DANIELA DA SILVA CASTIGLIONI

Os ciclos biológicos de duas espécies simpátricas de Hyalella Smith, 1874 (Crustacea, Peracarida, Amphipoda, Dogielinotidae)

Tese de Doutorado apresentada ao Programa de Pós-graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutor em Biologia Animal.
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Tese de doutorado aprovada em___________

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VIVER COMO UM RIO

Viver como um rio
que vence os obstáculos
e sempre deságua no mar.

Ser persistente como um rio
que seca na estiagem
e renasce, gota a gota,
nas chuvas do outono.

Ser gentil como um rio
que purifica os náufragos
acolhidos em suas águas.

Ser humilde como um rio
que esconde nas águas mansas
a vida efervescente.

Ser impetuoso como um rio
que rompe suas margens
arrastando a terra morta
para voltar tranqüilo ao leito
depois de semear a vida.

Ser generoso como o rio
que alimenta o homem faminto
como o fruto de suas entranhas.

Viver a vida como um rio
que parece desfazer-se no mar,
mas se renova a cada dia,
ao mesmo tempo frágil e eterno.

Clóvis Wannemacher

“Sempre que pensamos em mudar, queremos tudo o mais rápido possível. Não tenha pressa, pois as pequenas mudanças são as que mais importam. Por isso, não tenha medo de mudar lentamente, tenha medo de ficar parado.” (Provêrbio Chinês)

“O que vale na vida não é o ponto de partida, e sim a caminhada. Caminhando e semeando, no fim téras o que colher.” (Cora Coralina)

“Quanto maior a dificuldade, maior o mérito em superá-la.” (H. W. Beecher)

“As coisas simples são as mais extraordinárias.” (Paulo Coelho)

Dedico este trabalho com todo o meu amor e gratidão aos meus queridos pais Dirlam e Tânia, que me auxiliaram na conquista de mais essa etapa de minha caminhada profissional. Muito obrigada por terem me ensinado a ser íntegra, humilde e batalhadora. Amo vocês!

Ao amor da minha vida, Adriano Martins, por toda a compreensão, companheirismo e amor. Amo você!

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Prefácio

A presente Tese de Doutorado trata de vários aspectos da biologia de duas espécies simpátricas de anfípodos de água doce, *Hyalella pleoacuta* e *H. castroi*, as quais foram recentemente descobertas na região dos Campos de Cima da Serra, no município de São José dos Ausentes, RS.

A tese está constituída por uma Introdução Geral, Objetivos, Metodologia Geral, seguida por cinco artigos, redigidos em conformidade com as normas da revista (ver normas nos Anexos) em que os mesmos serão publicados e também pelas Considerações Finais.

Na Introdução Geral estão apresentadas algumas generalidades sobre o grupo animal de interesse (Crustacea, Peracarida, Amphipoda, Dogielinotidae) e também um panorama geral sobre o que se conhece sobre Amphipoda e mais especificamente sobre as espécies de *Hyalella* no Brasil. Posteriormente estão apresentados os Objetivos e a Metodologia Geral. Cabe salientar que as seções Introdução Geral, Objetivos e Metodologia Geral estão formatadas segundo as normas da Revista Brasileira de Zoologia.

No primeiro capítulo estão apresentados os resultados referentes à idade da primeira muda, a freqüência de mudas, a determinação do sexo e a maturidade sexual de *H. pleoacuta* e *H. Castroi* em condições de laboratório (19 °C ± 1 °C e 12 horas luz / 12 horas escuro).

O sucesso de pareamento e o sucesso reprodutivo de *H. pleoacuta* e *H. castroi* são abordados no segundo capítulo.

No terceiro capítulo estão apresentados os resultados da produção de ovos de ambas espécies de *Hyalella* em ambiente natural.

A duração do comportamento pré-copulatório, o tamanho de machos e fêmeas em pré-copula, o tamanho corpóreo das fêmeas ovígeras, a duração dos períodos embrionário e pós-embrionário, a fecundidade e a viabilidade (número médio de juvenis liberados) foram estimados para as duas espécies de *Hyalella* em condições de laboratório (19 °C ± 1 °C e 12 horas luz / 12 horas escuro), sendo que estes resultados estão apresentados no quarto capítulo da presente tese.

O quinto capítulo apresenta a estrutura populacional de *H. pleoacuta* e *H. castroi*, as quais foram amostradas mensalmente (Agosto de 2003 a Julho de 2004) na localidade do Vale das Trutas, no município de São José dos Ausentes, RS. Para a caracterização da estrutura populacional foram analisados a distribuição de freqüência em classes de tamanho, o tamanho corpóreo de machos e fêmeas, o tamanho na maturidade sexual, a proporção sexual, o período reprodutivo e o recrutamento das duas espécies.

Por fim, nas Considerações Finais estão apresentadas as conclusões gerais da presente Tese de Doutorado, sendo que esta seção está formatada segundo as normas de Revista Brasileira de Zoologia.

Resumo

Considerando a importância dos anfípodos como bioindicadores de qualidade ambiental e por serem espécies representativas da fauna de invertebrados aquáticos continentais, este trabalho teve por objetivo o estudo do ciclo biológico de duas espécies simpátricas, *Hyalella pleoacuta* González, Bond-Buckup & Araujo 2006 e *H. castroi* González, Bond-Buckup & Araujo 2006. Com vistas à caracterização da dinâmica populacional das duas espécies em campo foram avaliados o tamanho dos animais, a distribuição de freqüência em classes de tamanho, a maturidade sexual, a proporção sexual, o período reprodutivo, o recrutamento, a fecundidade e o sucesso de pareamento e o sucesso reprodutivo. Os espécimes foram coletados com auxílio de puçá, durante 12 meses, em 4 pontos distintos em 2 tanques de cultivo de trutas na localidade de Vale das Trutas, próximo as nascentes do Rio das Antas, município de São José dos Ausentes, RS (28°47'00"S - 49°50'53"O). O sexo e a condição ovígera das fêmeas foram registrados e todos os espécimes foram medidos quanto ao comprimento do cefalotórax (mm). Em laboratório as duas espécies foram cultivadas a 19 °C ± 1 °C e 12h luz / 12h escuro, identificando a duração do comportamento pré-copulatório, o tamanho de machos e fêmeas em pré-copula, o tamanho corpóreo das fêmeas ovígeras, o tempo médio de desenvolvimento embrionário e pós-embrionário, a fecundidade, a viabilidade (número médio de juvenis liberados), a duração do ciclo de muda e a longevidade. Em laboratório os animais foram alimentados com ração para peixe (43% de proteína), macrófitas (*Salvinia* sp. e *Callitriche rimosa*) e alga (*Ankistrodesmus* sp.). A idade da primeira muda, a freqüência de muda, a idade da determinação do sexo e a maturidade sexual foram similares entre as espécies de *Hyalella* em condições laboratoriais. Entretanto, o período de intermuda total, o tamanho corpóreo máximo e a longevidade diferiram entre *H. pleoacuta* e *H. castroi.* Foi observada correlação entre o tamanho dos machos e fêmeas nos casais em pré-copula, ou seja, machos grandes carregam fêmeas grandes em ambas espécies de *Hyalella.* O sucesso de pareamento e sucesso reprodutivo dos machos aumenta a medida que o seu tamanho corpóreo também aumenta em ambas espécies de *Hyalella*. Fêmeas de tamanho corpóreo intermediário apresentaram maior sucesso reprodutivo em ambas espécies de *Hyalella*. Tanto *H. pleoacuta* como *H. castroi* mostraram as mesmas estratégias reprodutivas, especialmente quanto a duração do comportamento de pré-copula, a duração do período embrionário, fecundidade e fertilidade. Com relação à produção de ovos, *H. pleoacuta* produziu mais ovos do que *H. castroi,* mas nesta última os ovos são maiores. Em ambas espécies foi observada diminuição no número de ovos ao longo do desenvolvimento. Ambas espécies de *Hyallela* apresentaram uma estrutura populacional muito similar, especialmente a distribuição de freqüência em classes de tamanho, o maior tamanho corpóreo dos machos em relação às fêmeas, o padrão de proporção sexual com predominância de fêmeas e a reprodução e recrutamento contínuos. Entretanto, as espécies diferiram quanto ao número de exemplares amostrados, sendo *H. pleoacuta* aproximadamente 3 vezes mais freqüente do que *H. castroi* e também pelo fato de *H. pleoacuta* apresentar um tamanho corpóreo menor do que *H. castroi*. Além disso, *H. pleoacuta* reproduz-se com maior intensidade no outono e *H. castroi* no inverno. Diante dos resultados obtidos pode-se inferir que a coexistência de *H. pleoacuta* e *H. castroi* nos tanques de cultivo de trutas é facilitida pelas diferenças observadas no ciclo de vida, especialmente no tamanho corpóreo e nas estratégias reprodutivas.

Abstract

Considering the importance of the amphipods as bioindicators of environmental quality and by being representative species of the aquatic invertebrate fauna of the continents, this work it was performed with the aim to analyze and to compare the biologic cycle of the sympatric species, *Hyalella pleoacuta* and *H. castroi*. Population dynamics was characterized by means of the frequency distribution in size classes, body size, size at sexual maturity, sex ratio, breeding period, recruitment, fecundity and pairing and reproductive success. The amphipods were collected monthly with nets from August 2003 through July 2004 in four distinct sites of two trout ponds at Vale das Trutas locality, São José dos Ausentes County, southern Brazil (28°47'00"S - 49°50'53"W). In the laboratory, the specimens were measured as cephalothorax length (mm), being the sex and ovigerous conditions checked. Two species of *Hyalella* were cultivated to 19 °C ± 1 °C and 12h light / 12h dark, being that the duration of the precopulatory mate-guarding phase, body size of males and females in precopulatory mating behavior, body size of ovigerous females, embryonic and postembryonic periods, fecundity, viability (mean number of juveniles liberated), time to first molt, intermolt duration, molt frequency, sex determination, sexual maturation, growth and longevity were estimated. At the laboratory cultures, the amphipods were feed with fish food (43% of protein), macrophytes (*Salvinia* sp. and *Callitriche rimosa*) and algae (*Ankistrodesmus* sp.). The time to first molt, the molt frequency, the age of the sex determination and the sexual maturation were very similar between species of *Hyalella* in laboratory culture. However, the total intermolt period, the maximum body size and the longevity differed between *H. pleoacuta* and *H. castroi*. Positive assortative mating by size was observed in both species, i.e., larger males tended to pair with larger females. Male pairing success increased sharply with size. In both species, reproductive success in males increased with body size; however, the females of intermediate size classes showed greater reproductive success. Both species of *Hyalella* showed the same reproductive strategies, specially the duration of the precopulatory mate-guarding behavior, the duration of embryonic period, fecundity and viability. Egg production was greater in *H. pleoacuta*, but their eggs are minor than eggs produced by *H. castroi*. The number of eggs carried by females decreased with developmental stage in both *H. pleoacuta* and *H. castroi*, suggesting that egg loss may have occurred. Both species of *Hyalella* showed a population structure very similar, specially the frequency distribution in size classes, the larger body size of males and the reproduction and recruitment continuous. However, the species differed as number of specimens sampled, being *H. pleoacuta* about 3 times more frequent than *H. castroi* and also by the fact of *H. castroi* showed a larger body size than *H. pleoacuta*. Besides, *H. pleoacuta* reproduces with more intensity at the autumn and *H. castroi* at the winter. Probably the differences in the life cycle of *H. pleoacuta* and *H. castroi*, especially the body size and the reproductive strategies, can facilite the coexistence of these two species in the trout pond.

Introdução Geral

Quem são as Hyalella?

Os crustáceos são um grupo dominante e de sucesso, visto o elevado número de espécies, exibindo dessa forma uma grande variedade de estilos de vida e ocupando diferentes habitats no ambiente marinho, terrestre e dulcícola. Esta ampla diversidade de habitats permite observar entre os crustáceos uma série de padrões de história de vida e estratégias reprodutivas distintas, direcionando e maximizando a sobrevivência das espécies em diferentes circunstâncias (SASTRY 1983, HARTNOLL & GOULD 1988).

A superordem Peracarida, juntamente com os Decapoda, representam a maioria dos Malacostraca e de fato a maioria dos crustáceos (MARTIN & DAVIS 2001). O marsúpio, local onde ocorre a fertilização e a incubação dos ovos, é uma característica diagnóstica única dos Peracarida (BOROWSKY 1991). Esta estrutura é formada pela superfície ventral dos segmentos torácicos e pelos oostegitos, os quais são placas formadas a partir da superfície mediana das coxas torácicas (MORRIT & SPICER 1996).

Os crustáceos Peracarida estão representados pelas ordens Spelaeogriphacea, Thermosbaenacea, Lophogastrida, Mysida, Mictacea, Amphipoda, Isopoda, Tanaidacea e Cumacea. Destas, os anfípodos são o grupo mais representativo dos ecossistemas aquáticos, sendo caracterizado por apresentarem o corpo lateralmente comprimido e os primeiros e segundos pares de pereiópodos diferenciados, os gnatópodos (MARTIN & DAVIS 2001).

Os anfípodos são comuns em ecossistemas límnicos e freqüentemente dominam a comunidade bentônica das zonas profundas dos lagos (ISHIKAWA & URABE 2002), podendo representar 50% da biomassa da fauna de macroinvertebrados e constituindo-se numa quantia substancial da produção secundária dos ecossistemas aquáticos (WATERS & HOKENSTROM 1980, MARQUES & BELLAN-SANTINI 1990, CUNHA *et al.* 2000 a). Estes crustáceos constituem-se importantes elos nas cadeias alimentares dos ecossistemas onde ocorrem, pois possibilitam a transferência de energia produzida pelas algas e vegetais superiores para os consumidores de níveis tróficos mais elevados (MOORE 1975, GOSS-CUSTARDE 1977, MATTHEWS *et al.* 1992, MUSKÓ 1993, PILGRIM & BURT 1993). Além disso, os anfípodos estão sendo utilizados como organismos testes em estudos ecotoxicológicos, devido a sua distribuição cosmopolita, a sensibilidade a distúrbios ambientais e a sua fácil adaptação a cultivos e experimentos laboratoriais (NEUPARTH *et al.* 2002).

Os anfípodos representam a segunda maior ordem de Peracarida, estando distribuídos em quatro subordens, Caprellidea, Hyperiidea, Ingolfiellidea e Gammaridea (BELLAN-SANTINI 1999). Destas, a subordem Gammaridea abriga cerca de 80% de todas as espécies de Amphipoda, possuindo 124 famílias das 154 conhecidas (MARTIN & DAVIS 2001). Diferentemente das demais subordens que são altamente especializadas e ecologicamente restritas, os gamarídeos estão amplamente distribuídos em uma grande variedade de ambientes terrestres e aquáticos, sendo considerados o grupo mais representativo de anfípodos nos ambientes límnicos do mundo (BOUSFIELD 1973, BELLAN-SANTINI 1999). Aproximadamente 130 gêneros de Gammaridea são restritos ao ambiente de água doce e também são os únicos representantes da ordem Amphipoda nas águas continentais das Américas (BELLAN-SANTINI 1999).

No Brasil há registro de ocorrência de 29 famílias de Gammaridea compreendendo 81 gêneros e 133 espécies (WAKABARA & SEREJO 1998). Entretanto nos ambientes marinhos e límnicos do Rio Grande do Sul são encontrados representantes de apenas 7 famílias de

Gammaridea, das quais destacam-se Corophiidae, Stenothoidae, Hyalidae, Ischyriceridae, Gammaridae, Talitridae e Hyalellidae (BENTO & BUCKUP 1999).

A família Hyalellidae Bulycheva, 1957 pertencia à superfamília Talitroidea Rafinesque, 1815 e abrigava os gêneros *Hyalella* Smith, 1874, *Parhyalella* Kunzel, 1910, *Allorchestes*, Dana, 1849, *Insula* Kunzel, 1910, *Chiltonia* Stebbing, 1899, *Phreatochiltonia* Zeidler 1991, *Afrochiltonia* Barnard, 1955 e *Austrochiltonia* Hurley, 1958 (ZEILDER 1991, BOUSFIELD 1996). Recentemente, em uma proposta cladística realizada por SEREJO (2004), a superfamília Talitroidea foi elevada para Infraordem Talitra e a família Hyalellidae e Najnidae foram sinonimizadas com Dogielinotidae e estas 3 famílias foram classificadas como subfamílias de Dogielinotidae. A partir da proposta de SEREJO (2004a) o gênero *Hyalella* antigamente pertencente a Hyalellidae agora está alocado em Dogielinotidae e na subfamília Hyalellinae.

O gênero *Hyalella* é o único encontrado nos ambientes límnicos do Brasil (BENTO & BUCKUP 1999), sendo bastante comum na América do Norte e ocorrendo também na América Central e América do Sul. As espécies do gênero *Hyalella* apresentam uma distribuição restrita às regiões biogeográficas neártica e neotropical, sendo que 51 espécies já foram descritas até o momento (GONZÁLEZ & WATLING 2002, PEREIRA 2004, GONZÁLEZ *et al.* 2006). Na América do Sul há registro de ocorrência de 39 espécies de *Hyalella*, conferindo a esta região a maior diversidade de espécies.

No Brasil a diversidade do gênero alcança 13 espécies: *H. gracilicornis* (Faxon, 1876), *H. warmingi* Stebbing, 1899, *H. longistila* (Faxon, 1899), *H. meinerti* Stebing, 1899, *H. curvispina* Shoemaker, 1942, *H. pampeana* Cavalieri, 1968, *H. caeca* Pereira, 1989, *H. brasiliensis* Bousfield, 1996, *H. montenegrinae* Bond-Buckup & Araujo, 1998, *H. pseudoazteca* González & Watling, 2001, *H. dielaii* Pereira, 2 004, *H. pleoacuta* González, Bond-Buckup & Araujo 2006 e *H. castroi* González, Bond-Buckup & Araujo 2006 (González & Watling 2001, González 2003, Pereira 2004, González *et al*. 2006).

No Rio Grande do Sul há registros de ocorrência de *H. pseudoazteca*, *H. montenegrinae* e *H. curvispina*, mas recentemente Bond-Buckup & Araujo (comunicação pessoal), registraram a ocorrência de *H. pampeana* e mais 2 novas espécies simpátricas, *H. pleoacuta* e *H. castroi*, as quais foram encontradas no município de São José dos Ausentes, RS, na localidade do Vale das Trutas.

As espécies de *Hyalella* são geralmente encontradas em diversos habitats de água doce, como reservatórios permanentes, lagos, tanques e riachos, estando muitas vezes aderidas a macrófitas, nadando na coluna d'água ou confinados ao sedimento, sendo importantes membros da fauna bentônica (Kruschwitz 1978, Wellborn 1995, Bento & Buckup 1999, Grosso & Peralta 1999). Entretanto algumas espécies ocorrem em cavernas, como *H. caeca* e uma única espécie, *H. rionegrina* Grosso & Peralta, 1999, é encontrada em solos úmidos (Grosso & Peralta 1999). As espécies de *Hyalella* são omnívoras comedoras de depósito, alimentando-se principalmente de algas e bactérias associadas ao sedimento e macrófitas aquáticas (Hargrave 1970) e também de animais mortos e material vegetal (Cooper 1965).

O dimorfismo sexual das espécies de *Hyalella* é caracterizado pela presença do segundo par de gnatópodos alargados nos machos e pelo marsúpio nas fêmeas. O segundo par de gnatópodos dos machos são usados para o manuseio das fêmeas durante o comportamento de cópula e os primeiros pares são utilizados para carregá-las durante a fase pré-copulatória (Kruschwitz 1978, Wellborn 2000).

Simpatria

Várias espécies de *Hyalella* são encontradas no mesmo corpo d'água. De acordo com RICKLEFS (1993), a simpatria refere-se às áreas de sobreposição nas distribuições de espécies e por esse motivo algumas espécies de *Hyalella* são consideradas como simpátricas. No caso de simpatria, pode-se inferir que a competição pode causar divergência quando duas espécies ecologicamente semelhantes diferem mais quando estão juntas do que nas partes não sobrepostas de seu território (alopatria) (RICKLEFS 1993).

As espécies simpátricas geralmente são resultantes da especiação simpátrica, na qual o isolamento das populações se dá através de meios ecológicos ou genéticos na mesma área. Estas espécies tornam-se geneticamente isoladas através de diferentes padrões comportamentais e reprodutivos (ODUM 1988). Segundo MAYR (1977) os mecanismos de isolamento são propriedades biológicas que impedem o cruzamento entre duas espécies potencialmente simpátricas e estes mecanismos de isolamento podem ser visuais, olfativos, táteis, químicos ou simplesmente épocas reprodutivas diferentes ou estruturas reprodutivas incompatíveis entre as diferentes espécies.

A coexistência de espécies pertencentes ao mesmo gênero pode ser decorrente de diversas adaptações seletivas a partir da competição entre estas espécies. Geralmente, a competição envolve o espaço e o alimento e como resultado desta interação, pode haver ajustes para manter o equilíbrio entre as duas espécies. Se a competição for muito intensa, uma das espécies pode substituir a outra, ou forçar a outra a utilizar outro recurso alimentar ou ocupar outro lugar. Organismos estreitamente aparentados, que possuem hábitos ou morfologia semelhantes, muitas vezes não ocorrem nos mesmos lugares e quando realmente ocorrem, freqüentemente exploram recursos diferentes, apresentando diferenciação de nicho, ou são ativos em horas diferentes, ou devem apresentar diferenças morfológicas ou comportamentais (ABRAMS 1983, ODUM 1988). Segundo FUTUYMA (1992) se as espécies simpátricas diferem na utilização de recursos mais do que ao acaso, a diferença pode ser atribuível a fatores ecológicos e/ou evolutivos.

Além disso, espécies simpátricas podem também coexistir por mecanismos de equilíbrio que requerem apenas diferenças sutis no fenótipo (LEIBOLD 1996, 1998). Algumas espécies simpátricas freqüentemente diferem em características tais como o tamanho e estas diferenças podem ter evoluído para reduzir a competição interespecífica.

Reprodução, desenvolvimento e cuidado parental

No comportamento reprodutivo de Amphipoda-Gammaridea pode-se identificar diferentes etapas, sendo estas distinguidas como: etapa 1 , localização do parceiro, o qual é auxiliado pela secreção de ferormônios; etapa 2, formação do casal, sendo estimulada pelo contato e estímulos devido à liberação de ferormônios; etapa 3, macho e fêmea permanecem juntos até a fêmea sofrer muda; etapa 4, cópula, a qual ocorre brevemente após a muda da fêmea e é provavelmente estimulada por ferormônios. A maioria das espécies estudadas apresenta a mesma fisiologia reprodutiva básica e as mesmas quatro fases. Entretanto, os comportamentos exibidos durante as primeiras três etapas podem diferir, estando estas diferenças relacionadas à adaptações a diferentes ambientes aos quais os anfípodos gamarídeos são encontrados (BOROWSKY 1991).

Após as espécies de anfípodos terem atingido a maturidade sexual ocorre a indução de um comportamento reprodutivo através da liberação de ferormônios, sendo este comportamento denominado de pré-copulatório ou amplexo. Durante esta fase do ciclo reprodutivo, os machos transportam as fêmeas por baixo deles durante dias, prendendo a sua região torácica nas placas coxais das fêmeas com seu primeiro par de gnatópodos (BOROWSKY 1991). O comportamento pré-copulatório nestes crustáceos é um indicativo de que machos e fêmeas encontram-se maduros, ou seja, aptos a reproduzirem-se. Como o esperma não pode ser estocado pelas fêmeas, os machos maduros permanecem pareados com as fêmeas até a ovulação e fertilização (BOROWSKY 1991). Devido ao fato deste comportamento possuir um elevado custo energético (CALOW 1979), os machos iniciam o pareamento com as fêmeas somente quando a ovulação está preste a ocorrer (IRIBARNE *et al.* 1995).

Na maioria dos anfípodos que realizam o comportamento pré-copulatório, geralmente existe correlação entre o tamanho dos machos e o tamanho das fêmeas, ou seja, os machos grandes tendem a transportar e parear com as fêmeas grandes e os machos pequenos com as fêmeas pequenas (BIRKHEAD & CLARKSON 1980, WARD 1985, ADAMS & GREENWOOD 1987, HATCHER & DUNN 1997, LEFEBVRE *et al.* 2005). Várias hipóteses foram lançadas para tentar explicar a correlação existente entre o tamanho dos machos e o das fêmeas, das quais destacam-se: *mechanical constraints* (CROZIER & SNYDER 1923), *loading constraint* (ADAMS & GREENWOOD 1983), *spatial segregation* (BIRKEHEAD & CLARKSON 1980), *male-male competition* (WARD 1983), *sexual selection* (WARD 1984), *guarding time* (ELWOOD *et al.* 1987, ELWOOD & DICK 1990, DICK & ELWOOD 1996), *microhabitat segregation* (WARD & PORTER 1993), sendo a *male-male competition* e *loading constraints* as mais aceitas. Segundo a hipótese *male-male competition*, os machos com maiores tamanhos possuem duas vantagens sob os machos de menor tamanho: eles possuem maior capacidade de tomar uma fêmea de outro macho e estes são menos susceptíveis a perder a sua parceira para outros machos durante a pré-cópula (WARD 1983). Na *loading constraints*, os machos são limitados a selecionar fêmeas muito menores que eles, pois estas podem ser facilmente carregadas durante o comportamento pré-copulatório (ADAMS & GREENWOOD 1983).

