **Bibiana Kaiser Dutra**
Fone: 9191.1954

AMOSTRA: Ração em Flocos para Peixes – Ração 1

ENSAIOS:

Umidade.....5,13g/100g
Cinzas.....11,02g/100g
Proteina 30,88g/100g
Lípidios 6,19g/100g
Fibra Bruta.....3,59g/100g
Carboidratos 43,19g/100g
VCT 351,99Kcal/100g

Prof. **Adriano Brandelli**
Coordenador Prestação de Serviços

Heloisa H. Chaves Carvalho
Nutricionista
CRN 1484

Referências: Ministério da Agricultura. Laboratório Nacional de Referência Animal. Métodos analíticos para análise e seus ingredientes. Brasília, 1981. V.2: Métodos físicos e químicos.

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RELATÓRIO DE ENSAIO

NÚMERO**: 136B/2006** **DATA ENTRADA: 28/11/2006**
DATA SAÍDA: 11/12/2006

SOLICITANTE: **Bibiana Kaiser Dutra**
Fone: 9191.1954

AMOSTRA: Ração em Flocos para Peixes - Ração 2

ENSAIOS:

Umidade.....7,26g/100g
Cinzas.....14,15g/100g
Proteina 39,78g/100g
Lípidios 4,99g/100g
Fibra Bruta.....4,83g/100g
Carboidratos 28,99g/100g
VCT 319,99Kcal/100g

Prof. **Adriano Brandelli**
Coordenador Prestação de Serviços

Heloisa H. Chaves Carvalho
Nutricionista
CRN 1484

Referências: Ministério da Agricultura. Laboratório Nacional de Referência Animal. Métodos analíticos para análise e seus ingredientes. Brasília, 1981. V.2: Métodos físicos e químicos.

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Válido somente para a(s) amostra(s) fornecida(s) pelo interessado.

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ANEXO II

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COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY - PART A: MOLECULAR & INTEGRATIVE PHYSIOLOGY

An International Journal
Please bookmark this URL: http://www.elsevier.com/locate/cbpa

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Part A. Molecular and Integrative Physiology deals with molecular, cellular, integrative, and ecological physiology. Topics include bioenergetics, circulation, development, excretion, ion regulation, endocrinology, neurobiology, nutrition, respiration, and thermal biology. Studies on regulatory mechanisms at any level or organization such as signal transduction and cellular interactions and control of behaviour are encouraged.

Part B. Biochemistry and Molecular Biology covers biochemical and molecular biological aspects of metabolism, enzymology, regulation, nutrition, signal transduction, promoters, gene structure and regulation, metabolite and cell constituents, macromolecular structures, adaptational

mechanisms and evolutionary principles.

Part C. Toxicology and Pharmacology is concerned with chemical and drug action at different levels of organization, biotransformation of xenobiotics, mechanisms of toxicity, including reactive oxygen species and carcinogenesis, endocrine disruptors, natural products chemistry, and signal transduction. A molecular approach to these fields is encouraged.

Part D. Genomics and Proteomics covers the broader comprehensive approaches to comparative biochemistry and physiology that can be generally termed as "-omics", e.g., genomics, functional genomics (transcriptomics), proteomics, metabolomics, and underlying bioinformatics. Papers dealing with fundamental aspects and hypotheses in comparative physiology and biohcemistry are encouraged rather than studies whose main focus is purely technical of methodological.

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During the submission proces, authors are asked to select an appropriate section of CBP and to provide names and address (including e-mail) of at least five researchers of recognized competence who may be considered as reviewers.

Review articles
Before writing their manuscripts, potential authors of review articles should contact one of the Editors who, after

consultations with the other editor and/or members of the Editorial Board, will provide feedback on suitability of the topic. Reviews should be topical, and serve as critical appraisals of areas of research. They should provide an up-to-date analysis of concepts and point out future directions. For manuscript preparation, follow the instructions below.

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Lindsley, J.E., Rutter, J., 2004. Nutrient sensing and metabolic decisions. Comp. Biochem. Physiol. B 139, 543-559.
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ANEXO III

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