Logo após o comportamento pré-copulatório, o casal separa-se brevemente para permitir a muda pré-puberal da fêmea (HYNES 1955). Os ovos são liberados diretamente dentro do marsúpio das fêmeas, onde ocorre a fertilização dos mesmos. Estes ovos apresentam desenvolvimento direto, sendo que os filhotes eclodem com a forma corporal semelhante a do adulto (STRONG 1972, BOROWSKY 1991, STEELE & STEELE 1991, MORRIT & SPICER 1996). Dessa maneira, o desenvolvimento dos anfípodos, assim como dos demais peracarídeos, pode ser dividido em duas etapas: 1) da ovulação a eclosão - período embrionário e 2) da eclosão a emergência da prole - período pós-embrionário ou juvenil (BOROWSKY 1980).

O marsúpio confere aos embriões e aos juvenis marsupiais proteção contra predação (SHILLAKER & MOORE 1987, THIEL 1999 a). Esta proteção fornecida pelo marsúpio durante o desenvolvimento embrionário pode resultar numa tendência das espécies produzirem poucos embriões, mas estes são maiores quando comparados às espécies que não apresentam nenhum cuidado com a prole (SHEADER 1977).

Enquanto em muitas espécies de Peracarida o cuidado com a prole finaliza logo após os juvenis emergirem do marsúpio, em algumas espécies este cuidado estende-se além deste estágio, sendo este comportamento denominado de cuidado parental prolongado (AOKI 1997, THIEL, 1998, THIEL 1999 a b). Na maioria das espécies de crustáceos Peracarida que realizam cuidado parental prolongado, a progênie é carregada sobre o corpo da mãe, freqüentemente em estruturas especiais, como por exemplo, no marsúpio ou aderidos ao corpo das fêmeas até a mesma alcançar o estágio juvenil (AOKI & KIKUCHI 1991, BOROWSKI 1991, AOKI 1999, THIEL 2003 a). Porém, em outras espécies, os pais podem cuidar de sua prole em moradias como tubos e tocas (THIEL 1999 c, MURATA & WADA 2002, THIEL 2003 b). Em geral, qualquer atividade realizada pelos pais em relação à prole, a qual representa um custo para os pais caracteriza-se como cuidado parental e o retorno deste comportamento é o aumento no crescimento e sobrevivência da prole (e.g. AOKI 1997, THIEL 1999 b, KOBAYASHI *et al.* 2002).

História de vida

Os aspectos da história de vida agem diretamente na reprodução e na sobrevivência de uma determinada espécie. Dentre os principais componentes da história de vida, destacam-se o tamanho ao nascer, o padrão de crescimento, a idade e o tamanho na maturidade sexual, o número e o tamanho dos juvenis, a proporção sexual, os investimentos reprodutivos idade e tamanho-específicos, a mortalidade e a longevidade. É a interação entre estes aspectos que irão determinar o *fitness* individual de uma determinada espécie (STEARNS 1992).

Existe uma ampla literatura sobre aspectos da história de vida de Amphipoda-Gammaridea, porém limitadas a uma ou duas espécies. Além disso, as revisões sobre padrões reprodutivos e ciclo de vida de gamarídeos levaram em consideração os ritmos reprodutivos, a longevidade, o tamanho das fêmeas reprodutivas, o número de embriões, o hábito de vida (MORINO 1978, NELSON 1980, VAN DOLAH & BIRD 1980, WILDISH 1982, SAINTE-MARIE 1991).

O pioneiro em estudos sobre a história de vida de anfípodos gamarídeos foi MORINO (1978), o qual propôs uma classificação simples baseada nos ritmos reprodutivos (sazonal ou contínuo) e na longevidade. Sugeriu-se que os tipos de história de vida estão distribuídos de acordo com a latitude-temperatura, sendo que populações semianuais são registradas em regiões tropicais e populações bianuais ou perianuais para as regiões polares.

Dois anos após MORINO (1980) ter realizado a sua classificação para a história de vida de anfípodos gamarídeos, NELSON (1980) comparou o comprimento médio das fêmeas reprodutivas e o número de embriões por coorte em várias espécies de anfípodos gamarídeos. Concluiu-se que as fêmeas eram maiores e o número de embriões era mais numeroso nas espécies de gamarídeos epibentônico *versus* endobentônico, nas estuarinas *versus* dulcícolas e marinhas e na família Gammaridea *versus* Ampeliscidae e Haustoriidae. Além disso, as espécies semélparas produzem mais embriões por prole do que as espécies iteróparas. Embora suas conclusões relacionadas à fecundidade tenham sido aceitas, elas foram questionadas devido ao fato deste autor se referir apenas ao tamanho da prole e não fazer uma relação entre tamanho da prole e tamanho da fêmea.

No mesmo ano em que NELSON (1980) publicou a sua classificação para a história de vida de anfípodos gamarídeos, VAN DOLAH & BIRD (1980) sugeriram que as espécies de gamarídeos aquáticos epibentônicos produzem mais embriões e menores do que as espécies endobentônicas e encontraram correlação positiva entre latitude e tamanho do embrião em populações de determinadas espécies. Hipotetizaram ainda que a mortalidade dos adultos estava correlacionada positivamente com o número de ovos e inversamente com o tamanho dos ovos produzidos.

Posteriormente, WILDISH (1982) reconheceu seis tipos básicos de história de vida para os gamarídeos, sendo estes: multivoltino (mais de uma geração por ano) semianual, univoltino (uma geração por ano) ou multivoltino anual, semélparo (prole única) bianual e semélparo ou iteróparo (múltiplas proles) perianual. Fêmeas de espécies ou populações semianuais crescem rapidamente, maturam mais cedo e são muito fecundas. Este tipo de ciclo de vida está associado com habitats quentes e tropicais ou com populações sujeitas a elevados níveis de predação. Em contraste, fêmeas de espécies ou populações bianuais ou perianuais tendem a crescer mais lentamente, maturam mais tarde e são menos fecundas. Este tipo de ciclo é característico de populações que vivem em lugares onde a mortalidade é influenciada por fatores físicos.

Mais recentemente, SAINTE-MARIE (1991) sugeriu que as classificações da história de vida dos gamarídeos realizadas por MORINO (1978) e WILDISH (1982) resultaram de poucas observações e necessitavam ser revisadas e testadas novamente. Então, SAINTE-MARIE (1991) se propôs a fazê-la utilizando representantes de 214 espécies pertencentes a 16 superfamílias de gamarídeos aquáticos. Este autor classificou a história de vida dos anfípodos gamarídeos em semélparos ou iteróparos e o ciclo de vida em semi-anual, anual, bianual ou perianual. Segundo este autor, estes padrões são influenciados pela latitude, profundidade e salinidade e variam de acordo com a superfamília. Geralmente, as espécies de baixas latitudes são caracterizadas por histórias de vida semi-anual ou anual, pequeno tamanho corpóreo e elevado potencial reprodutivo. No entanto, as espécies de latitudes mais elevadas possuem histórias de vida bianuais ou perianuais, grande tamanho corpóreo, diminuição do tamanho na maturidade sexual, uma ou poucas proles, mas com muitos embriões de grande tamanho e baixo potencial reprodutivo.

Além disso, quando se quer caracterizar uma determinada espécie quanto à história de vida deve-se levar em consideração que os aspectos da história de vida podem ser influenciados por características bióticas e abióticas do lugar onde as mesmas vivem. O tamanho dos animais na maturidade, o tamanho da prole, o tamanho dos embriões, o número de embriões por fêmea, a idade da maturidade sexual e a estação reprodutiva podem variar intraespecificamente com a temperatura, latitude, profundidade, salinidade ou à exposição a predadores (e.g. HYNES 1954, SEGESTRALE 1967, 1970, FISH & PREECE 1970, STRONG 1972, MORINO 1978, PINKSTER & BROODBAKKER 1980, KOLDING & FENCHEL 1981, SAINTE-MARIE

& BRUNEL 1983, SHEADER 1983, SKADSHEIM 1984, 1989, SIEGFRIED 1985, FREDETTE & DIAZ 1986, NAYLOR *et al.* 1988).

Estudos sobre ciclo de vida de espécies de Amphipoda são facilitados pela rápida adaptação destes crustáceos às condições de laboratório, com eventos reprodutivos num mesmo período e curto tempo de geração, pelas elevadas densidades sob as quais são encontrados e pela fácil determinação do sexo e tamanho dos espécimes vivos sob lupa. Além disso, estes animais possuem uma grande tolerância às manipulações experimentais as quais são submetidos em laboratório (KRUSHCWITZ 1978, PENNAK 1953, COOPER 1965, BOROWSKY 1991, DUAN *et al.* 1997).

Biologia reprodutiva

A reprodução é um evento crítico no ciclo de vida dos animais e ela está intimamente relacionada com a capacidade reprodutiva, onde uma porção da energia corporal é direcionada para esse propósito (LÓPEZ-GRECO & RODRÍGUEZ 1999). No entanto, o estudo da biologia reprodutiva em crustáceos compreende dois aspectos distintos: primeiro, os eventos relacionados com a corte, incluindo processos que antecedem e estendem-se até o comportamento de cópula; segundo, o ciclo reprodutivo de uma espécie, como a maturidade sexual, o desenvolvimento dos ovos e as relações entre reprodução e ciclo de muda (GONZÁLEZ-GURRIARÁN 1985).

O ciclo reprodutivo de uma dada espécie envolve uma série de eventos numa população. Em indivíduos que completam a fase juvenil, esses eventos incluem a proliferação de células gonadais (ativação da gametogênese), diferenciação e crescimento dos gametas até a maturação, comportamentos reprodutivos associados com acasalamento, liberação de gametas pela transferência de espermatóforos durante a cópula pelos machos e ovulação e ovoposição pelas fêmeas e desenvolvimento do embrião até a eclosão e liberação das larvas ou juvenis. Este ciclo é básico para todos os crustáceos que se reproduzem sexualmente, mas o tempo entre estes eventos e a duração desses eventos varia entre as diferentes espécies (SASTRY 1983).

As variações específicas na procriação podem ser explicadas como respostas genotípicas ao ambiente, para se obter sucesso reprodutivo sobre condições favoráveis. Dessa maneira, uma população pode apresentar reprodução contínua, se as fêmeas produzirem ovos com a mesma intensidade ao longo dos meses do ano, ou reprodução sazonal, se a desova é mais intensa durante um certo período do ano, onde as condições ambientais são favoráveis para o desenvolvimento da prole (SANTOS 1978, SASTRY 1983). Assim, a disponibilidade de alimento para a manutenção, crescimento e reprodução dos indivíduos adultos, e para o desenvolvimento, crescimento e sobrevivência dos estágios larvais e/ou juvenis pode ser um dos mais importantes fatores na sincronização e coordenação das atividades reprodutivas num determinado habitat e, também, na evolução da história de vida e estratégias reprodutivas (SASTRY 1983).

Em relação aos ciclos de vida, espécies que apresentam um curto período de vida, geralmente menos de um ano, podem produzir uma única ou várias desovas durante a curta fase adulta, o que é conhecido como 'semelparidade', caracteristicamente marcada por uma única estação reprodutiva durante toda a vida do animal. Por outro lado, animais com um tempo de sobrevivência maior, irão produzir uma ou mais desovas em cada uma das várias estações reprodutivas durante a sua vida adulta, sendo este caso chamado de 'iteroparidade' (veja SASTRY 1983 e HARTNOLL 1985 para revisão).

O estudo da biologia reprodutiva em Crustacea tem envolvido principalmente análises da maturidade sexual, do período reprodutivo e da fecundidade. No caso dos anfípodos gamarídeos, os principais aspectos reprodutivos que estão sendo analisados são: tamanho corpóreo na maturidade sexual, idade da maturidade sexual, estação reprodutiva, tamanho da prole, tamanho do ovo, número de proles por fêmea, fecundidade potencial (número de ovos produzidos pela fêmea durante toda a sua vida) e potencial reprodutivo (número de ovos produzidos pela fêmea por estação reproduvita) (HYNES 1955; FISH & PREECE 1970; MORINO 1978; NELSON 1980; VAN DOLAH & BIRD 1980; KOLDING & FENCHEL 1981; SHEADER 1983; POWELL & MOORE 1991; SAINTE-MARIE 1991; APPADOO & MYERS 2004). Através da análise destes aspectos reprodutivos, podem-se compreender as estratégias adaptativas e o potencial reprodutivo de cada espécie e também suas relações com o ambiente e outros animais.

Entende-se por maturidade sexual o conjunto de transformações morfológicas e fisiológicas mediante as quais os jovens ou imaturos alcançam a capacidade de produzir gametas, que podem fertilizar ou serem fertilizados (MANTELATTO 2003). Tal condição possibilita que estes animais passem a atuar diretamente no mecanismo de flutuação populacional. A maturidade sexual das fêmeas de espécies de Amphipoda, assim como as espécies em *Hyalella*, são facilmente identificadas pelos seus ovários desenvolvidos, os quais são externamente visíveis de coloração verde escuro e pela presença de um marsúpio e/ou ovos dentro do marsúpio (KRUSCHWITZ 1978). Por outro lado, a maturidade sexual dos machos é determinada pelo alargamento completo do própodo do segundo par de gnatópodos (KRUSCHWITZ 1978, NELSON & BRUNSON 1995). No caso dos anfípodos, o surgimento do comportamento pré-copulatório é o indicativo mais preciso de que machos e fêmeas encontram-se maduros, ou seja, aptos a reproduzirem-se (BOROWSKY 1991).

O período reprodutivo é o intervalo de tempo no qual as fêmeas de uma determinada população encontram-se ovígeras, podendo apresentar picos na freqüência destas fêmeas. Estes picos reprodutivos, geralmente estão associados às variações de fatores bióticos e abióticos tais como temperatura, luz e disponibilidade de alimento, entre outros (SASTRY 1983).

A duração da estação reprodutiva nos crustáceos está relacionada à distribuição geográfica dos mesmos. Espécies que vivem em regiões temperadas, as quais estão sujeitas a uma maior sazonalidade das variações climáticas, tendem a apresentar desovas em sincronia com os períodos de condições ambientais mais favoráveis, determinando com isto, épocas de quiescência. Por outro lado, espécies tropicais e subtropicais, sujeitas a uma sazonalidade climática menos pronunciada, podem produzir desovas durante longos períodos ou mesmo durante todo o ano. Estas espécies podem apresentar picos de intensidade na freqüência de fêmeas ovígeras, o que varia em diferentes espécies e em diferentes localidades, como resposta às pequenas variações ambientais (SASTRY 1983).

Por outro lado, a temperatura, a luz, a presença de um parceiro para o acasalamento e a latitude são apontados como os principais fatores exógenos que irão exercer influência na atividade reprodutiva dos anfípodos (GINN *et al.* 1976, KRUSCHWITZ 1978, STEELE 1981, WILLIANS 1985, BOROWSKY 1991, TAKEUCHI & HIRANO 1992, OBERLIN & BLINN 1997, WILHELM & SCHINDLER 2000, PÖCKL *et al.* 2003).

A fecundidade é o número de ovos produzidos por uma fêmea em uma simples desova ou durante um determinado período do seu ciclo de vida (BOURDON 1962, SASTRY 1983). De acordo com SASTRY (1983), a energia utilizada para a produção de gametas pode ser armazenada num número grande de ovos pequenos ou num pequeno número de ovos grandes e estas características podem variar de uma espécie para outra. Além disso, o número de ovos e a taxa na qual estes são produzidos pelas fêmeas pode ser característico da espécie e pode possuir significância tanto para a sua estratégia de história de vida como para a sua ecologia. Entretanto, o número de ovos produzidos por uma determinada espécie pode variar de acordo com a idade e o tamanho da fêmea e também por influência de fatores ambientais, especialmente temperatura e disponibilidade de alimento (PENNAK 1953, MORINO 1978, STEELE 1981, WILDISH 1982, SASTRY 1983, DU PREEZ & MCLACHLAN 1984, VAN SENUS 1985, WILLIAMS 1985, KURIS 1991, POWELL & MOORE 1991, MARANHÃO & MARQUES 2003). De acordo com VAN DOLAH & BIRD (1980), os anfípodos gamarídeos são organismos ideias para analisar e comparar a produção de ovos por várias razões: o desenvolvimento dos ovos é completado dentro do marsúpio, o desenvolvimento é direto, com a prole eclodindo com a forma de juvenil e o tamanho e número de ovos são facilmente mensurados.

De acordo com MANTELATTO (2003), é importante ressaltar que na produção de ovos, existem inúmeros fatores relacionados, como o tamanho e o número de ovos, o tipo de desenvolvimento larval, a viabilidade dos ovos, a sobrevivência, os cuidados na incubação e o grau de fertilidade. Neste sentido, a fecundidade é uma das ferramentas utilizadas para auxiliar na avaliação do potencial reprodutivo de uma espécie, uma vez que, para se entender tal mecanismo, é necessária uma averiguação de todas as etapas mencionadas anteriormente. Cabe ainda salientar que a fecundidade também tem sido utilizada como um importante pré-requisito para o entendimento de adaptações reprodutivas frente às condições ambientais.

Estrutura populacional

Uma população pode ser definida como sendo qualquer grupo de organismos da mesma espécie ou de outros grupos, dentro dos quais os indivíduos possam intercambiar a informação genética e que ocupam um espaço determinado, funcionando como parte de uma comunidade biótica (ODUM 1988). Uma população possui várias características ou propriedades exclusivas do grupo, que não são atributos dos organismos isoladamente, tais como densidade, natalidade, distribuição etária, potencial biótico, dispersão, recrutamento e forma de crescimento. Todas estas propriedades agrupadas irão reger a dinâmica de uma população natural (ODUM 1988). Os indivíduos que fazem parte de uma população interagem competindo por alimento, abrigo, parceiros reprodutivos, etc, sendo que o equilíbrio na comunidade é mantido pelas relações inter e intraespecíficas (FONTELES-FILHO 1989).

A caracterização da estrutura populacional é considerada informação de base e fornece subsídios ao conhecimento da estabilidade ecológica das populações tratando de assuntos relacionados com a abundância sazonal, densidade populacional, distribuição de freqüência em classes de tamanho dos animais, taxas de natalidade e mortalidade, migração, entre outros (HUTCHINSON 1981, SANTOS *et al.* 1995).

No caso dos anfípodos, a estrutura populacional tem sido analisada, principalmente, pela distribuição dos indivíduos em classes de tamanho, densidade populacional, distribuição etária, proporção sexual, período reprodutivo, recrutamento e crescimento (LA FRANCE & RUBER 1985, WILSON & PARKER 1996, MOORE & WONG 1996, COSTA & COSTA 1999, CUNHA *et al.* 2000, GONÇALVES *et al.* 2003, MARQUES *et al.* 2003, GUERAO 2003, DIAS & SPRUNG 2004, APPADOO & MYERS 2004, KEVREKIDIS 2004, 2005, SUBIDA *et al.* 2005). Estas informações populacionais fornecem subsídios para o conhecimento da estabilidade ecológica das espécies num determinado habitat, além de permitirem a compreensão da biologia de uma determinada espécie.

Ciclo de muda

A presença de um exoesqueleto rígido nos crustáceos exige que estes animais sofram trocas periódicas deste revestimento, visando o crescimento. Nos crustáceos ele pode ser dividido em uma série de fases, cada uma com um ou vários estágios, conferindo aos indivíduos características e mudanças peculiares quanto à morfologia e a fisiologia, além de outros aspectos como comportamento e alimentação (HARTNOLL 1982).

Entende-se por ciclo de muda, um processo completo caracterizado por várias mudanças de estágios, onde cada uma é marcada por um período que antecede (pré-muda), outro posterior (pós-muda) e um período de repouso entre duas mudas (intermuda). O número de mudas e a duração de cada estágio variam de acordo com a espécie e em relação ao tamanho e ao sexo dos animais (HARTNOLL 1982; 1985). Além disso, o ciclo de muda nos crustáceos não é um fenômeno isolado, pois fatores ambientais (temperatura, luz, salinidade) e fisiológicos (maturação, regeneração, alimentação) podem também interagir com fatores internos e determinar a freqüência da muda (HARTNOLL 2001).

Nos crustáceos, o estágio de intermuda é considerado como o período no qual ocorre o direcionamento das energias metabólicas para serem armazenadas, especialmente no hepatopâncreas, o que envolve o movimento cíclico das reservas orgânicas, alternadamente para a formação de uma nova epiderme e para o desenvolvimento das gônadas (ADIYODI & ADIYODI 1970, HARTNOLL 1985), o que representa um outro aspecto importante para a compreensão a respeito do predomínio da condição de intermuda nesses animais.

Bioindicação

Os crustáceos são freqüentemente utilizados como bioindicadores e biomonitores em vários ecossistemas aquáticos (RINDERHAGEN *et. al.* 2000). Uma razão para isto, é que eles são um grupo de animais bem sucedidos, distribuídos em diferentes habitats, incluindo os ambientes marinho, terrestre e dulcícola. Além disso, especialmente os crustáceos anfípodos possuem uma série de características que os tornam adequados como organismos experimentais: são coletados com facilidade, ocorrem em altas densidades, são facilmente mantidos em laboratório, apresentam ciclo de vida curto e grande sensibilidade a contaminantes (SAMPAIO 1988, KRUSCHWITZ 1978). Tais crustáceos poderiam atuar como indicadores da qualidade dos ecossistemas aquáticos. Para tal, se faz necessário um conhecimento aprofundado tanto da biologia e ecologia como também da fisiologia destes animais coletados em ambiente natural e em cultivo experimental o que permitirá o gerenciamento adequado dessas populações.

Alguns aspectos como as estratégias reprodutivas, podem ser altamente importantes para a interpretação de dados sobre estudos da bioindicação e para o desenvolvimento de estudos ecotoxicológicos. Não somente os aspectos reprodutivos, mas também outras respostas comportamentais dos crustáceos, como as mudanças na alimentação, locomoção ou comportamento pré-cópula podem demonstrar a sensibilidade destes animais perante a bioindicação de toxicidade em determinado ambiente (RINDERHAGEN *et al.* 2000).

O número de espécies padronizadas em testes de toxicidade permanece limitado e na sua maioria são utilizados organismos alóctones. Este número reduzido deve-se, primariamente, ao fato de que a maioria dos testes de toxicidade implica em cultivos contínuos de organismos-teste em estado saudável em número suficiente, o que limita em muito a seleção das espécies utilizadas (BRENDONCK & PERSOONE 1993). Por serem organismos bentônicos, os anfípodos dulcícolas mais utilizados em testes de toxicidade e bioensaios para avaliação da qualidade do sedimento dos ecossistemas aquáticos tem sido a espécie norte-americana *Hyalella azteca* (Saussure, 1858) e *Gammarus lacustris* Sars, 1863 (BUYLE 1989, NELSON & BRUNSON 1995, DUAN *et al.* 1997). O sedimento serve ao mesmo tempo, como depósito e fonte de matéria orgânica e inorgânica (WETZEL 1983) 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 (BURTON 1991).

Panorama geral sobre o conhecimento de Amphipoda no Brasil

Comparado aos grandes grupos de crustáceos, como os Decapoda, relativamente pouco é conhecido sobre os aspectos biológicos de Amphipoda-Gammaridea que ocorrem no Brasil, sendo que a grande maioria dos trabalhos é de cunho sistemático (MOREIRA 1903, WAKABARA & LEITE 1977, TARARAM *et al.*1978, LEITE *et al.* 1978, PEREIRA 1985, PEREIRA 1989, SEREJO 1995, SEREJO 1997, WAKABARA & SEREJO 1998, SEREJO 2001, 2003, RAYOL & SEREJO 2003, SEREJO 2004 a, PEREIRA 2004, LOPES & SETUKO 2004, SENNA & SEREJO 2005, VALÉRIO-BERARDO 2005). Além disso, estudos sobre a ocorrência e a distribuição de espécies também têm sido desenvolvidos no Brasil (TARARAM *et al.* 1986, SEREJO 1998 a b, WAKABARA & SEREJO 1999, SEREJO *et al.* 2001, LEITE 2002, JACOBUCCI & LEITE 2002, SEREJO & LICINIO 2002, TANAKA & LEITE 2003, SEREJO & WAKABARA 2003, TANAKA & LEITE 2004, SEREJO 2004 b, FREIRE & SEREJO 2004, LOPES & MASUNARI 2004, LEITE *et al.* in press).

Entretanto, trabalhos que abordam aspectos biológicos restringem-se a espécies marinhas (WAKABARA *et al.* 1983, LEITE *et al.* 1986, LEITE & WAKABARA 1989, CARDOSO & VELOSO 1996, LEITE 1996 a b c, DUBIASKI-SILVA & MASUNARI 1998, LEITE *et al.* 2002, JACOBUCCI *et al.* 2002, LEITE & GUTH 2003, JACOBUCCI *et al.* 2006).

Panorama geral sobre o conhecimento do gênero Hyalella

Nos últimos anos o conhecimento das espécies de *Hyalella* que ocorrem nas Américas vem crescendo, mas ainda necessita-se desenvolver pesquisas que tenham por objetivo o conhecimento das espécies que ocorrem no Brasil.

A maioria dos trabalhos desenvolvidos com as espécies de *Hyalella* trata de uma única espécie norte-americana, *H. azteca* que vem servindo como modelo experimental na maioria dos laboratórios dos Estados Unidos, Canadá, Alemanha e Brasil. Os trabalhos desenvolvidos com *H. azteca* abordam aspectos reprodutivos, populacionais, taxonômicos e sistemáticos e também sobre a influência de alguns fatores ambientais nos diversos aspectos biológicos da espécie além da utilização desta espécie em testes de toxicidade (GEISLER 1944, COOPER 1965, STRONG 1972, KRUSHWITZ 1978, BORGMANN *et al.* 1989, NEBEKER *et al.* 1992, EDWARDS & COWEEL 1992, FRANCE 1992, PILGRIM & BURT 1993, WEN 1992, 1993, WELLBORN 1994, NELSON & BRUNSON 1995, WELLBORN 1995, PICKARD & BENKE 1996, SEVERO 1997, GROSSO & PERALTA 1999, WELLBORN 2000, MORELLI 2001, OTHMAN & PASCOE 2001, CASSET *et al.* 2001, GONZÁLEZ & WATLING 2002, PORTELA 2002, ALCOCER *et al.* 2002, WELLBORN & COTHRAN 2004, WELLBORN & BARTHOLF 2005, WELLBORN *et al.* 2005).

No Brasil, a maioria dos trabalhos desenvolvidos com o gênero *Hyalella* trata da descrição de novas espécies (PEREIRA 1985, 1989, BOUSFIELD 1996, BOND-BUCKUP &

ARAUJO 1998, GROSSO & PERALTA 1999, GONZÁLEZ & WATLING 2002, 2003, GONZÁLEZ 2003, PEREIRA 2004, GONZÁLEZ *et al.* 2006). Entretanto, o ciclo de vida das espécies pertencentes ao gênero *Hyalella* que ocorrem no Brasil e especialmente no Rio Grande do Sul é pouco conhecido, sendo que apenas *H. curvispina* teve seu ciclo biológico estudado por SEVERO (1997), porém os resultados não foram publicados. MORELLI (2001), investigou a taxa de fecundidade e a morfologia dos ovários de *Hyalella* sp. proveniente de São José dos Ausentes, RS, mas seus resultados também não foram publicados.

As espécies

As espécies de *Hyalella* objetos do presente trabalho, *H. pleoacuta* e *H. castroi* foram recentemente descritas por González, Bond-Buckup & Araujo, 2006 (Figuras 1, 2 e 3), sendo que as mesmas ocorrem simpatricamente em tanques de cultivo de trutas na localidade do Sítio Vale das Trutas (28°47’00”S - 49°50’53”W), o qual constitui-se em um pesque-paque localizado nos Campos de Cima da Serra, no município de São José dos Ausentes, RS, Brasil.

Estas espécies diferenciam-se especialmente pelo tamanho corpóreo e pela presença de projeções (flanges) em alguns somitos abdominais em *H. pleoacuta.*

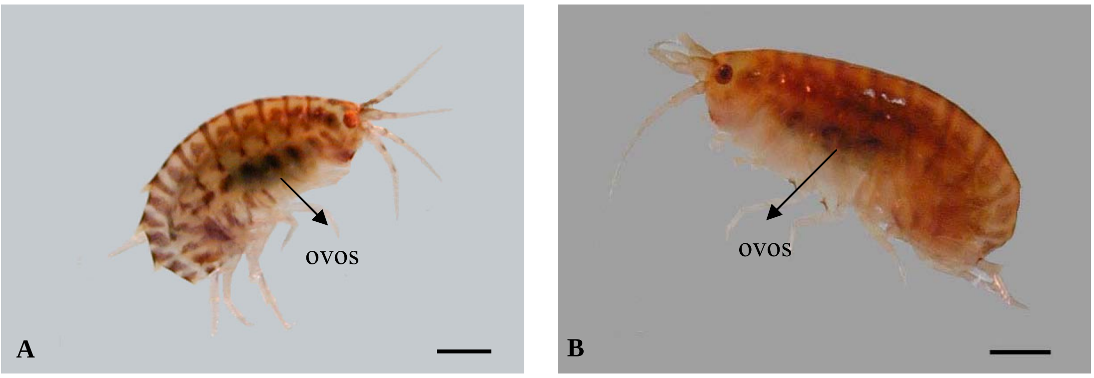

Figura 1. Exemplares fêmeas de *Hyalella pleoacuta* (A) e *Hyalella castroi* (B) mostrando os ovos no interior do marsúpio. Barra: 1 mm.

Figura 2. Exemplares fêmeas de *Hyalella pleoacuta* (A) e *Hyalella castroi* (B) mostrando os juvenis no interior do marsúpio. Barra: 1 mm.

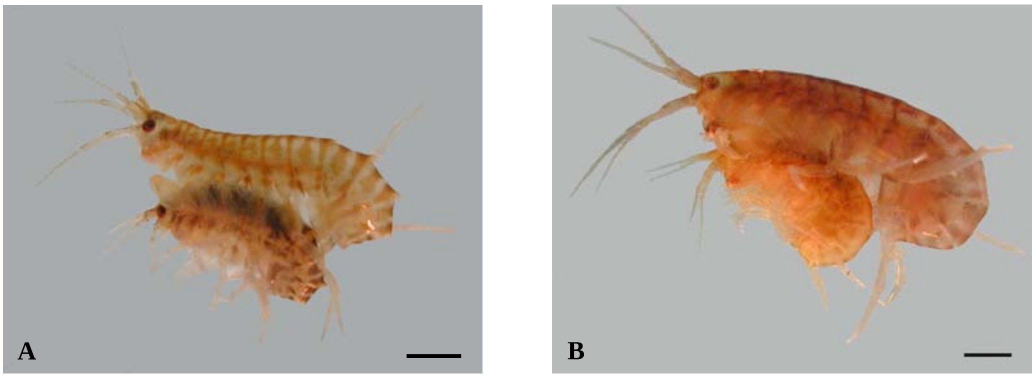

Figura 3. Casais em pré-copula de *Hyalella pleoacuta* (A) e *Hyalella castroi* (B). Barra: 1 mm.

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Objetivos

Várias espécies de *Hyalella* são encontradas no mesmo corpo d'água e a simpatria das mesmas pode ser facilitada, provavelmente devido a diferenças comportamentais e/ou ecológicas entre elas. Neste sentido este trabalho foi desenvolvido com o objetivo de responder a seguinte hipótese:

Os ciclos de vida de *Hyalella pleoacuta* e *H. castroi* apresentam características distintas que possibilitam a coexistência das mesmas.

Para responder esta questão, este trabalho caracterizou e comparou o ciclo de vida de duas espécies simpátricas de *Hyalella* que ocorrem na localidade Vale das Trutas em São José dos Ausentes, RS, *Hyalella pleoacuta* González, Bond-Buckup & Araujo, 2006 e *Hyalella castroi* González, Bond-Buckup & Araujo, 2006, sendo que no ambiente natural foram avaliados:

* sucesso de pareamento e sucesso reprodutivo.

* fecundidade.

* a estrutura populacional: distribuição de freqüência em classes de tamanho do comprimento do cefalotórax, tamanho mínimo, máximo, médio e mediano de machos e fêmeas, proporção sexual, recrutamento e período reprodutivo (freqüência de fêmeas ovígeras).

Além disso, em laboratório, em condições de cultivo, foram avaliados:

* a biologia reprodutiva: tempo de pareamento, tamanho de machos e fêmeas em pré-copula, tempo médio de desenvolvimento embrionário e pós-embrionário, fecundidade e fertilidade.

* o ciclo de muda: tempo de duração de cada estágio de muda desde o juvenil até a fase adulta, crescimento e longevidade.

Metodologia Geral

Os especimens das espécies simpátricas *Hyalella pleoacuta* (Figura 1A) e *H. castroi* (Figura 1B) foram amostrados mensalmente de Agosto de 2003 a Julho de 2004 em 2 tanques de cultivo de trutas (*Oncorhynchus mykiss*) (Walbaum, 1792), na localidade Sítio Vale das Trutas (Figura 2), próximo as nascentes do Rio das Antas no município de São José dos Ausentes, RS (28°47’00”S - 49°50’53”W) (Figura 3).

O Sítio Vale das Trutas foi criado em 1997 e constitui-se num pesque-pasque o qual é composto por 3 tanques de cultivo de trutas, sendo que o tanque localizado na região mais superior mede 30 m de comprimento e 15 m de largura; o tanque do centro mede 20 x 100 m e o inferior, 50 x 50 m. Ambos os tanques apresentam aproximadamente 2 m de profundidade e a água dos mesmos é proveniente do represamento da água do Rio das Antas. As trutas são provenientes do município de Curupá, SC.

A área de São José dos Ausentes, localizada no Planalto Sul Riograndense, região conhecida como Campos de Cima da Serra representa um ambiente de especial interesse ecológico, pois nesta região encontram-se as nascentes do Rio das Antas e do Rio Pelotas. O clima da região é caracterizado como mesotérmico médio, com invernos acentuados e verões brandos pelo efeito da altitude (1100 m) (NIMER 1989), sendo que a temperatura média anual durante o desenvolvimento do presente trabalho foi registrada entre 8,7 ºC e 18,4 ºC e no inverno a média das mínimas temperaturas diárias manteve-se abaixo de 6 °C. Durante o desenvolvimento do presente trabalho as temperaturas mínima e máxima no município de São José dos Ausentes foram registradas em Julho 2004 (3.4 °C) e Fevereiro 2004 (24.1 °C), respectivamente (PREFEITURA MUNICIPAL DE SÃO JOSÉ DOS AUSENTES) (Tabela I).

Cabe salientar que *H. pleoacuta* e *H. castroi* são endêmicas da região dos Campos de Cima da Serra, mais especificamente do município de São José dos Ausentes. A coexistência destas duas espécies de anfípodos de água doce não foi observada em outros rios da região. Apenas *H. castroi* foi amostrada no Rio das Antas, o qual fornece a água para os tanques de cultivo de trutas e também no Rio da Divisa e no Arroio Sepultura, ambos em São José dos Ausentes. Entretanto, *H. pleoacuta* não foi encontrada em rios da região até o presente mmento (BOND-BUCKUP, comunicação pessoal).

Inicialmente, em Maio de 2004 foi realizada uma saída de campo piloto para a padronização do método de amostragem das espécies de *Hyalella.* Nesta saída de campo, foram escolhidos dois tanques para a amostragem dos animais, devido a presença de macrófitas em suas margens, sob as quais as espécies de *Hyalella* são encontradas. Nesta amostragem piloto foi possível constatar que ambas as espécies e também todas as faixas etárias ocorrem aderidas as macrófitas. Em cada tanque de cultivo foram escolhidos dois pontos de amostragem (P1 e P2 localizados no tanque 1 e P2 e P3 localizados no tanque 2) sendo que estes pontos diferiam quanto à presença de distintas macrófitas, tais como *Senecio* sp. Linnaeus, *Ludwigia* sp. Linnaeus, *Hydrocotile* sp. Linnaeus e *Callitriche rimosa* Fasset, 1951. Os pontos de amostragens caracterizam-se como segue: P1: composto predominantemente por macrófita do gênero *Senecio* e alguns exemplares de *Callitriche rimosa*; P2: composto essencialmente por *Senecio*; P3: composto predominantemente por *Ludwigia* e algumas *Hydrocotile*; P4: composto na sua grande maioria por *Hydrocotile* e poucos exemplares de *Callitriche rimosa* (Figura 4). Cabe salientar que as macrótifas dos gêneros *Ludwigia* e *Hydrocotile* e também *Callitriche rimosa* apresentam raízes flutuantes, as quais não possuem contato com o substrato dos tanques de cultivo de trutas e *Senecio* apresenta raízes em contato com o substrato (observação pessoal).

Para a captura dos animais, as macrófitas presentes em cada ponto de amostragem eram retiradas cuidaddosamente com puçás (Figura 5) e as mesmas eram acondicionadas em sacos plásticos e colocadas em isopor com gelo para serem transportadas ao laboratório. Posteriormente, em laboratório, as plantas eram lavadas em água corrente e os animais ficavam retidos em peneiras. Os animais amostrados foram devidamente identificados e o sexo e a condição ovígera das fêmeas foi registrada. Cabe salientar que as fêmeas ovígeras e os casais em pré-cópula foram individualizados logo após a coleta, antes do acondicionamento e transporte ao laboratório.

Os animais foram agrupados em 4 categorias demográficas: juvenis, machos, fêmeas e fêmeas ovígeras (fêmeas com ovos ou juvenis dentro do marsúpio). Estas categorias foram estabelecidas após a determinação do tamanho da maturidade sexual para cada espécie. Posteriormente todos os espécimes foram mensurados quanto ao comprimento do cefalotórax (da margem anterior do rostro até a margem posterior do cefalotórax) sob uma ocular micrométrica de microscópio estereomicroscópio Stemi SV8 Zeiss. De acordo com EDWARDS & COWEEL (1992) e PICKARD & BENKE (1996) o comprimento do cefalotórax apresentou correlação positiva com o comprimento total em *Hyalella azteca* e dessa maneira o comprimento do cefalotórax pode ser usado para representar o tamanho desta espécie assim como de outras espécies do mesmo gênero. Correlação positiva entre o comprimento total e comprimento do cefalotórax foi observada também em outras espécies de anfípodos gamarídeos por SHEADER & CHIA (1970), WILDISH (1972), COSTA & COSTA (1999), LEITE (2002) e GUERAO (2003).

Adicionalmente, foram realizadas amostragens para a obtenção dos animais para serem cultivados em laboratório com a finalidade de caracterizar os aspectos reprodutivos, tais como duração do comportamento pré-copulatório, tamanho corpóreo das fêmeas ovígeras, duração dos períodos embrionário e pós-embrionário, fecundidade, viabilidade e também o ciclo de muda, crescimento e longevidade de ambas as espécies de *Hyalella.*

Após serem amostrados, os exemplares das espécies de *Hyalella* eram levadas para o laboratório e mantidas em aquários de 15 litros durante aproximadamente 15 dias para aclimatação. Os animais foram mantidos em condições controladas de temperatura (19 °C ± 1 °C), intensidade luminosa de cerca de 1.000 lux e fotoperíodo de 12/12 horas de luz/escuro. A água utilizada para o cultivo dos animais era proveniente dos tanques de cultivo de trutas dos quais os animais foram amostrados, sendo que a mesma não foi filtrada previamente antes dos experimentos. Alguns parâmetros físicos e químicos da água estão apresentados na tabela II e os mesmos foram quantificados pelo Laboratório de Análises da Faculdade de Agronomia, Departamento de Solos, Universidade Federal do Rio Grande do Sul. Os exemplares de ambas as espécies de *Hyalella* foram mantidas juntas durante o perído de aclimatação (15 dias) e alimentadas com ração para peixe (TetraDiskus® - 43% de proteina), complementada com macrófitas do gênero *Salvinia* e também com *Callitriche rimosa* trazida do local de amostragem.

Juntamente com a amostragem dos animais, foram mensurados alguns parâmetros ambientais como temperatura da água dos tanques de cultivo, oxigênio dissolvido na água e pH. A temperatura mínima e máxima do ar e a pluvisiodade foram fornecidas pela Prefeitura Municipal de São José dos Ausentes (Tabela I).

Após todos os procedimentos laboratoriais, os espécimens de *H. pleoacuta* e *H. castroi* foram depositados na Coleção Científica do Laboratório de Carcinologia da Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil.

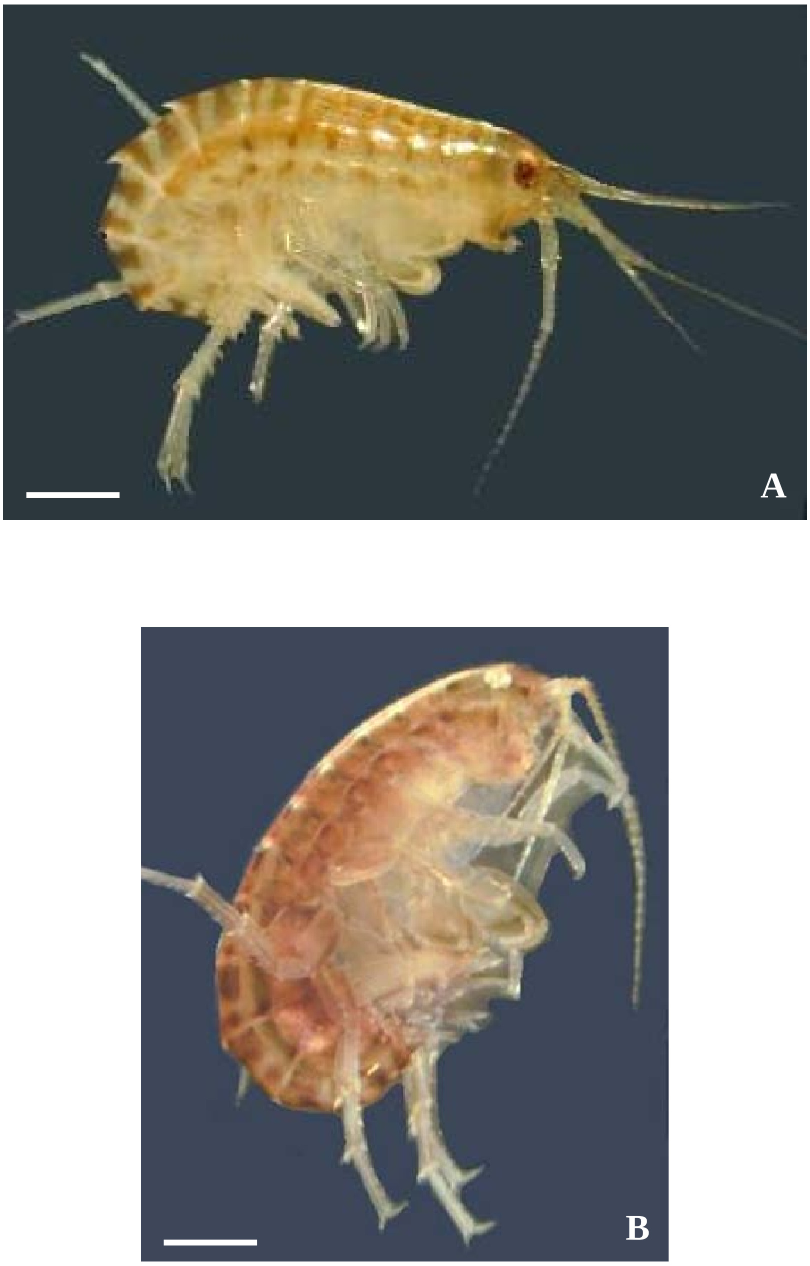

Figura 1. Exemplares fêmeas de *Hyalella pleoacuta* (A) e *H. castroi* (B). Barra: 1 mm.

Figura 2. Sítio Vale das Trutas, São José dos Ausentes, RS, Brasil.

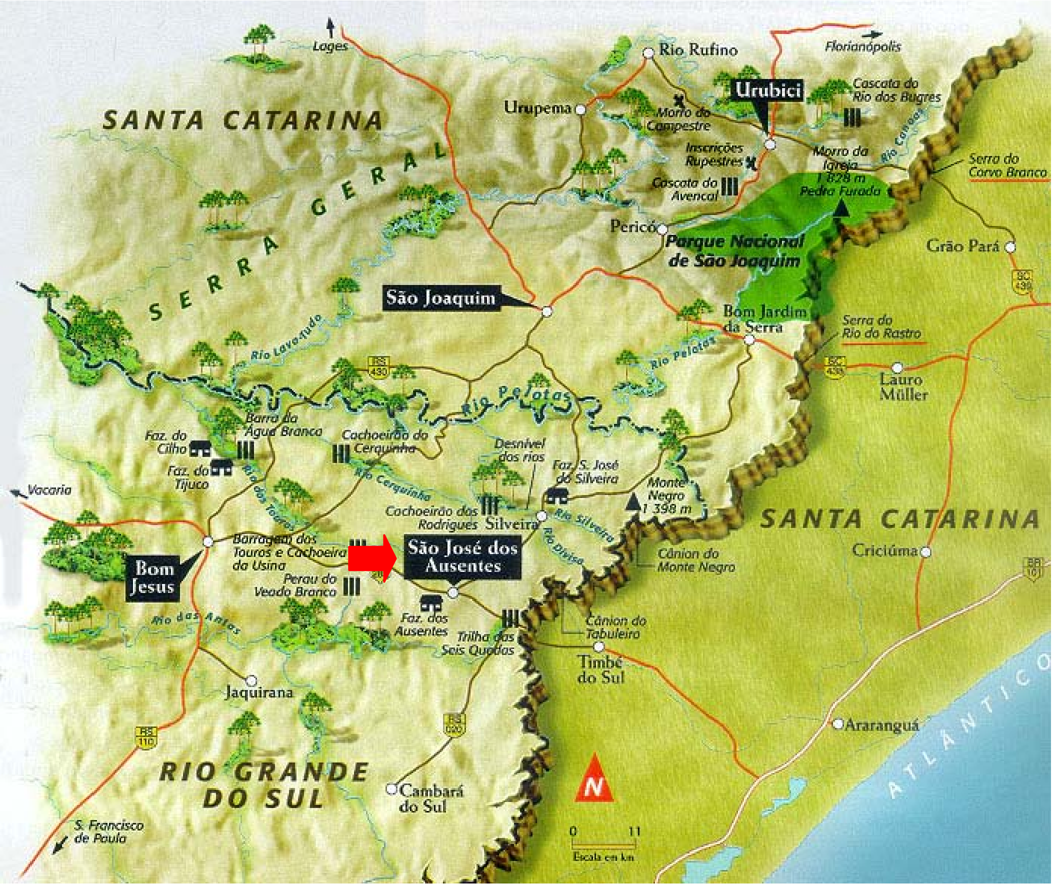

Figura 3. Localização do município de São José dos Ausentes, RS, Brasil.

Ponto 1

Ponto 2

Ponto 3

Ponto 4

Figura 4. Pontos de amostragem de *Hyalella pleoacuta* e *H. castroi*.

Figura 5. Amostragem das espécies de *Hyalella*.

Tabela I. Parâmetros ambientais da localidade do Vale das Trutas, São José dos Ausentes.

	O_2 (%)		**O_2 (mg/l)**		**pH**		**Temperatura da água**		**Temperatura mínima do ar***	**Temperatura máxima do ar***	**Pluviosidade***
	T1	T2	T1	T2	T1	T2	T1	T2			
Ago 03	67,0	71,0	6,90	7,70	8,0	6,60	7,3	9,1	3,6	15,8	36,1
Set 03	78,3	79,4	7,17	7,20	7,2	6,47	10,0	11,0	6,0	18,0	59,9
Out 03	88,0	107,1	8,23	9,23	6,9	7,04	15,6	15,4	8,1	18,6	163,4
Nov 03	77,1	78,2	6,18	6,22	7,0	6,90	20,8	21,4	9,9	20,4	113,2
Dez 03	74,9	84,2	6,30	6,96	7,0	7,42	18,3	18,1	13,9	22,9	205,1
Jan 04	82,1	78,5	6,99	6,64	6,9	6,93	19,5	19,5	13,2	23,6	110,4
Fev 04	83,4	80,8	6,93	6,67	7,0	6,87	18,1	18,6	12,6	24,1	69,8
Mar 04	64,0	69,1	5,09	5,24	6,8	6,70	21,4	21,7	9,8	21,8	69,4
Abr 04	93,1	98,4	7,42	8,12	6,9	6,88	18,7	18,7	10,0	20,9	24,9
Mai 04	87,0	87,2	5,60	5,60	6,4	6,35	14,3	14,5	5,6	16,2	243,1
Jun 04	55,7	50,5	5,17	5,65	7,7	8,90	13,3	13,4	6,4	17,4	41,2
Jul 04	79,0	85,0	7,10	7,60	5,0	5,07	14,3	14,5	3,4	13,9	159,3

Nota: os parâmetros ambientais estão expressos em valores médios; T1= tanque 1; T2= tanque 2 ; * dados fornecidos pela Prefeitura Municipal de São José dos Ausentes.

Tabela II. Parâmetros físicos e químicos da água dos tanques de cultivo de trutas utilizada nos cultivos em laboratório.

Parâmetros	Quantificações*
pH	6,4
Cond. Electric - µS/cm	29,3
Nitrogênio (TKN) - mg/L	0,7
Nitrogênio total - mg/L	1,0
Fósforo total - mg/L	0,16
Potássio total - mg/L	1,5
Cálcio total - mg/L	1,6
Magnésio total - mg/L	0,56
Cobre total – mg/L	< 0,02
Zinco total - mg/L	0,01
Ferro total – mg/L	2,2
Manganês total - mg/L	< 0,01
Sódio total - mg/L	2,4
Boro total - mg/L	0,01
Sulfato - mg/L	2,0
Dureza total - mg $CaCO_3$/L	3,7
$N\text{-}NH_4^+$ - mg/L	0,3
$N\text{-}NO_3^- + N\text{-}NO_2^-$ - mg/L	0,3

Nota: os valores estão expressos em média, as quais foram calculadas a partir de duas amostras de água (Abril 2005 e Abril 2006).

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Intermolt duration and postembryonic growth of two sympatric species of *Hyalella* (Amphipoda, Dogielinotidae) in laboratory conditions.

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Intermolt duration of two sympatric species of *Hyalella*

Artigo submetido a Nauplius

Abstract

This study was designed to test the hypothesis that two sympatric species of freshwater gammaridean, *Hyalella pleoacuta* and *H. castroi* 2006, might show differences at the intermolt duration and postembryonic growth in laboratory conditions. Ovigerous females were collected with a net in April 2005 and April 2006, in a trout culture pond in the Vale das Trutas, municipality of São José dos Ausentes, southern Brazil. These specimens were reared in the laboratory (19 °C ± 1 °C and 12-hour light-dark photoperiod), and examined for signs of release of juveniles at the same time each day. The culture water was changed twice a week. Juveniles that emerged from the brood pouch were separated and classified as stage I; the subsequent stages were identified by each successive molt. All the amphipods were observed twice a day until their death. There was no significant difference between the mean total intermolt period of males (9.68 days) and females (9.03 days) of *H. pleoacuta*. However, in *H. castroi*, the males showed a longer mean total intermolt period (12.18 days) than did females (10.68 days). Sexual dimorphism was observed at stage V in both species (*H. pleoacuta* - males: 25.6 ± 0.65 days and females: 24.7 ± 0.40 days; *H. castroi* - males: 28.4 ± 1.29 days and females: 27.1 ± 0.56 days). Sexual maturity (stage VIII) was attained after 52.3 ± 1.1 days in males and 51.5 ± 1.1 days in females of *H. pleoacuta*. In *H. castroi* the males and females attained sexual maturity after 56.7 ± 2.5 and 53.2 ± 1.3 days, respectively. Males and females attained a maximum cephalothorax length of 0.85 and 0.79 mm, respectively in *H. pleoacuta*, and 0.89 and 0.86 mm, respectively in *H. castroi*. The mean longevity of males and females was 124.8 ± 10.32 and 121.8 ± 9.31 days, respectively in *H. pleoacuta*, and 164.0 and 139.5 days, respectively in *H. castroi*

Key words. Intermolt duration; molt frequency; sexual maturation; growth; *Hyalella*.

Introduction

The bodies of crustaceans are limited by rigid exoskeletons that determine a peculiar type of growth. The growth appears as a discontinuous phenomenon that takes place in staggered increments, whose most evident manifestation is the molting process or ecdysis. Each ecdysis is followed by a post-molt uptake of water and, as consequence, a rapid increase in body size during the short soft-skinned period (Hartnoll, 1982). The increase in weight and size after each molting event obeys to an increase in the volume of the body fluids and the subsequent tissue growth. The neurohormonal regulation of the molt cycle is affected by diverse external and internal factors, being temperature and photoperiod the most relevant ones among the first group, and age, sex, and gonad maturity the most important ones among the second group (Hartnoll, 1982; Lipcius, 1985). Besides, these animals allocate energy between growth and reproduction many times throughout adult life (Stearns, 1992).

The genus *Hyalella* Smith, 1874 occurs in freshwater environments in southern Brazil. Most of the *Hyalella* species are usually found associate to macrophytes, being that others species are found swimming at the water column or burrowing at the sediment of permanent reservoir, lakes and streams (Kruschwitz, 1978; Wellborn, 1995). These species are important members of the benthos, because they are used as food by aquatic birds, fishes and other crustaceans (Kruschwitz, 1978; Wellborn, 1995).

Development of the species of *Hyalella* occurs in the marsupium (Cooper, 1965). These crustaceans, as well all others peracrids, have direct development, and their eggs hatch into juveniles that have the general adult morphology, although they still lack secondary sex characteristics (Strong, 1972; Borowsky, 1991; Steele and Steele, 1991). Development in these species is divisible into an embryonic period, extending from ovulation to hatching and a postembryonic period (juvenile period), extending from hatching to emergence from the marsupium (Borowsky, 1980 a b).

The life cycles of the species of *Hyalella* that occur in Brazil are not known. The majority of studies on *Hyalella* in Brazil have emphasized the taxonomy of the group (Pereira, 1989; Bond-Buckup and Araujo, 1998; Pereira, 2004; González *et al.*, 2006). However, the biological traits of species of *Hyalella* were recently investigated by Castiglioni and Bond-Buckup (submitted a b c d). This work was developed with the aim to test the hypothesis that two sympatric species of freshwater gammaridean, *Hyalella pleoacuta* González, Bond-Buckup and Araujo, 2006 and *H. castroi* González, Bond-Buckup and Araujo, 2006 might show differences at the intermolt duration and postembryonic growth in laboratory conditions. For these, the time to first molt, intermolt duration, molt frequency, age of the sex recognition, age of the sexual maturation and growth were analyzed in males and females of these two sympatric species of *Hyalella*.

Material and Methods

Ovigerous females of two *Hyalella* species were collected with a net in April 2005 and April 2006 in one culture pond holding trout - *Oncorhynchus mykiss* (Walbaum, 1792) in the Vale das Trutas near the headwaters of the Rio das Antas in the municipality of São José dos Ausentes, State of Rio Grande do Sul, Brazil (28°47’00”S - 49°50’53”W). Both species were found together, living under the roots of aquatic macrophytes of the genera *Senecio* Linnaeus, *Hydrocotile* Linnaeus, *Ludwigia* Linnaeus and *Callitriche* Linnaeus along the sides of the ponds. The regional climate is characterized as medium mesotherm**,** with cold winters and mild summers because of the altitude (1,100 m) (Nimer, 1989).

The amphipods collected were placed in plastic bags and kept in a cooler during transportation to the laboratory where the animals were identified. The ovigerous females were cultured in the laboratory at a temperature of 19 °C with a 12-hour light-dark photoperiod. During the embryonic and postembryonic periods, the specimens of both species were kept indiviadualy in containers with a volume of water of 100 ml each. The water used in laboratory cultures was collected together with the amphipods, from the trout culture pond without previous filtering process. *Salvinia* sp. Séguier was added to the container to serve as a substrate as well as food for both species of *Hyalella*. Every other day, fish food (TetraDiskus® - 43% protein) was added *ad libitum* to the cultures as a food supplement for the females. Ovigerous females were examined for signs of release of juveniles at the same time each day. Juveniles that emerged from the brood pouch were placed individually into 50 ml containers, and classified as stage I. The subsequent stages were identified by each successive molt. These amphipods were fed twice a week with three drops of the algae *Ankistrodesmus* sp. Corda, 1838 (3.5 x 10^6 cells/ml) and a small leaf (about 0.009 g) of the macrophyte *Callitriche rimosa* Fasset, 1951. This macrophyte was collected together with the ovigerous females in the trout culture ponds. The water in the cultures was changed twice a week. After the 8th stage, the specimens were transferred to 100 ml containers. All the amphipods were observed twice a day until their death.

The duration (days) of each molt stage was calculated for each amphipod. Next the total mean time between each molt (± SE) was calculated for males and females individually, for each species. Analyses of variance, complemented by a Bonferroni test, were used to compare the mean time between molt stages of each sex of species of *Hyalella* ($\alpha = 0.05$) (Zar, 1996). Each molt stage was compared between males and females of *H. pleoacuta* and *H. castroi* (ANOVA; Bonferroni *test*; $\alpha = 0.05$) (Zar, 1996).

The stages of life cycle were classified based on secondary sexual characters, being considered as: (1) immature: individuals that no have secondary sexual character developed; (2): juvenile: males with rudimentary second gnathopods and females with nosnsetose and rudimentary oostegites; (3) adults ou matures: males with fully developed second gnathopod and females with fully developed oostegites or carrying eggs or juveniles in the maruspium.

Some amphipods were measured for cephalothorax length using a micrometer eyepiece in a stereoscopic microscope (0.01 mm), two days after each molt during the life cycle. This measurement was performed to determine the growth and molt increment in each molt stage of males and females of both species. The molt increment in each molt stage of males and females of each species was compared by analysis of variance (ANOVA) complemented by a Bonferroni test ($\alpha = 0.05$) (Zar, 1996). We also compared the molt increment between males and females of each species, and between males and females of both species (ANOVA; Bonferroni *test*; $\alpha = 0.05$) (Zar, 1996).

The longevity (days) was recorded for each specimen. Next the mean longevity of males and females for each species was calculated. The mean longevity (days) of amphipods was compared between sex and species, using a *t* test ($\alpha = 0.05$) (Zar, 1996).

Results

The life cycle of *H. pleoacuta* and *H. castroi* can be divided into an immature stage (consisting of the first 4 stages), a juvenile stage (stages V, VI and VII) and an adult stage (stage VIII and older). The duration of each stage differed between species (Table I).

Mortality in the first juvenile stages was high: 107 (51.2%) and 83 (35.3%) juveniles died before their sex could be determined, in *H. pleoacuta* and *H. castroi*, respectively. The first molt of the juveniles occurred at 7.1 ± 0.13 days (mean ± SE) (n= 152) in *H. pleoacuta* and 6.8 ± 0.09 days (n= 103) in *H. castroi*.

There was no significant difference between the mean total intermolt period (± SE) of males (9.68 ± 0.31 days) and females (9.03 ± 0.21 days) of *H. pleoacuta* (t= 1.749; p<0.05). However, in *H. castroi*, the males showed a longer mean total intermolt period than the females (males: 12.18 ± 0.50 days; females: 10.68 ± 0.29 days) (t= 2.628; p<0.05). Males and females of *H. castroi* showed a longer mean intermolt period than did males and females of *H. pleoacuta* (males: t= -4.268; females: t= -4.675; p<0.05). Males and females of *H. pleoacuta* molted 10.1 ± 0.38 and 10.1 ± 0.38 times (means ± SE), respectively, whereas males and females of *H. castroi* molted 11.3 ± 0.41 and 10.5 ± 0.35 times along of life cycle, respectively.

The duration (days) of each stage of males and females of *H. pleoacuta* and *H. castroi* is shown in Table I. Males and females molted with more frequency at the 5th stage in *H. pleoacuta* (p<0.05) and at the 6th stage in *H. castroi* (p<0.05). However, males and females of both species showed a longer mean intermolt period in the later stages than in the first stages (p<0.05).

Sexual dimorphism, identified by the enlarged second gnathopods of males and by the oostegites of females, was first observed at the 5th stage in both species of *Hyalella*. This stage was reached after about 25.6 ± 0.65 and 24.7 ± 0.40 days in males and females, respectively, in *H. pleoacuta*. In *H. castroi* sexual dimorphism was attained after 28.4 ± 1.29 days in males and 27.1 ± 0.56 days in females. The sex could be determined at sizes of about 0.35 and 0.37 mm CL in *H. pleoacuta* and *H. castroi*, respectively. Stage VIII (sexual maturity) was attained after 52.3 ± 1.1 days (mean ± SE) and 51.5 ± 1.1 days in males and females, respectively, in *H. pleoacuta*. In *H. castroi* the males and females attained sexual maturity after 56.7 ± 2.5

days and 53.2 ± 1.3 days, respectively. The males and females of *H. pleoacuta* attained sexual maturity at 0.51 and 0.48 mm CL, respectively. In *H. castroi*, males and females were sexually mature at 0.55 and 0.53 mm CL, respectively.

The molt increment in each molt stage of males and females of both species is shown in Figure 1. Although the males showed a high molt increment, there was no significant difference in the molt increment in each molt stage of males and females of both species (p>0.05). Besides, there was no observed significant difference in the molt increment between males and females of both species. In Figure 2 indicates the cephalothorax length of males and females of *H. pleoacuta* and *H. castroi* at each stage. The cephalothorax length of males and females of both species is represented by a sigmoid curve, but males became larger than females with each successive stage, mainly in *H. pleoacuta*. The males and females attained a mean maximum size (cephalothorax length) of 0.85 and 0.79 mm, respectively in *H. pleoacuta*, and 0.89 and 0.86 mm in *H. castroi*. Figure 3 represents the growth curve of males and females of *H. pleoacuta* and *H. castroi* constructed by integrating data on molt increment and intermolt duration.

The longevity of males of *H. pleoacuta* ranged from 58 to 259 days, and that of females from 51 to 266 days. There was no significant difference between the mean longevity (± SE) of males (124.8 ± 10.32 days; n= 33) and females (121.8 ± 9.31 days; n= 42) of *H. pleoacuta* (t= 0.014; p>0.05). In *H .castroi*, the longevity of males ranged from 62 to 240 days and that of females from 56 to 262 days, i.e., males showed greater mean longevity than females (males: 164.0 ± 42.5 days; n= 21; females: 139.5 ± 10.39 days; n= 46) (t= 1.230; p<0.05). Males and females of *H. castroi* showed greater mean longevity than did males and females of *H. pleoacuta* (males: t= 2.509; females: t= -1.296; p<0.05).

Discussion

The stages of the life cycle identified in *H. pleoacuta* and *H. castroi* were similar as observed in *H. azteca* Saussure, 1858 by Cooper (1965). However, the duration of each stage and the age at sexual maturity of the species of *Hyalella* analyzed in the present work differed from *H. azteca*. Probably the duration of each stage of life cycle is directly influenced by the environmental conditions under which the animals are raised or exposed, especially the temperature and photoperiod (Bovee, 1950; Cooper, 1965; Strong, 1972; Kruschwitz, 1978). The intermolt periods in females of *H. pleoacuta* and *H. castroi* were very similar to that observed in *H. azteca* (11 days) by Othman and Pascoe (2001). However, the total intermolt period in males of these two species was lower than that of *H. azteca* studied by Kruschwitz (1978) (17.17 days) and by Othman and Pascoe (2001) (20 days).

Sexual dimorphism is often pronounced in amphipods. The sexes of gammarideans can usually be distinguished by differences in secondary sexual characters, including body size, gnathopods (Moore and Wong, 1996), oostegites (Kruschwitz, 1978), and antennae (Tsoi and Chu, 2005). Development of these secondary sexual characters reflects sexual differentiation and maturation (Hartnoll, 1982). The time of sex recognition in *H. pleoacuta* and *H. castroi* was very similar to the results reported by Nelson and Brunson (1995) and Othman and Pascoe (2001) in *H. azteca* (see Table II). Sex recognition in both species of *Hyalella* could be observed by the development of the second gnathopods in males and by development of the oostegites in females.

The age of sexual maturity of males and females of *H. pleoacuta* was similar to *H. castroi*. However, *H. azteca* became sexually mature at different ages than the species of *Hyalella* analyzed in the current work, as observed in Table II. It should be noted, however, that these variations in the age of sexual maturity may be attributed to the different ambient or laboratory conditions to which different species or populations were exposed. In the case of *H. azteca*, its growth and development are affected by several environmental factors, particularly temperature (Bovee, 1950; Cooper, 1965; De March, 1977; Kruschwitz, 1978), but also photoperiod (De March, 1977; Kruschwitz, 1978), dissolved oxygen (Nebeker *et al.*, 1992), pH (Pilgrim and Burt, 1993) and food quantity and quality (Hargrave, 1970; Moore and Farrar, 1996; Wellborn, 1994). For example, this species reared at lower temperatures can take longer to mature and grow larger than animals reared at higher temperatures.

The growth of individuals of *H. pleoacuta* and *H. castroi* was found to be continuous throughout life, in laboratory conditions. Nevertheless, growth rates were higher in the early phases (juvenile phases) in both species, when it is commonly accepted that individuals grow exponentially. These crustaceans allocate all energies to maintenance and somatic growth at the first phases of their life cycle. After sexual maturity, certain amount of their energies was assimilated to the gonads, in competition with the interrelated processes of maintenance and somatic growth, making it difficult for them to continue to grow at the same rates as juvenile phases (Hartnoll, 1982; Stearns, 1992). Similar growth pattern were reported by Othman and Pascoe (2001) for *H. azteca* and by Maranhão and Marques (2003) for *Echinogammarus marinus* (Leach, 1815), also in laboratory conditions.

Males and females of *H. pleoacuta* and *H. castroi* molted about the same time until sexual maturity. However, in adult males, the intermolt period was longer than in females. This observation is similar to those of Geisler (1944), Strong (1973), Kruschwitz (1978), and Othman and Pascoe (2001), who found that adult females molted more frequently than adult males of *H. azteca*. This is probably because the female are involved in producing and releasing the juveniles, and so they need to molt more frequently than the males.

In the case of gammaridean amphipods, the females have no mechanism for sperm storage and typically produce a clutch of eggs at each molt (Cooper, 1965; Strong, 1973). One to a few days prior to the female molt, males use their anterior gnathopods to grasp the female's second coxal segment, in a pre-copulatory mate-guarding behavior (Borowsky, 1984). Pairs remain attached in this way (male dorsal to female) throughout the female's molt, at which time the first-instar offspring are released and the new clutch of eggs is fertilized by the guarding male as eggs pass into the marsupium. Pairs separate after fertilization (Cooper, 1965; Strong, 1973). The pre-copulatory mate-guarding behavior guarantees that males and females are together during this phase of the female life cycle. The males of *H. pleoacuta* and *H. castroi* carry the females for about 6 days (Castiglioni and Bond-Buckup, submitted c). The embryonic and postembryonic periods take about 15 days in *H. pleoacuta* and 16 days in *H. castroi* (Castiglioni and Bond-Buckup, submitted c). Adding the time that the females of *H. pleoacuta* and *H. castroi* remain together with the males, to the time the females require to incubate the eggs and carrying the hatching juveniles in the marsupium, sums to about 20 days of their reproductive cycle. Thus, the somatic growth of the females is compromised, and they do not molt during this life phase. Afterwards, the females need to molt more frequently than males to compensate for the increase in body size. Besides, it must be taken into account that the body size of the female is correlated with the number of eggs produced, that is the larger the female, the more eggs that she will can produce and carry in the marsupium during the course of embryonic development (Castiglioni and Bond-Buckup, submitted a).

The body sizes at which sex can be identified, and maximum body size attained by individuals of *H. pleoacuta* and *H. castroi* under laboratory conditions were all smaller than in field conditions (see Castiglioni and Bond-Buckup, submitted d). Hargrave (1970) and Moore and Farrar (1996) observed that diet quality and quantity are clearly important in determining growth and reproduction in *H. azteca*. Probably the food quality and quantity influence the growth of *H. pleoacuta* and *H. castroi*. A smaller body size under laboratory conditions was also observed in the amphipod *Echinogammarus marinus* by Maranhão and Marques (2003). In addition to the influence that the quality of the food may have had on these results, the lower body size reached by individuals under laboratory conditions could also be attributable to a decrease in the scope for growth in response to stress. Chen *et al.* (1990) recorded higher mortalities and lower growth rates of penaeid shrimps in culture, because of the deterioration of water quality caused by metabolic wastes, mainly ammonia and nitrite, which can be toxic to crustaceans. It is possible that water-quality problems caused by wastes since the food was supplied *ad libitum* and the water was changed only twice per week, may have influenced the present results for growth and survivor of *H. pleoacuta* and *H. castroi*.

The time to first molt, molt frequency, sex determination, and sexual maturation were very similar between these sympatric species, *H. pleoacuta* and *H. castroi*. However, the species differed in the total intermolt period, maximum body size and longevity of males and females in laboratory conditions. Probably these differences in the life cycle observed in laboratory conditions can facilite the coexistence of these two species in the trout pond. Besides, the knowledge of these biological traits is essential to understanding of the life cycle of these freshwater gammarideans, and for their future utilization in tests of environmental quality.

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Table I. Duration (days) (mean ± SE) of each stage of males and females of *Hyalella pleoacuta* and *H. castroi*.

Stages	***Hyalella pleoacuta***		***Hyalella castroi***	
	Juveniles		**Juveniles**	
I	7.1 ± 0.13 (213) e		6.8 ± 0.09 (200) d	
II	6.0 ± 0.13 (200) d		6.7 ± 0.11 (168) d	
III	6.0 ± 0.11 (152) d		6.8 ± 0.19 (145) d	
IV	6.6 ± 0.17 (152) de		7.1 ± 0.17 (124) d	
	Males	**Females**	**Males**	**Females**
V	8.1 ± 0.27 (58) c	7.7 ± 0.25 (94) de	7.4 ± 0.49 (25) c	7.7 ± 0.26 (78) e
VI	8.6 ± 0.31 (57) c	9.0 ± 0.38 (87) cd	9.1 ± 0.74 (25) c	8.5 ± 0.38 (68) de
VII	10.1 ± 0.40 (46) c	10.9 ± 0.44 (66) bc	11.1 ± 0.90 (22) bc	10.4 ± 0.61 (58) d
VIII	12.9 ± 0.86 (34) bc	12.6 ± 0.75 (42) b	15.0 ± 1.28 (21) b	14.3 ± 1.02 (47) c
IX	17.3 ± 2. 11 (19) ab	17.5 ± 2.23 (25) a	20.4 ± 1.55 (19) a	19.4 ± 1.64 (29) b
X	19.2 ± 1.74 (17) a	18.1 ± 2.01 (20) a	20.3 ± 1.78 (16) a	22.8 ± 1.72 (25) ab
XI	21.4 ± 1.93 (14) a	17.4 ± 1.12 (15) a	20.6 ± 2.30 (14) a	24.2 ± 1.83 (22) a
XII	22.0 ± 3.55 (10) a	19.8 ± 2.17 (12) a	25.5 ± 2.79 (11) a	24.9 ± 1.70 (18) a
XIII	22.3 ± 4.37 (4) a	20.8 ± 2.23 (9) a	25.6 ± 2.69 (8) a	25.4 ± 1.22 (13) a
XIV	25.7 ± 4.26 (3) a	23.6 ± 3.23 (7) a	29.0 ± 1.00 (2) a	28.4 ± 1.51 (7) a
XV	26.0 (1) a	23.7 ± 0.88 (3) a		
XVI		26.0 ± 6.00 (2) a		

Note: Juvenile stages I to IV were not separated into males and females, since these juveniles could not be sexed because their secondary sexual characters are not yet developed. Numbers between parentheses are number of individuals analyzed. Small letters correspond to the comparisons of each sex of each species of *Hyalella*. Values with at least one letter in common did not differ statistically ($\alpha = 0.05$).

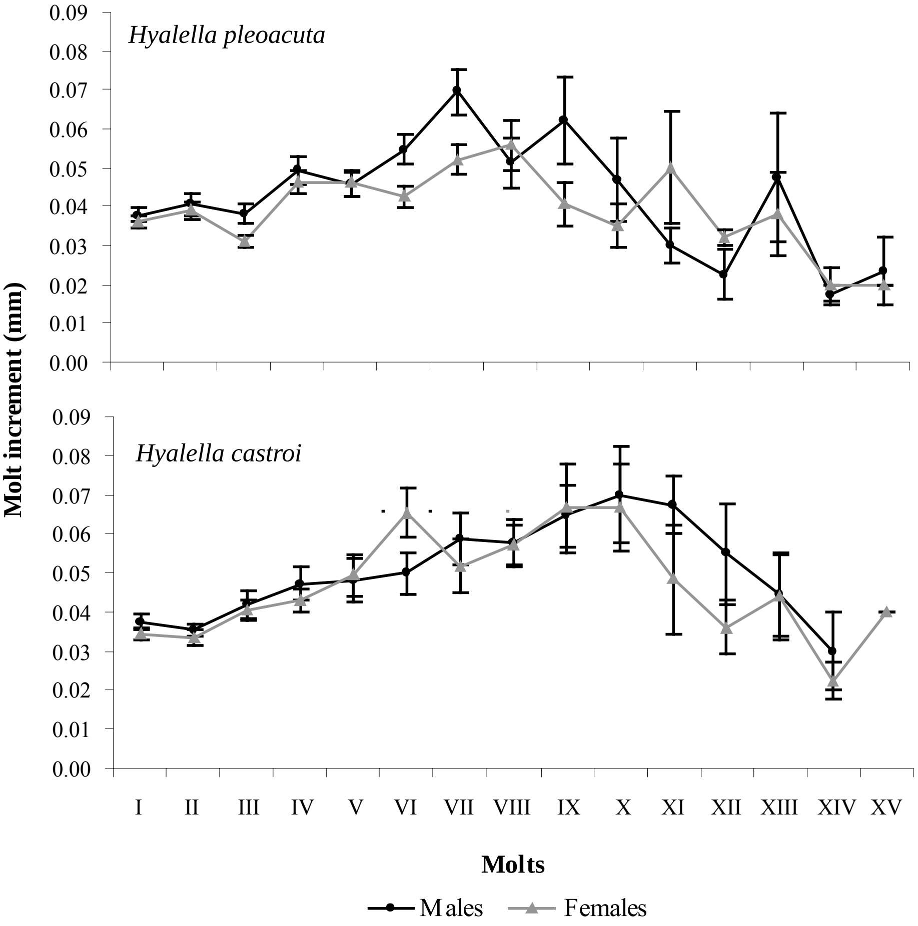

Figure 1. Mean molt increment (± SE) of males and females of *Hyalella pleoacuta* and *H. castroi* in each development stage.

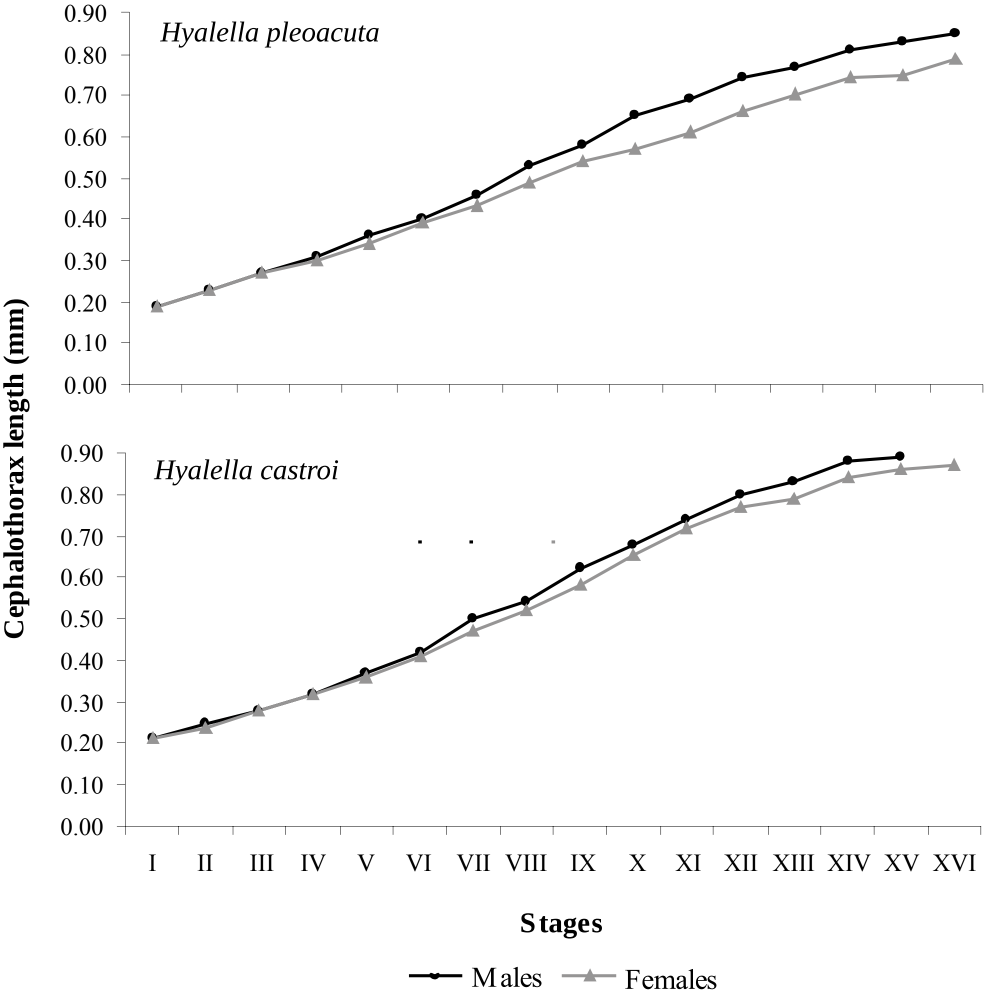

Figure 2. Mean cephalothorax length of males and females of *Hyalella pleoacuta* and *H. castroi* at each stage of their life cycle.

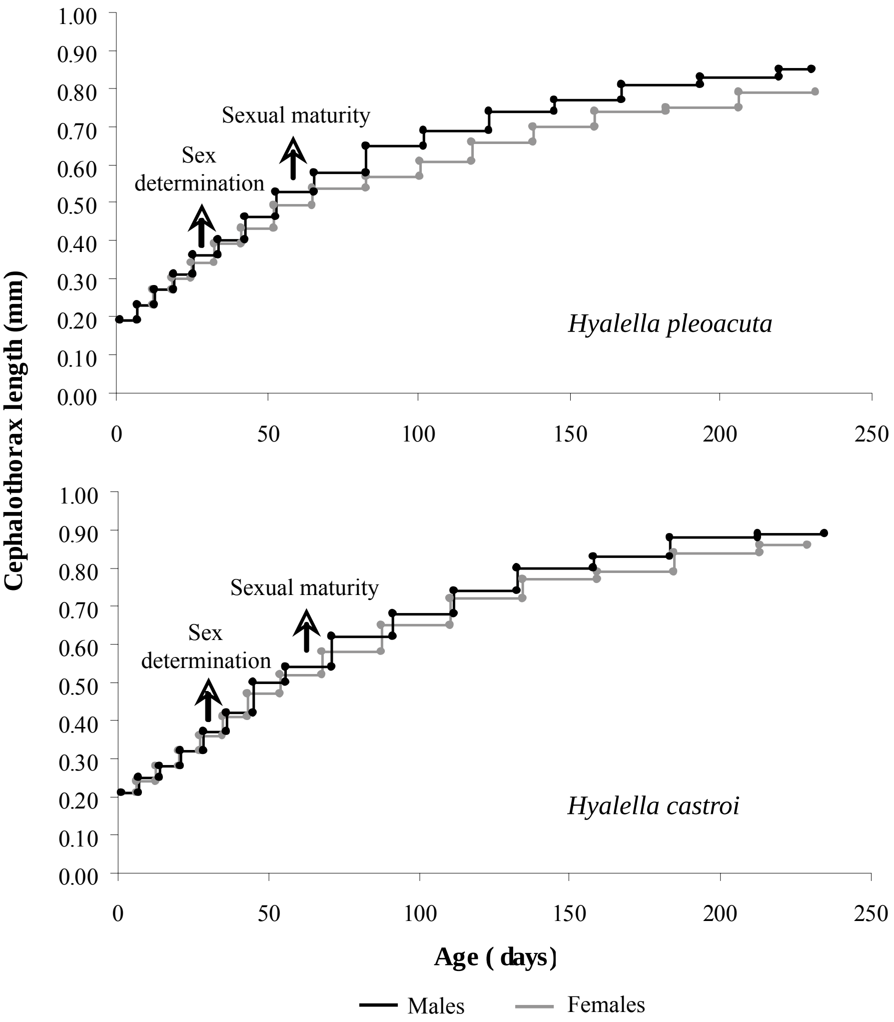

Figure 3. Growth curve of males and females of *Hyalella pleoacuta* and *H. castroi* in laboratory conditions constructed by integrating data on molt increment and intermolt duration.

Table II. Comparison of age at sex recognition and age at sexual maturity of some species of *Hyalella.*

	Age of the sex recognition (days)	**Age of the sexual maturity (days)**	**Laboratory conditions (temperature and photoperiod light/dark)**	**Authors**
Hyalella azteca	_	60 - 65	20 ºC - 22 ºC	Bovee (1950)
Hyalella azteca	_	26 - 41	26 ºC - 28 ºC	Bovee (1950)
Hyalella azteca	_	36	20 ºC and 15 h / 9 h	Cooper (1965)
Hyalella azteca	_	24	20 ºC and 16 h/ 8 h	Nelson and Brunson (1995)
Hyalella azteca	19-21	23	22 ºC and 16 h / 8 h	Othman and Pascoe (2001)
Hyalella pleoacuta	Males: 24.7* Females: 25.6*	Males: 52.3* Females: 51.5*	19 ºC and 12 h / 12 h	Present work
Hyalella castroi	Mals: 27.1* Females: 28.4*	Males: 56.7* Females: 53.2*	19 ºC and 12 h / 12 h	Present work

* = means

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Pairing and reproductive success in two sympatric species of *Hyalella* (Crustacea, Amphipoda, Dogielinotidae) from southern Brazil.

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Abstract

Reproductive success is determined by an individual's ability to form precopulatory pairs with receptive mates, and also by the number of eggs fertilized in a typical mating. This study aimed at characterizing pairing and reproductive success in relation to male and female sizes of *Hyalella pleoacuta* and *H. castroi* from southern Brazil. These amphipods exhibit precopulatory mate guarding, in which a male will carry a potential mate beneath its ventral surface, guarding the female for several days until it molts and lays its eggs. The specimens were collected monthly with nets, from November 2003 to July 2004 in two trout aquaculture ponds in the Vale das Trutas. The precopulatory pairs and ovigerous females were identified and separated in the field. In the laboratory, they were measured (cephalothorax length in mm), using a micrometer eyepiece in a stereoscopic microscope. Pairing success was estimated from the proportion of mating males and females related to their respective background individuals by size classes. Reproductive success was estimated from egg production. The mean size of paired males was larger than that of the background males and females, and did not differ between the species. Positive assortative mating by size was observed in both species; i.e., larger males tended to pair with larger females. Male pairing success increased sharply with size. In both species, reproductive success in males increased with body size; however, the females of intermediate size classes showed greater reproductive success. This result supports the hypothesis that loading constraints play a part in structuring size-assortative pairing in these species.

Key words: assortative mating; pairing success; reproductive success; *Hyalella*.

1. Introduction

The genus *Hyalella* Smith, 1874 is known only from the Nearctic and Neotropical biogeographical regions; 51 species have been described (González and Watling, 2002; Pereira, 2004; González et al., 2006). Members of the genus are found in a variety of freshwater habitats such as lakes, ponds and streams, clinging to vegetation and burrowing in bottom sediments, where they are important members of the benthic fauna (Kruschwitz, 1978; Wellborn, 1995; Grosso and Peralta, 1999).

Sexual dimorphism in *Hyalella* consists of the enlarged second gnathopods of the male, which are about 15 times larger than in females (Kruschwitz, 1978; Wellborn, 2000). These large second gnathopods are used for maneuvering the female into the carrying and copulatory positions. The small first gnathopods are used in carrying the female (Kruschwitz, 1978); this appendage can influence reproductive success in some populations of the *H. azteca*-complex (Wellborn, 1995, 2000; Wellborn and Bartholf, 2005).

Precopulatory mate-guarding behavior is common among species in which mating is confined to a short period in the female's reproductive cycle (Crespi, 1989), as observed in *H. azteca* (Saussure, 1858) (Wen, 1992). A mature female amphipod is available for fertilization after molting, when the cuticle is sufficiently flexible to allow release of the eggs through the genital pores into the brood pouch. A male has only this short time between molting and ovulation to deposit sperm into her brood pouch (Birkhead and Clarkson, 1980; Sheader, 1983; Borowsky, 1988). Males of different amphipod taxa exhibit a variety of mating behaviors and secondary sexual changes at maturity (Bousfield, 1979; Borowsky, 1988). According to Conlan (1991), gammaridean amphipod males use one of two strategies for mating: either they mate-guard by carrying or attending their mates until the females are ready to molt and be fertilized, or they do not guard, instead searching benthically or swarming pelagically at the time that the females are ready to molt.

Mating-guarding by carrying has been documented for species of the superfamilies Gammaroidea, Talitroidea, and Hadzioidea (Conlan, 1991). In these amphipods, the male guards a potential mate by carrying her beneath his ventral surface for several days before she becomes available for mating. Pairs remain attached in this way (male dorsal to female) through the female's molt, and the new clutch of eggs is fertilized by the guarding male as the eggs pass into brood pouch. The pair separates after fertilization (Wellborn, 1995). The function of mate-guarding behavior is to ensure that the male is present during the short period when the female is receptive, and also secures the female against predatory attack by other males while her cuticle is soft after molting (Strong, 1973; Lewbel, 1978; Borowsky, 1984; Dick et al., 1990).

Pairing in amphipods is size-assortative: a linear relationship between male and female size has been found in field and laboratory studies, i.e., large males usually pair with large females, and small males with small females (Birkhead and Clarkson, 1980; Ward, 1985; Adams and Greenwood, 1987; Hatcher and Dunn, 1997; Lefebvre et al., 2005).

In *H. azteca*, the females appear to have some control over pair formation, and are generally more receptive to male mating attempts during the later stages of their molt interval (Strong, 1973; Wellborn, 1995). Females do not store sperm and usually produce a new clutch of eggs at each molt (Cooper, 1965; Strong, 1973). The duration of the precopulatory mate-guarding phase is variable within the species-complex, with species co-occurring with *Lepomis* Rafinesque, 1819 pairing for a shorter duration than those species found in habitats where these predators are absent (Strong, 1973; Wellborn, 2001; Cothran, 2002).

In this study, pairing success and reproductive success in relation to male and female sizes of two *Hyalella* species from southern Brazil were estimated.

2. Material & Methods

2.1. Collection of mating pairs and background individuals

Specimens of *Hyalella pleoacuta* González, Bond-Buckup and Araujo, 2006 and *H. castroi* González, Bond-Buckup and Araujo, 2006 were collected with a net, monthly (November 2003 to July 2004) in two artificial ponds used to raise trout (*Oncorhynchus mykiss*) (Walbaum, 1792) in the Vale das Trutas, near the headwaters of the Antas River in the Municipality of São José dos Ausentes, southern Brazil (28°47’00”S - 49°50’53”W). Both species of *Hyalella* are found together, living under the roots of plants in the genera *Senecio* Linnaeus, *Hydrocotile* Linnaeus, *Ludwigia* Linnaeus, and *Callitriche* Linnaeus around the edges of the culture ponds.

In the field, mating (precopulatory pairs) and ovigerous females were identified and separated. In the laboratory, all amphipods (included the unpaired amphipods) were measured (cephalothorax length, CL, in mm) using a micrometer eyepiece in a stereoscopic microscope.

In order to minimize the effects of embryo loss and mortality within the brood pouch, egg production in both species was analyzed using only data obtained from females carrying eggs in the brood pouch (stage I + stage II + stage III) (Costa and Costa, 1999). All eggs were removed from the brood pouch and counted under a stereoscopic microscope.

2.2. Data analysis

For each month, the percentage of adults in the field that were found in precopulatory pairs in relation to unmated adults was estimated. The minimum size of adults included in the calculation was the smallest size observed in pairs (0.53 mm CL for males and 0.48 mm for females in *H. pleoacuta,* and 0.70 mm CL for males and 0.67 mm for females in *H. castroi*).

To assess the extent to which *Hyalella* species mate assortatively by size, we examined the correlation between male and female cephalothorax length (CL) for pairs in the field. The mean sizes (cephalothorax length) of unpaired and paired males were compared with the mean sizes of unpaired and paired females, by means of a *t* test ($\alpha = 0.05$) (Zar, 1996).

The mean cephalothorax lengths of paired and unmated amphipods were compared, to assess whether pairing success was influenced by body size (Analysis of Variance; $\alpha = 0.05$) (Zar, 1996). An index of relative pairing success adapted from an index of relative feeding bias described by Manly (1974) was used to evaluate the form of the relationship between pairing success and body size for males: $\beta_i = (r_i / n_i) / \Sigma\, rj / nj$

where,

β_i is the relative pairing success in size class *i*, and r_i and n_i are the proportions of males of size *i* in pairs and in the background sample, respectively). The denominator sums the ratio across all *m* adult size classes. The minimum size class used in the analysis was the minimum size of males and females observed in precopulatory pairs. This index of relative pairing success was modified by Wellborn (1995) and used for both species. Female pairing success was independent of body size, as described below.

According to Wellborn (1995), reproductive success will depend not only on pairing success, but also on the number of eggs fertilized during mating. Reproductive success was therefore defined as:

$$R_{M,i} = \beta_i e_i / \Sigma \beta_i e_i$$

where,

$R_{M,i}$ is reproductive success of males in size class *i*. The expected number of eggs fertilized by paired males in size class *i*, e_i was determined by calculating the expected fecundity of each paired female using regressions of female fecundity on female body size (*H. pleoacuta:* $F= 63.843\ CL^{1.8544}$; *H. castroi*: $F= 41.948\ CL^{1.6523}$). The denominator sums quantities across all *m* adult size classes.

Because pairing success does not depend on body size in females, a female's reproductive success derives simply from egg production:

$$R_{F,i} = f_i / \Sigma f_j$$

where,

$R_{F,i}$ is the reproductive success of females in size class *i*, f_i is the mean observed fecundity (number of eggs in the brood pouch) of background females in size class *i*, and the denominator sums fecundity values across all *m* adult female size classes. The minimum size class was the smallest observed in mating pairs.

3. Results

For males and females, respectively, the percentage of individuals in precopulatory pairs was 16.46% and 14.35% in *H. pleoacuta*, and 12.79% and 10.71% in *H. castroi*. Species did not differ in the proportion of paired individuals for either sex ($p>0.05$). Figure 1 illustrates the frequencies of paired males and females and background individuals during the year.

The mean cephalothorax length of paired males was greater than that of unpaired males in *H. pleoacuta* ($t= 14.290$; $p<0.05$) and *H. castroi* ($t= 8.194$; $p<0.05$) (Table 1). However, the mean cephalothorax length of paired females did not differ significantly from the background females (*H. pleoacuta*: *t*= 2.749; *H.* castroi: *t* = 0.045; p>0.05) (Table 1). Paired and unpaired males of both species were larger than the females (*p*<.0.05) (Table 1).

A propensity to mate assortatively by size was observed in both species, with large males generally paired with large females, and small males with small females. The size-assortative pairing was more pronounced in *H. pleoacuta* (r= 0.52) than in *H. castroi* (r= 0.42) (*p*<0.05) (Fig. 2).

Pairing success increased sharply with body size for males in both species: a male in the largest size class was about 20 times more likely to be paired than an individual in one of the smallest size classes in both *H. pleoacuta* and *H. castroi* (Fig. 3).

Reproductive success increased with body size for males in both species. However, the females of the intermediate classes showed greater reproductive success in both *H. pleoacuta* and *H. castroi*. The pattern of reproductive success observed in males and females was similar for both species (Fig. 4).

4. Discussion

According to Crespi (1989), size-assortative mating, one of the most common mating patterns in natural populations, can be explained by three hypotheses: (1) mate choice, whereby large males, large females or large individuals of both sexes choose large mates because they benefit reproductively and are differentially capable of exercising choice; (2) mate availability, whereby large females or large individuals of both sexes are differentially available for mating because of size-differential variation in mating probability or timing that is unrelated to mate choice; and (3) mating constraints, whereby relative size differences between males and females cause physical or energetic difficulties with courtship, pairing or mating. In the majority of gammarideans, assortative mating may be explained by mating constraints, because the males carry females during the precopulatory mate-guarding phase, as observed in the two species analyzed in the present work. Distinguishing among hypotheses based on mate choice, mate availability and mating constraints is crucial to understanding the roles of natural selection and sexual selection in causing assortative mating.

The low frequency of mates sampled throughout the year can be explained by the disturbance created by the sampling process, which led to separation of some pairs prior to collection (personal observation; see also Ward, 1988 and Wellborn, 1995). Wellborn (1995) also observed that fewer than 10% of adults were found in precopulatory pairs in two populations of *Hyalella* in Michigan, U.S.A.

Sexual selection is selection in relation to access to mates (Arnold, 1983). Thus, a difference in the mean sizes of paired and unpaired animals can be used to estimate the intensity of sexual selection on body size. The paired males showed a greater size than unpaired males in both *Hyalella* species analyzed in the present study. Lefebvre et al. (2005) and Wellborn and Bartholf (2005) made similar observations for another freshwaters amphipods, *Paracalliope fluviatilis* (Thomson, 1879) and *H. azteca* complex, respectively. According to Ward (1983) and Dick and Elwood (1990), the larger males may be less likely to lose their mates during the precopulatory mate-guarding phase which is typical of freshwater amphipods, and may be more likely to succeed in attempts to take over a guarded female.

However, body size did not affect pairing success in females: both paired and background females showed the same mean body size in *H. pleoacuta* and *H. castroi.* This was also observed by Wellborn (1995) in *Hyalella* spp., Wellborn and Bartholf (2005) in *H. azteca* complex and by Ward (1988) in *Gammarus pulex* (Linnaeus, 1758), suggesting that females of all sizes find mates, and male availability does not limit female reproductive success. However, in *G. duebeni* Lilljeborg, 1851 studied by Hatcher and Dunn (1997), very large females had a lower probability of pairing than smaller ones: the mean weight of paired females was significantly lower that that of the background individuals.

Amphipod males generally expend most of their reproductive effort in mating, while females allocate relatively more time and energy to parental effort, especially the production of large nutritive gametes. It is probably this reproductive difference that leads to the distinct dimorphism between males and females in shape (size): generally, mean male size was greater than females. In *H. pleoacuta* and *H. castroi* the males were, on average, significantly larger than the females, which are in accordance with previous observations by Geisler (1944), Wen (1992) and Othman and Pascoe (2001) for *H. azteca.* Adams and Greenwood (1983) noted that males of *Gammarus pulex* were larger than the females, because of the mechanical demands of carrying their partner. Ward (1983) suggested that large *G. pulex* males have two advantages over small males: they are better able to successfully take over a female in a competition with another male, and better able to resist such takeover attempts themselves while paired. According to Adams and Greenwood (1983) and Adams et al. (1985), a larger male-to-female size ratio may allow the male to carry the female more easily during the precopulatory mate-guarding phase.

In both *Hyalella* species investigated in the present study, there was a significant positive relationship between the size of females and the size of males holding them in precopula: relatively larger males tended to hold relatively larger females in precopula. Wellborn (1995) observed size-assortative mating for one specie of the *Hyalella azteca* complex, assessed as the correlation between male and female size (head length). However, no significant relationship was found between male and female size in precopulatory pairs in *H. azteca* studied by Wen (1992) and Othman and Pascoe (2001). In laboratory experiments, Wen (1992) found that both large and small males preferentially select large females, presumably because of their fecundity, and that in competition between males, larger males usually win this contest. This pairing behavior is probably evolutionarily favored because large females are more fecund than small ones, and mating with large females enhances the reproductive success of the males.

Positive assortative pairing has also been found in other gammaridean amphipods, such as *Gammarus troglophilus* Hubricht and Mackin, 1940 (Jenio, 1980), *G. duebeni* (Ward, 1985; Hartcher and Dunn, 1997), *G. pulex* (Birkhead and Clarkson, 1980; Elwood and Dick, 1990; Ward and Porter, 1993; Hume et al., 2002; Bollache and Cézilly, 2004), *G. leopoliensis* Jazdzewski and Konopacka, 1989 (Zielinski, 1998) and *Paracalliope fluviatilis* (Lefebvre et al., 2005). Although various hypotheses have been advanced to explain assortative pairing by size in amphipods (mechanical constraints: Crozier and Snyder, 1923; loading constraint: Adams and Greenwood, 1983; spatial segregation: Birkhead and Clarkson, 1980; male-male competition: Ward, 1983; sexual selection: Ward, 1984; guarding time: Elwood et al., 1987; Elwood and Dick, 1990; Dick and Elwood, 1996; microhabitat segregation: Ward and Porter, 1993; see Crespi, 1989 for review), one of the most widely accepted views involves a large male advantage in the male-male competition for access to larger, more fecund females (Ward, 1983; Ward, 1986; Ward, 1988; Elwood et al., 1987; Crespi, 1989; Dick and Elwood, 1996; Bollache et al., 2000), and physical constraints (Adams and Greenwood, 1987; Adams and Greenwood, 1989; Adams et al., 1989; Hatcher and Dunn, 1997). In laboratory experiments, Wen (1992) observed that precopulatory behavior in *H. azteca* supports the male-male competition hypothesis of Ward (1983) and Elwood et al. (1987).

For males of *H. pleoacuta* and *H. castroi*, pairing success and reproductive success increased with body size, indicating that larger males have a greater chance of encountering a suitable female to mate. Wellborn (1995), analyzing the reproductive success in two species of

Hyalella which differed substantially in adult body size and other life-history traits, and occupied ecologically dissimilar habitats, observed that the form and degree of the relative mating advantage for males appeared to differ between these populations. In the marsh species, male pairing success increased throughout the range of variation in male size; and in the lake species, small males had lower pairing success than intermediate-sized and large males, but large males did not have greater pairing success than males of an intermediate size.

Female reproductive success increased with body size in two species of *Hyalella* from Michigan (U.S.A.) studied by Wellborn (1995). However, in the present study, the larger females showed lower reproductive success than females of intermediate sizes. The results support the prediction that very large females should have a lower probability of pairing than smaller ones, although larger females are more fecund. In this context, the larger females have a smaller chance of encountering a suitable male with which pair formation is feasible. Hence, these females are constrained to pairing with a limited subset of males, whereas the smaller females would have ab initio a higher pairing success because they are able to pair with a higher proportion of the males. This supports the hypothesis that loading constraints play a part in structuring size-assortative pairing in both *Hyalella* species investigated in the present study. Similar observations were made for *G. duebeni* by Hartcher and Dunn (1997). According to Adams and Greenwood (1983) and Greenwood and Adams (1984), the males may nevertheless pair preferentially with larger (more fecund) females, within the limits of size constraints. Therefore, a further study on precopulatory behavior in the laboratory is needed in order to more fully understand the causes of pairing patterns in *H. pleoacuta* and *H. castroi.*

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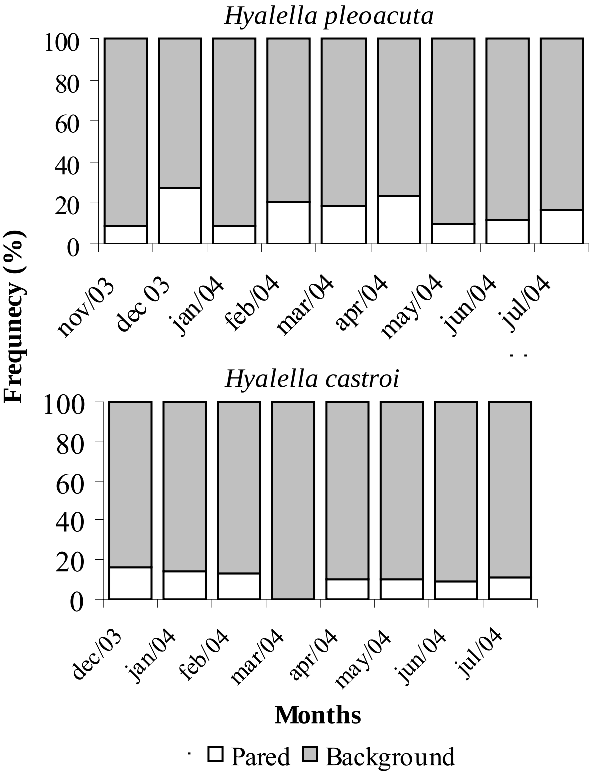

Fig. 1. Relative frequency of precopulatory mating pairs and background individuals in each month of sampling.

Table 1. Mean (± standard error) body size (cephalothorax length in mm) of mated and background individuals of *Hyalella pleoacuta* and *H. castroi*.

	Sex	Mate	Background
Hyalella pleoacuta			
	Males	0.80 ± 0.004 (491)	0.74 ± 0.002 (2471)
	Females	0.68 ± 0.003 (491)	0.67 ± 0.001 (2892)
Hyalella castroi			
	Males	0.95 ± 0.01 (77)	0.86 ± 0.004 (588)
	Females	0.80 ± 0.008 (77)	0.80 ± 0.003 (679)

Note: the number of amphipods in each category is gives in parentheses.

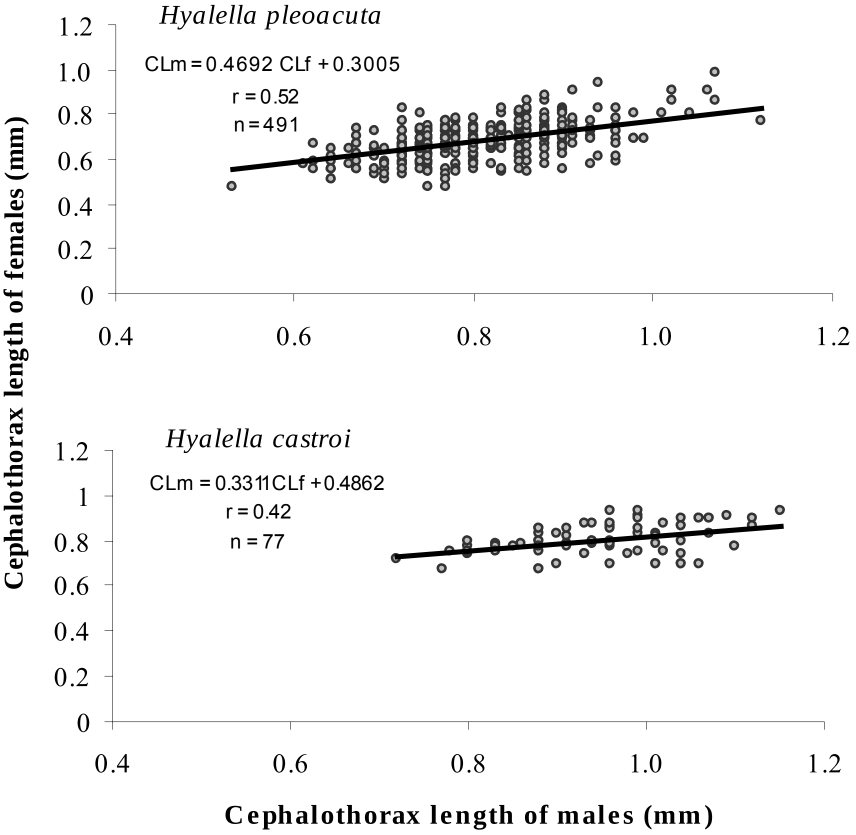

Fig. 2. Assortative mating assessed as the relationship of male and female body size (cephalothorax length in mm) for pairs in *Hyalella pleoacuta* and *H. castroi*. Clm= cephalothorax length of males; CLf= cephalothorax length of females; n= number of individuals.

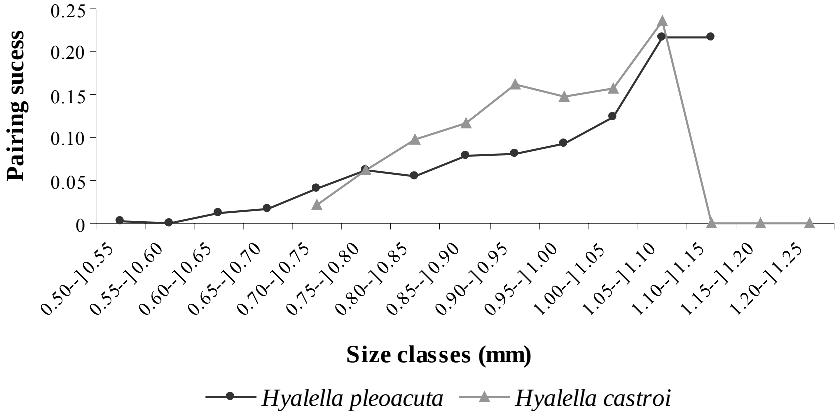

Fig. 3. The relationship of pairing success (*β*) and body size (cephalothorax length in mm) for males in *Hyalella pleoacuta* and *H. castroi*.

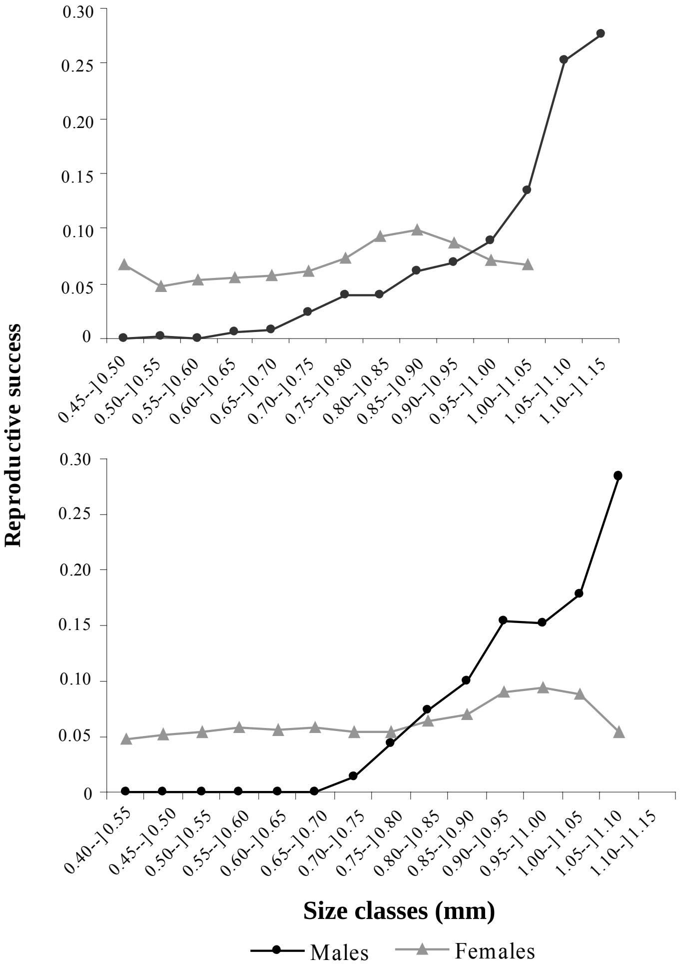

Fig. 4. Relative reproductive success of males ($R_{M,i}$) and females ($R_{F,i}$) expressed as a function of body size (cephalothorax length in mm) in *Hyalella pleoacuta* and *H. castroi*.

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Reproductive strategies of two sympatric species of *Hyalella* Smith, 1874 (Amphipoda, Dogielinotidae) in laboratory conditions.

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Abstract

This study was performed in order to test theoretical predictions that sympatric species might show different reproductive strategies to facilitate their coexistence. The duration of precopulatory mate-guarding behaviour, body size of ovigerous females, embryonic and postembryonic development time, fecundity, and viability of the sympatric amphipod species *Hyalella pleoacuta* and *H. castroi* were investigated under laboratory conditions (19 °C ± 1 °C and 12-hour light-dark photoperiod). Specimens were collected with nets in Autumn and Spring 2004, and Summer and Winter 2005 in the Vale das Trutas, São José dos Ausentes County, southern Brazil, and transported to the laboratory. Both species of *Hyalella* showed similar reproductive strategies, especially in respect to the duration of the precopula, duration of the embryonic period, and fecundity. However, these species differed in body size of ovigerous females, duration of the post-embryonic period, viability, and body size of juveniles that remained in the marsupium and those released by females. These differences in the reproductive traits of *H. pleoacuta* and *H. castroi* may facilitate their coexistence in nature.

Keywords: *Hyalella*, precopulatory mate-guarding time, embryonic and postembryonic periods, fecundity, viability.

Introduction

Freshwater amphipods of the genus *Hyalella* Smith, 1874 are widely distributed throughout North America, and occur also in Central and South America (González and Watling 2002). The life cycles of the species of *Hyalella* that occur in Brazil are not known. The majority of studies on *Hyalella* in Brazil have emphasised the systematic of the group (Pereira 1989; Bond-Buckup and Araujo 1998; Pereira 2004; González et al. 2006).

Amphipods are frequently used as bioindicators and biomonitors in a variety of aquatic ecosystems because they are found in marine, terrestrial, and freshwater environments (Borgmann et al. 1989; Buyle 1989; Nelson and Brunson 1995; Duan et al. 1997; Rinderhagen et al. 2000). Some features of the life cycle of these crustaceans, in particular their reproductive strategies, may be appropriate to interpret the results of ecotoxicological studies that utilise these organisms as bioindicators, especially in the species of *Hyalella* native to southern Brazil. In Brazil, as in the United States and Canada, ecotoxicological studies have used the North American species *Hyalella azteca* to evaluate the effect of different heavy metals on sediment quality of freshwater environments (Hogg et al., 1998). Assessment of the impacts caused by pollutants in a certain environment should use species endemic to the locale to be evaluated, because the effects may be under- or over-estimated when exotic species, for example *H. azteca*, are used.

Most of the species of *Hyalella* are found in a variety of permanent freshwater habitats, from large lakes to small ponds and marshes, clinging to vegetation, swimming in open water, or burrowing in bottom sediments (Kruschwitz 1978; Wellborn 1995; Grosso and Peralta 1999). However, some species are sympatric, such as *Hyalella pleoacuta* González, Bond-Buckup and Araujo, 2006 and *Hyalella castroi* González, Bond-Buckup and Araujo, 2006; others live in caves, such as *H. caeca* Pereira, 1989; and one species, *H. rionegrina* Grosso and Peralta 1999, is found in wet soils (Grosso and Peralta 1999). The main aim of this study was to test theoretical predictions that sympatric species might show different reproductive strategies to facilitate their coexistence. The precopulatory mate-guarding time, the duration of embryonic and postembryonic development, the body size of ovigerous females, fecundity, and viability were investigated under laboratory conditions, to aid in understanding the reproductive strategies of *H. pleoacuta* González, Bond-Buckup and Araujo, 2006, and *H. castroi* González, Bond-Buckup and Araujo, 2006.

Materials and methods

Collection of amphipods

Specimens of the two species of *Hyalella* were collected with a net (Autumn and Spring 2004 and Summer and Winter 2005) in two ponds used for culturing trout, *Oncorhynchus mykiss* (Walbaum, 1792). The ponds are located in the Vale das Trutas, near the headwaters of the Antas River in São José dos Ausentes County, southern Brazil (28°47'00"S - 49°50'53"W). Both species are found together, living under the roots of aquatic macrophytes of the genera *Senecio* Linnaeus, *Hydrocotile* Linnaeus, *Ludwigia* Linnaeus, and *Callitriche* Linnaeus, on the edges of the ponds. The local climate is medium mesotherm**,** with cold winters and mild summers, because of the altitude of 1,100 m (Nimer 1989).

Laboratory procedures and data analysis

The amphipods were transported to the laboratory, where they were identified. They were maintained in the laboratory at 19 °C and a 12-hour light/dark photoperiod. During a 15-day acclimation period, the specimens of both species were kept in two aquariums, each with a final volume of 15 liters of water. During this period, plants of *Salvinia* Séguier were added to the aquarium, to provide substrate and food. Every other day, fish food (TetraDiskus® - 43% protein) was added *ad libitum* as a food supplement. The water used in laboratory cultures was collected together with the amphipods, from the trout-culture pond, without previous filtering. Some physical and chemistry water parameters are shown in Table I.

To determine the length of the precopulatory period, about 120 males and 100 females of *H. pleoacuta* and about 70 males and 50 females of *H. castroi* were kept together in a container in 1 liter of water. *Salvinia* was provided as food and substrate, and fish food was added every other day. The containers were checked twice a day. Precopulatory pairs were transferred from the containers to an aquarium that had six connected compartments. The pairs were observed twice a day until they separated. Ovigerous females were separated from the males and observed daily to determine the embryonic and postembryonic development time. During the same period, another container with males and females was set up, to obtain more ovigerous females in order to characterise the embryonic and postembryonic periods. The embryonic development time was recorded as the period from oviposition until the release of juveniles into the marsupium; the postembryonic development time was recorded as the period from the release of juveniles into the marsupium until their liberation from the marsupium.

To assess the extent to which *Hyalella* species mate assortatively by size, the correlation between male and female head length (HL) was examined for mating pairs in the laboratory. The mean size of paired males and females was compared by a *t* test ($\alpha = 0.05$) (Zar 1996). The head length (tip of the rostrum to the posterior head margin in lateral view) was measured with a dissecting microscope and an ocular micrometer (0.01 mm).

The contents of the marsupium of ovigerous females were characterised as one of three embryonic developmental stages, based on Hynes (1955) and Subida *et al.* (2005), and one postembryonic stage (juvenile remaining in the marsupium). These stages were distinguished easily by means of a stereoscopic microscope, and recognised as follows: stage I, with little or no cleavage, orange coloration, and yolk occupying the entire egg space; stage II, the beginning of the cellular cleavage is visible, but the body parts are not defined; stage III, the yolk is consumed, reduced to a tiny spot on the embryo, and the eyes are easily visible; stage IV, with hatching juveniles present in the marsupium (see Castiglioni & Bond-Buckup, submitted *a* for more details).

The fecundity (number of eggs) and viability (number of juveniles released by females) were determined under laboratory conditions (19 °C and 12/12 hours light/dark) for *H. pleoacuta* and *H. castroi*. About 250 males and 200 females of each species were kept together in an aquarium with a final volume of 15 liters of water. After they were fertilised, the females containing eggs at different stages of embryonic development were killed, and the number of eggs and/or juveniles was counted by means of a stereomicroscope. The mean number of eggs and the body size of ovigerous females were compared between *H. pleoacuta* and *H. castroi* using a *t* test ($\alpha = 0.05$) (Zar 1996). Analyses of variance, complemented by a Bonferroni test, were used to compare the number of eggs produced in each embryonic developmental stage and the number of juveniles remaining in the marsupium in each species ($\alpha = 0.05$) (Zar 1996).

We carried out regression analyses of egg numbers for each embryonic developmental stage, the number of juveniles remaining in the marsupium (y), and the head length (mm) of ovigerous females (x) ($y = ax - b$). Pearson's correlation coefficient (r) was calculated for each relationship (Zar 1996). To determine whether the egg production at each embryonic developmental stage differed in size-specific fecundity, the embryonic stages were compared by covariance analysis, with the number of eggs and/or juveniles as the response variable, head length as the covariate, and the embryonic developmental stage as the grouping variable ($\alpha = 0.05$) (Zar 1996).

After the juveniles hatched, some females were isolated in containers with a final water volume of 100 ml, in order to determine the mean number of released juveniles (viability). The mean number of released juveniles was compared with the mean number of juveniles remaining in the marsupium during the postembryonic development for each species, by means of a *t* test ($\alpha = 0.05$) (Zar 1996). The mean number of released juveniles was compared between the species, by means of a *t* test ($\alpha = 0.05$) (Zar 1996).

The head length was measured in juveniles born from ovigerous females (40 juveniles born from 8 females), with a dissecting microscope and an ocular micrometer and (0.01 mm). Student's *t* test for mean comparisons ($\alpha = 0.05$) was used to compare the mean size of juveniles inside the marsupium of females with those released by females (Zar 1996).

Results

Precopulatory mate-guarding time

The duration of the precopulatory mate-guarding phase is shown in Table II. The *t* test indicated no significant differences in the duration of this behaviour between *H. pleoacuta* and *H. castroi* ($t = 0.585$; $p > 0.05$).

Paired males of both species were significantly larger than the females (*H. pleoacuta*: $t = 13.618$; *H. castroi*: $t = 16.744$; $p < 0.05$) (Table III). A propensity to mate assortatively by size was observed in *H. pleoacuta* ($r = 0.52$) ($p < 0.05$), with large males generally paired with large females, and small males with small females. However, size-assortative mating in *H. castroi* was less pronounced ($r = 0.23$) ($p < 0.05$) than in *H. pleoacuta* (Figure 1).

Duration of embryonic and postembryonic development

The duration of embryonic and postembryonic development is shown in Table IV. The length of the embryonic period was not significantly different between the two species ($t = 0.629$; $p > 0.05$). However, the postembryonic period was significantly longer in *H. castroi* than in *H. pleoacuta* ($t = -5.741$; $p < 0.05$). The total embryonic period was significantly longer in *H. castroi* (16.1 ± 0.21) than in *H. pleoacuta* (15.3 ± 0.20) ($t = -2.966$; $p < 0.05$).

A total of 59 females of *H. pleoacuta* were observed after fertilisation. Of these females, 66.1% carried the young until they completed embryonic development, 13.6% aborted, 5.1% ate the juveniles after they left the marsupium, and 15.3% died during the incubation period. In *H. castroi*, 35 females were observed after fertilisation. Of these, 65.7% carried the young until they completed their development, 20% aborted, and 14.3% died during the incubation period.

During the time when juveniles were inside the female marsupium, no more-developed parental care was observed in both species, as for example, feeding the juveniles, except for aeration by beating their pleopods and sometimes by flexing the uropods. The juveniles did not return to the marsupium after they were released.

Fecundity and viability

Eggs within a single brood developed almost synchronously, and all eggs hatched on the same day in both species (observations performed in the laboratory). The number of eggs (stage I + stage II + stage III) produced by ovigerous females of *H. pleoacuta* in the laboratory ranged from 10 to 59 (n = 147), and the number of juveniles from 15 to 40 (n = 35). The egg production of *H. castroi* ranged from 7 to 42 (n = 57), and the number of juveniles from 16 to 36 (n = 29). There was no significant difference between the mean number of eggs produced by *H. pleoacuta* (26.6 ± 0.75) and *H. castroi* (26.1 ± 1.10) ($t = 0.477$; $p > 0.05$). There was no significant difference between the species in the mean number of juveniles that remained in the marsupium (*H. pleoacuta*: 25.1 ± 1.05; *H. castroi*: 24.9 ± 0.94; $t = -0.464$; $p < 0.05$). There was no significant difference between the mean number of eggs in each embryonic developmental stage and the mean number of juveniles remaining in the marsupium during the embryonic period in either species ($p > 0.05$) (Table V). Furthermore, females were not observed moulting during the embryonic and postembryonic period in both species of *Hyalella.*

Ovigerous females of *H. pleoacuta* had a smaller mean head length ± SE (0.75 ± 0.001 mm) than did females of *H. castroi* (0.86 ± 0.001 mm) ($t = -9.594$; $p < 0.05$). The females of *H. pleoacuta* carrying juveniles in the marsupium had a smaller mean size (0.75 ± 0.001 mm) those of *H. castroi* (0.85 ± 0.001 mm) ($t = 5.947$; $p < 0.05$).

There was a positive linear correlation between the number of eggs in all embryonic developmental stages and size (head length) of females of *H. pleoacuta* and *H. castroi* ($p<0.05$). There was also a correlation between the number of juveniles remaining in the marsupium and the head length of females in both *H. pleoacuta* (Figure 2) and *H. castroi*

(Figure 3) ($p < 0.05$). Table VI shows the results of the analysis of covariance of the number of eggs or juveniles produced by *H. pleoacuta* and *H. castroi* at the different embryonic developmental stages. There was no significant difference between the number of eggs and juveniles in either *Hyalella* species ($p > 0.05$).

The mean number of juveniles that hatched in the laboratory (± SE) was higher in *H. pleoacuta* (24.9 ± 1.13 juveniles; n = 64) than in *H. castroi* (23.8 ± 1.46 juveniles; n = 40) ($t = 2.588$; $p < 0.05$). The mean number of hatching juveniles did not differ from the mean number of juveniles remaining in the marsupium during postembryonic development in either species of *Hyalella* ($p > 0.05$).

The mean head length (± SE) of juveniles carried by females in the marsupium and juveniles liberated by females was 0.19 ± 0.001 mm in *H. pleoacuta* and 0.22 ± 0.002 mm in *H. castroi*. These results showed that juveniles of both species did not moult during the time that they remained inside the marsupium after hatching. The juveniles that remained in the marsupium and those released by females of *H. castroi* showed a greater mean head length than juveniles of *H. pleoacuta* ($t = 9.644$; $p < 0.05$).

Discussion

Precopulatory mate-guarding time

The reproductive behaviour of *H. pleoacuta* and *H. castroi*, like that of *H. azteca* Saussure, 1858 (Strong 1973; Wen 1992; Nelson and Brunson 1995), involves a precopulatory mate-guarding phase, during which the male holds and carries the female. The large second gnathopods of the male are used for manoeuvring the female into a carrying and copulatory position, while the small first gnathopods are used to carry the female during the precopulatory mate-guarding phase. A precopulatory pair remains attached, with the male dorsal to the female, until the female moults; the male continues to guard as the eggs pass into the marsupium, and the pair separates after fertilisation occurs. Precopulatory mate-guarding ensures that the male is present during the short period when the female is receptive, and also secures the female against other males while her cuticle is soft after moulting (Strong 1973; Borowsky 1984; Dick et al. 1990). The duration of the precopulatory mate-guarding phase in the species of *Hyalella* analysed in the present study was similar to that observed by Othman and Pascoe (2001) for *H. azteca* and Strong (1973) for *H. azteca* from Hunter Hot Springs. However, Geisler (1944) reported that pairs of *H. azteca* remained attached for 7 days, and Strong (1973) reported that pairs of *H. azteca* from Siltcoos Lake and Suttle Lake remained together for 3.29 and 1.05 days, respectively (at 22 ºC). The duration of the precopulatory mate-guarding phase can vary with temperature and salinity (Hartnoll and Smith 1978), predation intensity (Strong 1973), the number of competitors, and the number of available females (Ward 1983). The duration of mate guarding also depends on the length of the period in which females can be mated after moulting, and the ability of the females to delay ovulation (Conlan 1991). In *Gammarus pulex* Linnaeus, 1758, females cannot be mated after 12 hours following moulting (Birkhead and Clarkson 1980). On the other hand, in *Orchestia gammarellus* (Pallas, 1766), mating is still possible up to 72 hours after the female moults (Campbell-Parmentier 1963).

Paired males of both *Hyalella* species were larger than paired females. This sexual dimorphism is considered essential for male performance during precopulatory guarding (Sexton 1924, 1928; Vlasbom 1969), because the larger the male, the more easily it can carry the female (Adams and Greenwood 1983; Adams et al. 1985). Furthermore, large males have two advantages over small males: (1) they are better able to successfully "take over" females in competition with another male, and (2) they are better able to resist such take-over attempts themselves while paired (Ward 1983).

Both *Hyalella* species investigated in the present study showed size-assortative pairing, with larger males carrying larger females during the precopulatory mate-guarding phase. Probably, this size-assortative pairing is explained by loading constraints (Adams and Greenwood 1983; Greenwood and Adams 1984) and the male-male competition hypothesis (Ward 1983). According to the male-male competition hypothesis, larger males have two advantages over smaller males: they are better able to affect a takeover, and they are able to resist takeover attempts while paired (WARD 1983). Under the loading constraints hypothesis, males are constrained to select females much smaller than themselves because only those females can be easily carried during precopulatory mate guarding (Adams and Greenwood 1983). In laboratory experiments, Wen (1992) observed that precopulatory behaviour in *H. azteca* supports the male-male competition hypothesis of Ward (1983) and Elwood et al. (1987). Size-assortative pairing was also observed in *H. pleoacuta* and *H. castroi* in field conditions (Castiglioni and Bond-Buckup, submitted *b*), and in *Gammarus troglophilus* Hubricht and Mackin, 1940 (Jenio 1980), *G. duebeni* Lilljeborg, 1851 (Ward 1985; Hatcher and Dunn 1997), *G. pulex* (Linnaeus, 1758) (Birkhead and Clarkson 1980; Elwood and Dick 1990; Ward and Porter 1993; Hume et al. 2002; Bollache and Cézilly 2004), *G. leopoliensis* Jazdzewski and Konopacka, 1989 (Zielinski 1998), and *Paracalliope fluviatilis* (Thomson, 1879) (Lefebvre et al. 2005).

Duration of embryonic and postembryonic development

Embryonic development, from ovulation to hatching, of *H. pleoacuta* and *H. castroi* requires about 12 days at 19 ºC. This is similar to periods reported for *H. azteca* by Strong

(1972), and Edwards and Cowell (1992), in which the embryonic period lasted 10.9 to 13.4 days at 20 ºC and 11.1 days at 25 ºC, respectively. However, Geisler (1944) and Cooper (1965) observed that embryonic development in *H. azteca* was 21 days at 25 ºC and 9.3 days at 25 ºC, respectively. The duration of embryonic and postembryonic development in amphipods is strongly influenced by the temperature and photoperiod to which the females are submitted during this period (Strong 1972; Kruschwitz 1978; De March 1978; Borowsky 1980 a; Shillaker and Moore 1987; Takeuchi and Hirano 1992; Maranhão and Marques 2003). Another factor that may cause differences in estimates of the duration of the embryonic period is the possibility of the existence of cryptic species. For instance, although *H. azteca* has been traditionally considered a single, widely distributed species, but recent molecular genetic studies have demonstrated that it is a species complex (Thomas et al. 1997; Hogg et al. 1998; McPeek and Wellborn 1998; Witt and Hebert 2000).

The juveniles of both species of *Hyalella* investigated in the present study remained in the marsupium after the embryonic period of 1.0 to 6.0 days. Geisler (1944) reported a similar period of 3 days in the marsupium for *H. azteca*. While the juveniles were in the marsupium, no parental care was observed in either species of *Hyalella*. Juveniles of both species did not moult during their development inside the marsupium. This was confirmed by the similar head length observed in juveniles inside the marsupium and those liberated by the females. Juvenile permanence inside the marsupium occurs in other amphipod species, such as *Parallorchestes ochotensis* Brandt, 1851, in which the females display no parental care (Kobayashi et al. 2002), *Gammarus palustris* Bousfield, 1969 (Borowsky 1980 b), and *G. pulex* (Welton et al. 1983). Extended parental care in amphipods can improve the survival and growth rates of offspring, which has been confirmed by experimental studies (Aoki 1997; Thiel 1999; Kobayashi et al. 2002).

Fecundity and viability

Females of *H. pleoacuta* and *H. castroi* could produce consecutive broods (iteroparity) under laboratory conditions, which were confirmed by the presence of developed (dark-green) ovaries in females that were carrying eggs or juveniles in the marsupium. Taking into account the results for the duration of embryonic development, and the extrapolated values for the time of female maturation and the estimated female life-span (Castiglioni and Bond-Buckup, submitted *c*), one should expect more than 2 generations per year in both species (multivoltine life cycle). Multivoltinism has been reported in other species of gammarideans, such as *Pseudorchestoidea brasiliensis* (Dana, 1853) (Cardoso and Veloso 2001), *Gammarus locusta* (Linnaeus, 1758) (Costa and Costa 1999), *Echinogammarus longisetosus* Pinkster, 1973 (Guerao 2003), and *G. chevreuxi* Sexton, 1913 (Subida et al. 2005).

Ovigerous females of *H. pleoacuta* produced more eggs than *H. castroi* in field conditions, even though the females of the former species are larger. The lower egg production observed in *H. castroi* may be compensated by the production of larger eggs and also by low brood mortality (Castiglioni and Bond-Buckup, submitted *a*). However, these species produced fewer eggs under laboratory conditions, compared to females in the natural environment (Castiglioni and Bond-Buckup, submitted *a*). Temperature and photoperiod may influence egg production, and therefore the females may produce fewer eggs under laboratory conditions (Kinne 1961; Steele and Steele 1969; Steele 1981). Laboratory experiments suggest that the quantity and quality of food is also important in determining brood size, as has been suggested in studies on other amphipods (Fish and Preece 1970; Van Dolah et al. 1975; Sheader 1983, France 1992; Muskó 1993).

No decrease in egg number during development was observed in either *H. pleoacuta* and *H. castroi*, as occurs under natural conditions in both species (Castiglioni and Bond-

Buckup, submitted *a*), as well as in other species of amphipods (Beare and Moore 1996; Dick et al. 1998; Cunha et al. 2000; Dick et al. 2002; Kevrekidis 2004, 2005). This may be related to the available space in the marsupium for incubating eggs, which is sufficient to maintain the number of eggs from the initial development stage to the moment of hatching. The absence of predators in the laboratory expriments may also account for the lack of any decrease in egg number.

There was a significant correlation between the number of eggs or juveniles and the size of ovigerous females in both species, as also observed in wild populations (Castiglioni and Bond-Buckup submitted *a*). Brood size in gammarideans is often reported as being proportional to body size of females (e.g., Beare and Moore 1996; Costa and Costa 1999; Persson 1999; Cunha et al. 2000; Othman and Pascoe 2001; Alcocer et al. 2002; Maranhão and Marques 2003; Kevrekidis 2004, 2005).

Conclusions

Both species of *Hyalella* showed similar reproductive strategies under laboratory conditions, especially in respect to the duration of the pre-copula and the embryonic period and fecundity. However, these species differed in the body size of ovigerous females, duration of the post-embryonic period, viability, and body size of juveniles which remained in the marsupium and those liberated by females. These differences in the reproductive traits of *H. pleoacuta* and *H. castroi* might also facilitate their coexistence in nature. This is the first account of the reproductive strategy of endemic species of *Hyalella* in Brazil. Further studies on reproductive behaviour, feeding, and sensitivity to contaminants will increase our understanding of the reproductive strategies of these freshwater amphipods and their possible future use as bioindicators and biomonitors.

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Table I. Physical and chemical parameters of the water of the culture pond holding trout used in laboratory cultures.

Parameters	Quantifies
O_2 (mg/L)	6.9
O_2 (%)	78
pH	6.4
Cond. Electric - µS/cm	29.3
Nitrogen (TKN) - mg/L	0.7
Nitrogen total - mg/L	1.0
Phosphor total - mg/L	0.16
Potassium total - mg/L	1.5
Calcium total - mg/L	1.6
Magnesium total - mg/L	0.56
Copper total – mg/L	< 0.02
Zinc total - mg/L	0.01
Ferro total – mg/L	2.2
Manganese total - mg/L	< 0.01
Sodium total - mg/L	2.4
Boron total - mg/L	0.01
Sulphate - mg/L	2.0
Hardness total - mg $CaCO_3$/L	3.7
$N\text{-}NH_4^+$ - mg/L	0.3
$N\text{-}NO_3^- + N\text{-}NO_2^-$ - mg/L	0.3

Note: values are express in mean obtained of two water sampled (April 2005 and April 2006).

Table II. Duration (days) of the precopulatory mate-guarding phase in *Hyalela pleoacuta* and *H. castroi*.

	Hyalella pleoacuta	*Hyalella castroi*
Minimum – Maximum	4.0 – 12.0	2.0 – 11.0
Mean ± SE	5.6 ± 0.36	5.4 ± 0.40
Number of mating pairs	31	20

Table III. Body size (head length in mm) of paired males and females of *Hyalella pleoacuta* and *H. castroi*.

	Hyalella pleoacuta		*Hyalella castroi*	
	Males	**Females**	**Males**	**Females**
Minimum – Maximum	0.61 – 1.12	0.58 – 0.94	0.80 – 1.25	0.66 – 0.99
Mean ± SE	0.88 ± 0.009	0.72 ± 0.007	0.99 ± 0.009	0.80 ± 0.007
Number of individuals	100	100	92	92
t test	13.618		16.744	

Table IV. Duration (days) of the embryonic and postembryonic periods in *Hyalella pleoacuta* and *H. castroi*.

	Hyalella pleoacuta			*Hyalella castroi*		
	EP	**PP**	**TP**	**EP**	**PP**	**TP**
Min – Max	11 – 15	01– 05	13 – 20	10 – 14	03 – 06	14 – 18
Mean ± SE	12.2 ± 0.12	3.2 ± 0.12	15.3 ± 0.20	12.0 ± 0.23	4.1 ± 0.13	16.1 ± 0.21
N	39	39	39	23	23	23

Note: EP = embryonic period; PP = postembryonic period; TP = total embryonic period; Min = minimum; Max = maximum; N = number of ovigerous females.

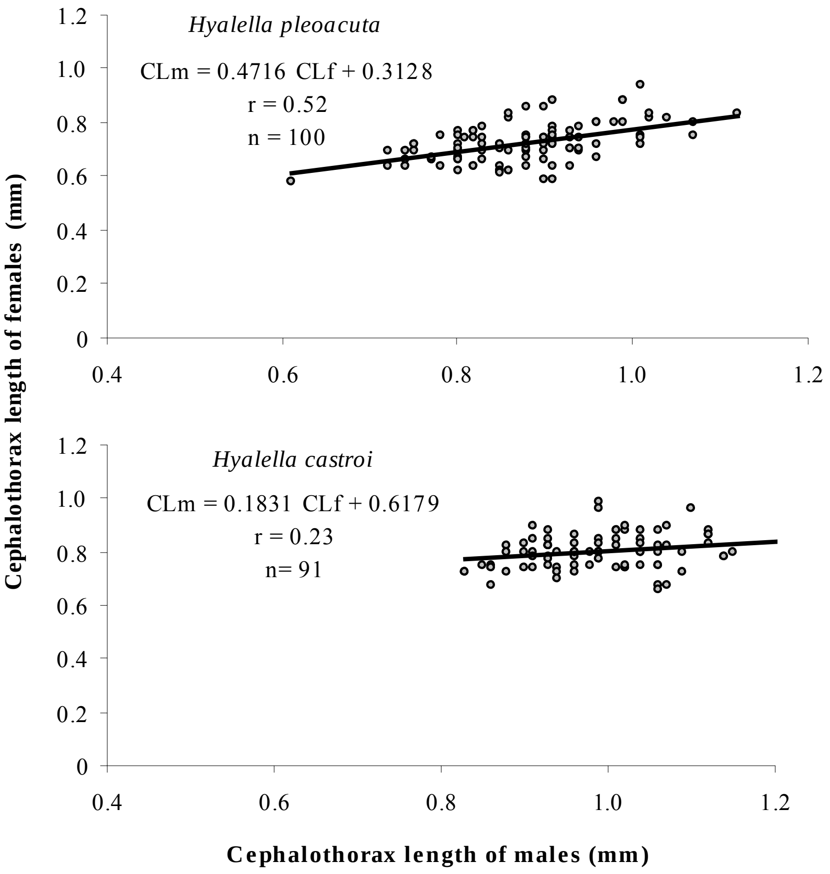

Figure 1. Assortative mating, estimated as the relationship of male and female body size (head length in mm) for precopulatory mating pairs in *Hyalella pleoacuta* and *H. castroi*. Clm= cephalothorax length of males; CLf= cephalothorax length of females; n= number of individuals.

Table V. Comparison of the mean number of eggs in each embryonic developmental stage and number of juveniles remaining in the marsupium of *Hyalella pleoacuta* and *H. castroi*.

	Hyalella pleoacuta			*Hyalella castroi*		
Development stage	**Min – Max**	**Mean ± SE**	**N**	**Min – Max**	**Mean ± SE**	**N**
Stage I	12 – 46	26.0 ± 1.12	54	16 – 45	26.3 ± 0.93	48
Stage II	16 – 36	25.5 ± 0.89	35	17 – 40	26.0 ± 1.03	34
Stage III	16 – 38	25.3 ± 1.03	32	15 – 45	25.4 ± 1.60	28
Stage IV	15 – 40	25.1 ± 1.05	35	16 – 36	24.9 ± 0,94	29

Note: N = number of ovigerous females; min = minimum number of eggs; max = maximum number of eggs.

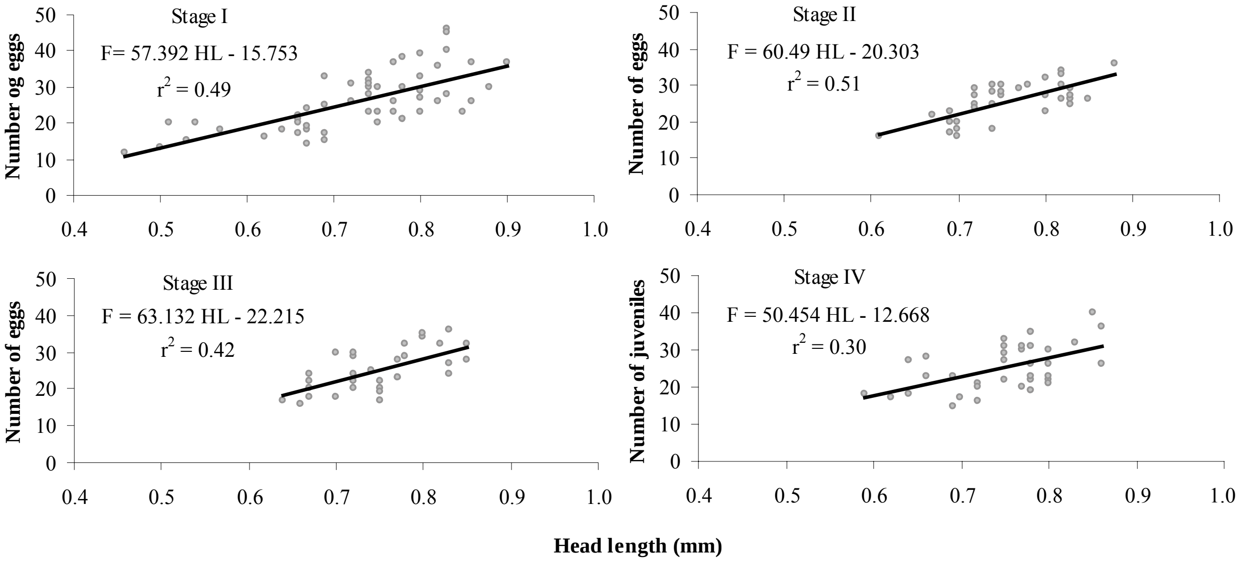

Figure 2. Regression analyses between body size of ovigerous females (head length - HL) and number of eggs in embryonic developmental stages and number of juveniles of *Hyalella pleoacuta*. F = fecundity.

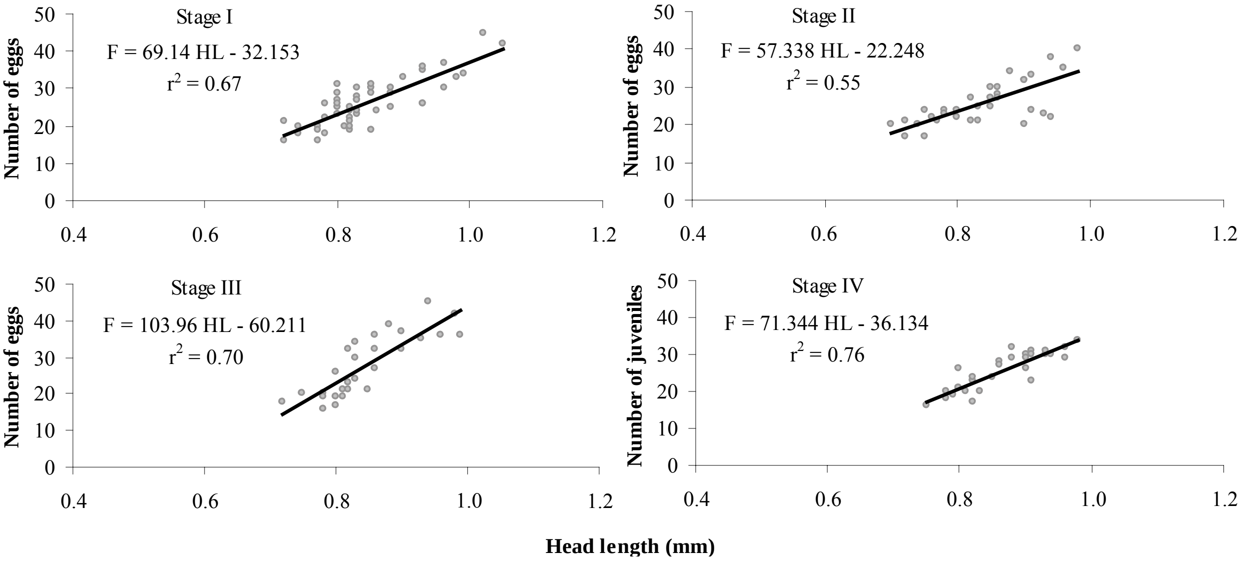

Figure 3. Regression analyses between body size of ovigerous females (head length - HL) and number of eggs in embryonic developmental stages and number of juveniles in *Hyalella castroi*. F = fecundity.

Table VI. Results of the analysis of covariance of size-specific fecundity among embryonic developmental stages of *Hyalella pleoacuta* and *H. castroi*.

	Slope (Parallelism)		**Intercept**	
	F	*p*	F	*p*
Hyalella pleoacuta				
Stage I x Stage II*	0.84	0.36	5.43	0.02
Stage I x Stage III	2.16	0.14	3.83	0.05
Stage I x Stage IV	3.32	0.07	3.53	0.06
Stage II x Stage III	0.44	0.50	0.17	0.67
Stage II x Stage IV	0.85	0.35	0.19	0.66
Stage III x Stage IV	0.10	0.74	0.004	0,94
Hyalella castroi				
Stage I x Stage II	0.06	0.80	0.03	0.84
Stage I x Stage III	3.33	0.07	0.36	0.54
Stage I x Stage IV*	0.003	0.95	7.11	0.009
Stage II x Stage III	2.87	0.09	0.09	0.76
Stage II x Stage IV*	0.03	0.85	6.08	0.01
Stage III x Stage IV*	3.49	0.06	10.11	0.002

Note: * = significant difference ($p < 0.05$).

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Egg production of two sympatric species *Hyalella* Smith, 1874 (Crustacea, Amphipoda, Dogielinotidae) in aquaculture ponds in southern Brazil

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Abstract

This study compares the egg production of two sympatric species, *Hyalella pleoacuta* and *H. castroi*, in the Vale das Trutas, São José dos Ausentes County, southern Brazil. The specimens were collected monthly with nets from August 2003 through May 2004 in two trout ponds. In the field, the ovigerous females and females carrying juveniles in the brood pouch were sorted. In the laboratory, they were measured (cephalothorax length, in mm). All eggs were removed from the female's brood pouch, counted, and classified according to their developmental stage (stage I, II and III), or the presence of juveniles in the brood pouch. In both species, the number of eggs in all embryonic developmental stages and the number of juveniles were positively correlated with female size (cephalothorax length in mm) ($p<0.05$). Although ovigerous females of *H. pleoacuta* had a smaller mean size (0.71 ± 0.08 mm) than *H. castroi* (0.83 ± 0.09 mm), *H. pleoacuta* females produced more eggs (36.1 ± 13.5 eggs) than *H. castroi* (31.4 ± 10.7 eggs). Egg production continued throughout the year, with the highest number of eggs produced during the winter and spring by *H. pleoacuta,* and winter, spring, and autumn by *H. castroi.* The number of eggs carried by females decreased with developmental stage in both *H. pleoacuta* and *H. castroi*, suggesting that egg loss may have occurred. Both species showed higher fecundity rates than other gammaridean amphipods.

Keywords. Egg production; embryonic developmental stages; *Hyalella* species.

Introduction

Reproductive output of crustaceans has been generally determined by counting the number of fertilized eggs ovulated and carried by the female as an egg mass, in an ovisac or a brood chamber (Sastry 1983). However, other aspects such as larvae development, egg viability, embryo survival, care during incubation, and degree of fertility must be also determined in order to evaluate the reproductive potential of a species.

Bourdon (1962) and Sastry (1983) defined fecundity as the number of eggs produced by a female in a single egg batch or during a given period of its life cycle. The number of eggs can be correlated with size or weight of the ovigerous females (Ogawa and Rocha 1976; Du Preez and McLachlan 1984), age of female (Pennak 1953; Kuris 1991), and the survival rate of larvae (Branco and Avilar 1992).

Egg production of gammaridean amphipods has been studied by Sheader and Chia (1970), Fish (1975), Sheader (1977), Williams (1978), Fish and Mills (1979), Sheader (1983), Powell (1992), Sheader (1996), Beare and Moore (1996), and Cardoso and Veloso (2001). However, the life cycles and fecundity of most freshwater amphipods, especially *Hyalella* Smith, 1874 species, are little known; most studies have been done with *H. azteca* Saussure, 1858, which occurs throughout Central and North America (Cooper 1965; Strong 1972; Kruschwitz 1978; France 1992; Wellborn 1994; Othman and Pascoe 2001; Alcocer *et al.*, 2001; Wellborn *et al.*, 2005).

The genus *Hyalella* encompasses exclusively freshwater species, widespread in temperate and tropical American regions, totaling 51 described species. Of these, 13 occur in Brazil (González and Watling 2002; Pereira 2004; González *et al.* 2006). Species of *Hyalella* are found across the variety of permanent freshwater types, from large lakes to small ponds and marshes, clinging to vegetation, swimming in the water column, or burrowing in bottom sediments. They are important members of the benthos (Kruschwitz 1978; Wellborn 1995).

The brood pouch or marsupium is a unique characteristic of the peracaridan female, and is the site where fertilization and incubation of eggs occur (Borowsky 1991). Amphipods have direct development: the eggs hatch into juveniles with the general adult morphology, although they lack secondary sex characteristics (Strong 1972; Borowsky 1991; Steele and Steele 1991; Morrit and Spicer 1996; Aoki 1997; Thiel 1999*a*). The brood pouch "roof" is formed by the ventral surface of the thoracic segments, and the "floor" is formed by the oostegites, plate-like processes that arise from the medial surfaces of the thoracic coxae (Morrit and Spicer 1996). The brood pouch is a porous basket that confines the eggs and juveniles, but permits free flow of water around them during development (Borowsky 1980*a*). In general, two main types of oostegites can be distinguished in amphipods: broad, with relatively short marginal setae that extend almost to the base (the primitive type); and narrow, with long marginal setae (Steele 1991). Amphipod females with broad oostegites tend to have smaller eggs than those with narrow oostegites (Steele 1991; Steele and Steele 1991).

The development of amphipods (and other peracaridans) occurs in the brood pouch and is divisible into an embryonic period, extending from ovulation to hatching; and a juvenile period (postembryonic period), extending from hatching to emergence from the brood pouch (Borowsky 1980 *a*, *b*). However, some species perform extended parental care, during which the juveniles remain in the female's brood pouch or in her burrow, or cling to her body for certain periods after hatching (Aoki 1997; Thiel 1998; Thiel 1999*b*).

Amphipods are frequently used as bioindicators and biomonitors in a variety of aquatic systems, because they occur in widely different habitats including marine, terrestrial, and freshwater environments (Rinderhagen *et al.* 2000). Some aspects of the life cycle of these crustaceans, such as embryonic development and fecundity, may be important for interpretation of results of ecotoxicological studies that utilize these organisms as bioindicators, especially in the *Hyalella* species native to State of Rio Grande do Sul, Brazil. This study compares the fecundities of two recently described species of *Hyalella*. We compared the fecundity among three embryonic developmental stages and the number of juveniles carried by the female in the brood pouch of both species.

Material and methods

Study site

Specimens of the two species of *Hyalella* were collected monthly with a net (August 2003 to July 2004) in two culture ponds holding trout, *Oncorhynchus mykiss* (Walbaum, 1792), in the Vale das Trutas near the headwaters of the Rio das Antas in the municipality of São José dos Ausentes, State of Rio Grande do Sul, Brazil (28°47'00"S – 49°50'53"W). Both species were found together, living under the roots of aquatic macrophytes of the genera *Senecio* Linnaeus, *Hydrocotile* Linnaeus, *Ludwigia* Linnaeus and *Callitriche* Linnaeus, along the sides of the ponds. São José dos Ausentes is located in the South Riograndense Highlands, and is of special ecological interest because it contains headwaters of two rivers, Rio das Antas and Rio Pelotas. The regional climate is medium mesotherm**,** with cold winters and mild summers because of the altitude (Nimer 1989).

Sampling and laboratory procedures

The collected amphipods were placed in plastic bags and transported in a cooler to the laboratory, where they were identified. Ovigerous females and females carrying juveniles were separated in the field. In the laboratory, the eggs and juveniles were removed from the brood pouch, counted, and the developmental stage was determined by use of a dissecting microscope. The cephalothorax length (CL, in mm) of ovigerous females and females carrying juveniles in the brood pouch was measured using a micrometer eyepiece in a stereoscopic microscope. Only the number of eggs per brood from ovigerous females with undamaged brood pouches was counted (Subida *et al.* 2005).

The contents of the brood pouch of the females were characterized as one of three embryonic developmental stages, based on Hynes (1955) and Subida *et al.* (2005), and one postembryonic stage (juvenile remaining in the brood pouch). These stages were distinguished easily by means of a stereoscopic microscope, and recognized as follows: stage I, with little or no cleavage, orange coloration, and yolk occupying the entire egg space; stage II, the beginning of the cellular cleavage is visible, but the body parts are not defined; stage III, the yolk is consumed, reduced to a tiny spot on the embryo, and the eyes are easily visible; stage IV, with hatching juveniles present in the brood pouch. These stages correspond to stages 1-2, 3, 4-5, and 6, respectively, as described by Sheader and Chia (1970) for *Gammarus obtusatus* Dahl, 1938. The term "egg" is used in this report synonymously with "embryo", representing all developmental stages before hatching.

Statistical analysis

The mean fecundity in each month and season was calculated for both species. The results were submitted to an analyses of variance (ANOVA), complemented by a Bonferroni test ($\alpha = 0.05$) (Zar 1996). In order to minimize the effects of embryo loss and mortality within the brood pouch, the analyses of monthly and seasonal egg production were done using only data obtained from females carrying eggs in the brood pouch (stage I + stage II + stage III) (Costa and Costa 1999). Student's *t* test was used to assess for significant differences between the overall mean fecundity of *H. pleoacuta* and *H. castroi* ($\alpha = 0.05$) (Zar 1996). Analyses of variance, complemented by a Bonferroni test, were used to compare the number of eggs produced in each embryonic developmental stage and the number of juveniles remaining in the brood pouch in each species ($\alpha = 0.05$) (Zar 1996).

Because the eggs become ellipspoid during development, an average of their length and width was taken as a measurement of their size (Maranhão *et al.* 2001). The eggs in all embryonic developmental stages were measured using a micrometer eyepiece in a stereoscopic microscope. The egg volume was quantified by the formula (Subida *et al.* 2005):

$$V=4/3\pi R_{max}(R_{min})^2$$

where R_{max} and R_{min} are the maximum and minimum egg radii, respectively.

Next, the mean diameter of eggs and egg volume in embryonic developmental stages was calculated for each species, and the means were compared using Analysis of Variance (ANOVA) and the Bonferroni test ($\alpha = 0.05$) (Zar 1996).

The cephalothorax length (mm) of hatching juveniles that remained in the brood pouch was measured using a dissecting microscope in each *Hyalella* species (5 juveniles from 8 different females). A *t* test was used to compare the mean size (cephalothorax length) of hatching juveniles of *Hyalella pleoacuta* and *H. castroi* ($\alpha = 0.05$) (Zar 1996).

Regression analysis of egg number in each embryonic developmental stage and the number of juveniles remaining in the brood pouch (y) and cephalothorax length (mm) of ovigerous females (x) was carried out ($y = ax - b$). Subsequently the Pearson's correlation coefficient (r) for each relationship was determined (Zar 1996). To assess whether the egg production in each embryonic developmental stage differed in size-specific fecundity, the embryonic developmental stages were compared using analysis of covariance, with egg number and/or juvenile number as the response variable, cephalothorax length as the covariate, and the embryonic developmental stage as the grouping variables ($\alpha = 0.05$) (Zar 1996).

Results

Total cephalothorax length in ovigerous females of *H. pleoacuta* ranged from 0.46 to 1.04 mm, and in females of *H. castroi* from 0.44 to 1.06 mm. Females of *H. pleoacuta* had a smaller mean size (0.71 ± 0.004 mm standard error) than females of *H. castroi* (0.83 ± 0.005 mm) ($p<0.05$). The size of females of *H. pleoacuta* carrying juveniles in the brood pouch ranged from 0.44 to 0.92 mm (0.73 ± 0.008 mm), and of females of *H. castroi* from 0.74 to 1.09 mm (0.87 ± 0.014 mm). The ovigerous females and females carrying juveniles of *H. castroi* were larger than those of *H. pleoacuta* ($p<0.05$).

The number of eggs (stage I + stage II + stage III) produced by ovigerous females of *H. pleoacuta* ranged from 13 to 86 eggs, and the number of juveniles from 10 to 60; the egg production of *H. castroi* ranged from 13 to 60 eggs, and the number of juveniles from 15 to 56. The mean (± standard error) number of eggs produced by *H. pleoacuta* was significantly higher (36.1 ± 0.65 eggs) than the number of eggs produced by *H. castroi* (31.4 ± 0.74 eggs) ($p<0.05$). However, there was no significant difference between the mean numbers of juveniles remaining in the brood pouch of females of either species (*H. pleoacuta*: 29.2 ± 1.12; *H. castroi*: 27.9 ± 1.58; $p>0.05$).

The mean number of eggs produced by *H. pleoacuta* and *H. castroi* in each embryonic developmental stage and the mean number of juveniles remained in the brood pouch are shown in Table 1. In both species there was a decrease in the number of eggs produced during embryonic development, and the mean number of eggs in the embryonic stage I was significantly greater than the number of juveniles in the brood pouch ($p<0.05$). Estimated mortality in the brood pouch between stage I (36.0 eggs/brood) and stage IV (29.2 juveniles/brood) was 18.9% in *H. pleoacuta.* In *H. castroi*, the estimated percentage brood mortality between stage I (32.9 eggs/brood) and stage IV (27.9 juveniles/brood) was 15.2%. The mortality rate was significantly higher in *H. pleoacuta* than *H. castroi* ($p<0.05$). The stage I and II of the embryonic development were more frequent than other stages in both species (Table 1).

During embryonic development, egg size (mean diameter) significantly increased in both species: stage III eggs enlarged 27.3% in *H. pleoacuta* and 18.4% in *H. castroi.* Stage III eggs were significantly larger in *H. castroi* than in *H. pleoacuta* ($p<0.05$) (Table 2). Egg volume significantly increased in both species, being that stage III egg increased 86.4% in *H. pleoacuta* and 84.4% in *H. castroi* ($p<0.05$) (Table 3).

The mean number of eggs produced by females was positively correlated with the size of ovigerous females in both species throughout the year (*H. pleoacuta*: $r^2= 0.61$; *H. castroi*: $r^2= 0.74$; $p< 0.05$), i.e., the number of eggs and the size of ovigerous females decreased during the warmer months of the year (Fig. 1).

The frequency variation of egg in different developmental stages confirmed that sexual activity takes place throughout the year in both species. In fact, eggs in all stages of embryonic development were found throughout the study period (Fig. 2).

The mean number of eggs produced by females of *H. pleoacuta* was significantly higher in winter than in other seasons ($p<0.05$) (Fig. 3A). In *H. castroi,* egg production remained high in fall, winter, and spring, when the mean number of eggs did not differ significantly ($p>0.05$) (Fig. 3B).

The hatching juveniles which remained in the brood pouch of female *H. castroi* had a mean cephalothorax length greater (0.22 ± 0.001 mm) than that of juveniles of *H. pleoacuta* (0.19 ± 0.002 mm) ($p<0.05$).

There was a positive linear correlation between the number of eggs in all embryonic developmental stages and size (cephalothorax length) of females of both species ($p<0.05$). There was also a correlation between the number of juveniles remaining in the brood pouch and the cephalothorax length of females in *H. pleoacuta* (Fig. 4) and *H. castroi* (Fig. 5) ($p<0.05$). The size-specific fecundity differed significantly between stage I and stage IV and stage II and stage IV in both *Hyalella* species as demonstred by analysis of covariance at the table 4. These results evidence the utilization of the embryonic developmental stages I, II and III to characterize the fecundity of *H. pleoacuta* and *H. castroi.*

Discussion

In most crustaceans, the eggs in a brood are synchronized at the same stage of embryonic development, indicating that they were fertilized at the same time (Green 1965). This pattern was observed in *H. pleoacuta* and *H. castroi* in the present study, as previously reported for *Marinogammarus obtusatus* (Dahl, 1936) (Sheader and Chia 1970), *Gammarus duebeni* Lilljeborg, 1851 (Sheader 1983), *G. insensibilis* Stock, 1966 (Sheader 1996), *Pseudorchestoidea brasiliensis* (Dana, 1853) (Cardoso and Veloso 2001), *Corophium insidiosum* Crawford, 1937 (Kevrekidis 2004), and *C. orientale* Myers, 1982 (Kevrekidis 2005).

In both *Hyalella* species, all embryo developmental stages were found during this study in distinct ovigerous females of different sizes. This suggests that embryonic development is a continuous process, in accordance with their continuous reproductive activity. This pattern was also observed for the gammaridean amphipods *Pseudorchestoidea brasiliensis* at Prainha Beach, Brazil (Cardoso and Veloso 2001) and *Corophium orientale* Schellenberg, 1928 in the Monolimni Lagoon, Greece (Kevrekidis 2005).

Peracarid crustaceans are ideally suited for analysis of clutch mortality, because reproductive females brood their eggs, and embryogenesis is synchronous (Kuris 1991). According to Sheader (1983), egg mortality can be recognized either by the presence of deteriorating or moribund eggs within the brood pouch, or as a decrease in egg number during embryonic development. The reduction in the number of eggs per brood with progressive embryonic development in the *Hyalella* species analyzed in the present work is common in amphipods, such as *Bathyporeia pilosa* Lindstrom, 1855 (Fish, 1975), *Talitrus saltator* (Williams, 1978), *Corophium volutator* (Pallas, 1766) and *C. arenarium* Crawford, 1937 (Fish and Mills 1979), *Pontocrates arenarius* (Bate, 1858) and *P. altamarinus* Bate, 1858 (Beare and Moore 1996), *Crangonyx pseudogracilis* Bousfield, 1958 (Dick *et al.* 1998), *C. multisetosum* Stock, 1952 (Cunha *et al.* 2000), *Apherusa jurinei* (Milne-Edwards, 1830) (Dick *et al.* 2002), *C. insidiosum* Crawford, 1937 (Kevrekidis 2004), and *C. orientale* (Kevrekidis 2005). The decrease in available space in the brood pouch with increasing egg volume may cause the premature loss of some eggs. This is caused by the apparent production of more eggs than the brood pouch can support during the final embryonic development stage (Koch 1990). The reduction in egg numbers during embryonic development may also be caused by maternal cannibalism, as reported for *Gammarus duebeni*, in which the ovigerous females remove and consume their eggs when food is scarce (Sheader 1983). This was observed in *H. pleoacuta* and *H. castroi* in laboratory conditions, when two ovigerous females of each species were not fed and their eggs disappeared from the brood pouch over a period of one or two days. Environmental factors such as cold temperatures, salinity changes, and desiccation can kill broods in the brood pouch of amphipods in nature (Vlasblom 1969; Sheader 1983). Parasites present in the brood pouch might be expected to act by either reducing egg numbers or by causing deterioration in egg condition (Sheader 1983; Kuris 1991). Besides, in some species, the females oviposit even if no copulation occurs. However, these eggs never develop. Unfertilized broods generally dissapear from the marsupium within a few days, most probably eater by the female (Borowsky 1991).

According to Costa and Costa (1999), in the Gammaridae, providing that temperature is not a limiting factor, reproduction takes place throughout the year, although with seasonal fluctuations as is the case for the species studied in the present work. *H. pleoacuta* and *H. castroi* have their lowest egg production during the summer. These fluctuations in reproductive intensity appear to be related to the macrophyte cover, which is much depleted during the warmer months. This not only reduces the food and shelter available for breeding females, but may also contribute to increased mortality of recruits in the Vale das Trutas. A similar situation was observed for the estuarine gammaridean amphipod *Gammarus locusta* (Linnaeus, 1758) in Sado estuary, Portugal (Costa and Costa 1999).

A similar reproductive strategy, characterized by the production of small broods during summer, has been observed in other species of amphipods such as *Gammarus duebeni,* studied by Kinne (1959) and Steele and Steele (1969), *Corophium volutator* and *C. arenarium,* studied by Fish and Mills (1979), and *C. bonnellii* (Milne-Edwards, 1830) and *Bathyporeia pilosa,* studied by Powell (1992). According to Nelson (1980), high temperatures may lead to low availability of food and an increase in predation, consequently affecting the survivorship of adults and resulting in a small number of juveniles during summer. Fish and Mills (1979) argued that the larger brood size of overwintering females compared to summer females of the amphipods *Corophium volutator* and *C. arenarium* may reflect different food levels in each season; or alternatively, high summer temperatures might result in the reduction of nutrient transfer to the gonads because of increased metabolic utilization. Dutra *et al.* (submitted) observed a clear seasonal variation in stocking and mobilization of energy metabolites such as glycogen, proteins, lipids, and triglycerides in both *H. pleoacuta* and *H. castroi.* These results may suggest an increase in energy demand for gamete production in summer, incubation and laying of eggs in autumn and winter, and parental care in spring, respectively.

Production of many, small eggs in the summer and few, large eggs in the winter is a common amphipod reproductive strategy (Steele and Steele 1969; Sheader 1978; Kolding and Fenchel 1981; Powell 1992), reflecting both seasonal change in the reproductive investment of females that may be related to seasonal changes in food availability, and female and offspring mortality (Price 1974; Smith and Fretwell 1974). However, *H. pleoacuta* and *H. castroi* showed a contrary reproductive pattern to most species, producing fewer eggs during the summer, but the egg size was no monthly analyzed in the present study.

Gammarid amphipod species exhibit diverse life-history strategies, allowing adaptation to a wide range of environments through variation in reproductive parameters such as egg size and number, the duration of embryonic development, the size of hatched offspring, and the body length of adults at maturity (Sheader 1983; Steele and Steele 1991). However, these various reproductive parameters are correlated, so that a change in one brings about changes in many others. Thus, concomitantly with an increase in egg size, the duration of embryonic development, the size of the hatched offspring, and the size of adults increase while fecundity decreases. Therefore, opposing tendencies may be produced by a simple change in egg size. Even though *H. castroi* is larger than *H. pleoacuta*, the former produces fewer eggs. This lower egg production in *H. castroi* may be compensated by the production of larger eggs, and also by the low brood mortality. This result was also observed in two sympatrics species of

Hyalella in Michigan, U.S.A. by Wellborn and Cothran (2004). According to Wellborn and Cothran (2004), these differences in the tradeoff between cluth size and egg size may be ecologically relevant. Life history theory suggest that production of more smaller eggs is favoured by conditions that promote enhanced survival or growth rate of juveniles, such as low predation risk for juveniles or high resource levels that allow rapid juvenile growth (Stearns 1992; Roff 1992).

The low frequency recorded for stages III and IV in *H. pleoacuta* and *H. castroi* may indicate that the duration of these stages is much shorter than that of the earlier developmental stages, and/or that females may migrate to more secure places in the trout culture ponds, because the females are more vulnerable to predation when the juveniles are hatching. In the amphipods *Pseudorchestoidea brasiliensis* and *Corophium insidiosum* studied by Cardoso and Veloso (2001) and Kevrekidis (2004), respectively, ovigerous females with eggs in early embryonic developmental stages were sampled more often, which may be the result of a longer developmental duration of these stages than others. Koch (1990) attributed the lower frequency of ovigerous females of *Traskorchestia traskiana* (Stimpson, 1857) carrying eggs in advanced stages to brood mortality. On the other hand, the low frequency of females carrying juveniles in *Gammarus chevreuxi* Sexton, 1913 may indicate a short residence time in the brood pouch and little or no maternal care, the combination of which enables rapid brood sucession (Subida *et al.* 2005).

The number of eggs produced by a female may be related to the size or weight of the animals (Ogawa and Rocha 1976; Du Preez and McLachlan 1984; Powell 1992), environmental factors (Jensen 1958), geographical variations (Jones and Simons 1983), and the rate of survivorship of larvae and/or juveniles (Branco and Avilar 1992). Several studies of gammaridean amphipods have shown that fecundity is strongly correlated with the size of females (Kinne 1961; Strong 1972; Williams 1985; France 1992; Powell 1992; Muskó 1993; Wellborn 1995; Cardoso and Veloso 1996; Zielinski 1998; Moore and Wong 1996; Costa and Costa 1999; Persson 1999; Wilhelm and Schindler 2000; Othman and Pascoe 2001; Alcocer *et al.* 2002; Maranhão and Marques 2003; Kevrekidis 2004; Kevrekidis 2005). In Vale das Trutas locality the number of eggs of *H. pleoacuta* and *H. castroi* was also directly related to body size of ovigerous females.

The increase in egg size with advancing embryonic development seen in *H. pleoacuta* and *H. castroi* is a common process among gammaridean amphipods, such as *Marinogammarus obtusatus* (Sheader and Chia 1970), *Bathyporeia pilosa, Corophium bonnellii*, *Ampelisca macrocephala* Lilljeborg, 1852, *Stegocephaloides christianiensis* (Boeck, 1871), *Gammaropsis nitida* (Stimpson, 1853) and *Lembos websteri* Bate, 1857 (Powell 1992), *Orchestia gammarellus* (Pallas, 1766) (Jones and Wigham 1993), *Orchomene nanus* Kröyer, 1846 (Moore and Wong 1996), *Gammarus insensibilis* (Sheader 1996), *G. lacustris* Sars, 1863 (Wilhelm and Schindler 2000), *Pseudorchestoidea brasiliensis* (Cardoso and Veloso 2001), and *Apherusa jurinei* (Dick *et al.* 2002). This increase in egg size with advancing embryonic development is related to water absorption and conversion of reserve yolk into body structures, mainly the major body regions - the head, pereon and pleon (Sheader and Chia 1970; Koch 1990; Powell 1992; Sheader 1996).

The mean size (cephalothorax length) of hatching juveniles in *H. pleoacuta* and *H. castroi* was similar to that of *H. azteca* studied by Strong (1972) (Sutle – 0.22 mm; Hunter – 0.19 mm and Siltcoos – 0.19 mm). *H. castroi* produces larger eggs than *H. pleoacuta*, and consequently the hatching juveniles who remained in the brood pouch in females of *H. castroi* were larger than juveniles of *H. pleoacuta*.

According to the review by Nelson (1980) of the reproductive patterns of gammaridean amphipods, freshwater species produce fewer eggs than do marine and estuarine species. The eggs of freshwater crustaceans, such as the decapods, are generally larger than those of marine species, and this is often related to a reduction of larval stages (Rabalais and Gore 1985). However, freshwater amphipods also tend to have larger eggs than marine species (Steele and Steele 1991) even though this cannot be related to the mode of embryonic development, since all amphipods have direct development without any larval stages. A more general explanation for the larger eggs of freshwater crustaceans is that they will be subject to less osmotic stress than small eggs, because of their smaller surface area/volume ratio. According to Sastry (1983), the large eggs produce larger juveniles, better adapting them for feeding and providing greater competitive ability. However, egg production in *H. pleoacuta* and *H. castroi* is greater than in other freshwater gammarideans, especially *H. azteca* (Table 5). This high egg production can be related to the abundant food available in the study location, where the females are able to obtain sufficient energy to produce a high number of eggs. Another possible explanation for this large number of eggs is that the mortality of the early juvenile stages is high and therefore the females maximize egg production in an attempt to optimize juvenile survival (see Castiglioni and Bond-buckup submitted a b). It becomes evident that each species is able to develop a specific reproductive strategy in accordance with its environment, in order to ensure survival of its offspring under different physical and biological conditions.

The egg production characteristics found for the populations of *H. pleoacuta* and *H. castroi* in southern Brazil are similar to those observed in other gammaridean amphipods, especially the decrease in number and increase in size of eggs in the course of embryonic development. However, both of these species differ from the North American species

H. azteca, mainly in the number of eggs produced. Other aspects, such as egg size during the year and the influence of biotic and abiotic factors that influence egg development, are still in need of elucidating for a more complete understanding of egg production in *Hyalella* species.

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Table 1. Comparison of the mean number of eggs in each embryonic developmental stage and the number of juveniles in the brood pouch of *Hyalella pleoacuta* and *H. castroi.*

	N	**Mean ± SE**	**Min – Max**
Hyalella pleoacuta			
Stage I	176	36.0 ± 0.97 ab	14 –79
Stage II	166	35.3 ± 1.06 ab	13 – 86
Stage III	100	34.6 ± 1.23 bc	15 – 79
Stage IV	103	29.2 ± 1.13 c	10 – 60
Hyalella castroi			
Stage I	109	32.9 ± 1.07 a	13-56
Stage II	63	30.9 ± 1.23 a	14-57
Stage III	35	28.6 ± 1.90 a	10-60
Stage IV	35	27.9 ± 1.58 a	12-48

Note: N= number of individuals; SE= standard error; min= minimum; max= maximum. Values with at least one letter in common did not differ statistically (ANOVA and Bonferroni; α= 0.05).

Table 2. Size (diameter) of eggs at the different embryonic developmental stages in *Hyalella pleoacuta* and *H. castroi.*

Development stage	***Hyalella pleoacuta***		***Hyalella castroi***	
	N	Mean ± SE	N	Mean ± SE
Stage I	35	0.33 ± 0.03 c B	18	0.38 ± 0.01 b A
Stage II	45	0.39 ± 0.04 b A	17	0.40 ± 0.02 b A
Stage III	37	0.42 ± 0.03 a B	25	0.45 ± 0.03 a A

Note: Lowercase letters correspond to comparison among the egg size in different embryonic developmental stages in each specie; uppercase letters correspond to comparisons between the egg size same embryonic developmental stages but between species. Values with at least one letter in common did not differ statistically (ANOVA and Bonferroni; α= 0.05). N= number of eggs; SE= standard error.

Table 3. Egg volume (mm^3) at the different embryonic developmental stages in *Hyalella pleoacuta* and *H. castroi.*

Development stage	***Hyalella pleoacuta***		***Hyalella castroi***	
	N	Mean ± SE	N	Mean ± SE
Stage I	35	0.133 ± 0.01 c B	18	0.186 ± 0.01 c A
Stage II	45	0.235 ± 0.01 b A	17	0.229 ± 0.01 b A
Stage III	37	0.248 ± 0.01 a B	25	0.343 ± 0.02 a A

Note: Lowercase letters correspond to comparison among the egg size in different embryonic developmental stages in each specie; uppercase letters correspond to comparisons between the egg size same embryonic developmental stages but between species. Values with at least one letter in common did not differ statistically (ANOVA and Bonferroni; α= 0.05). N= number of eggs; SE= standard error.

Table 4. Results of the analysis of covariance of size-specific fecundity among embryonic developmental stages and juveniles of *Hyalella pleoacuta* and *H. castroi.*

	Slope (Parallelism)		**Intercept**	
	F	*p*	F	*p*
H. pleoacuta				
Stage I x Stage II	1.728	0.1895	2.268	0.1329
Stage I x Stage III	0.219	0.6401	1.890	0.1697
Stage I x Stage IV*	1.937	0.16468	10.19	0.0014
Stage II x Stage III	0.1602	0.6892	0.833	0.3621
Stage II x Stage IV*	0.1421	0.7064	8.8523	0.0032
Stage III x Stage IV	0.0028	0.9577	2.6595	0.1045
Stage I x Stage II*				
Stage I x Stage III	0.0933	0.7603	11.2109	0.0010
Stage I x Stage IV	0.0365	0.8486	2.8820	0.0917
Stage II x Stage III	0.1971	0.6576	26.697	0.000001
Stage II x Stage IV	0.0097	0.9213	1.2294	0.2703
Stage III x Stage IV	0.7987	0.3737	13.584	0.00038
Stage I x Stage II*	0.5104	0.4774	16.218	0.00014

Note: * = significant difference ($p<0.05$).

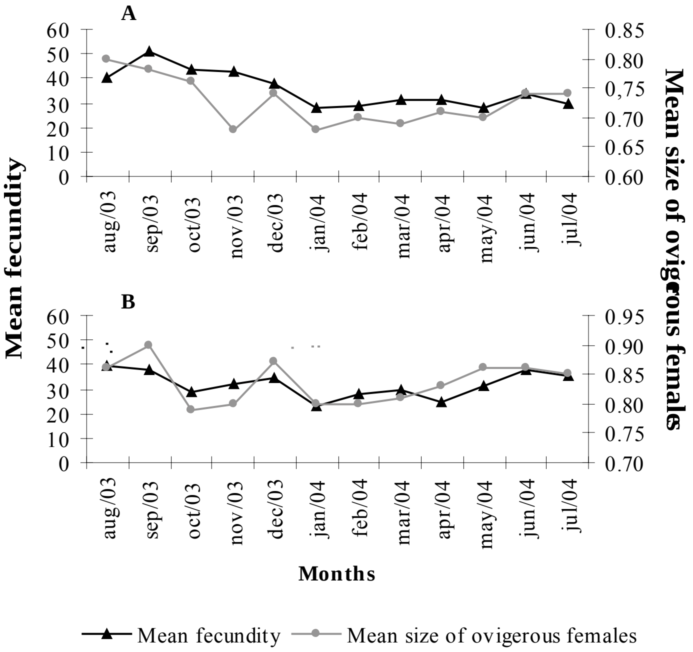

Fig. 1. Monthly mean fecundity and mean size of ovigerous females. Only females carrying eggs (stage I, II and III) were considered in the analysis. *Hyalella pleoacuta* (A) and *H. castroi* (B).

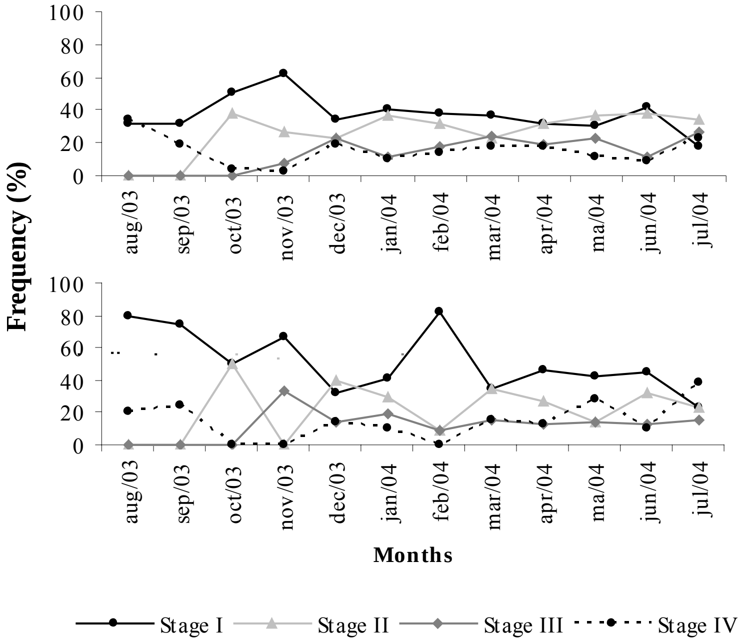

Fig. 2. Relative frequency (%) of the different embryonic and postembryonic developmental stages during the year in *Hyalella pleoacuta* (A) and *H. castroi* (B).

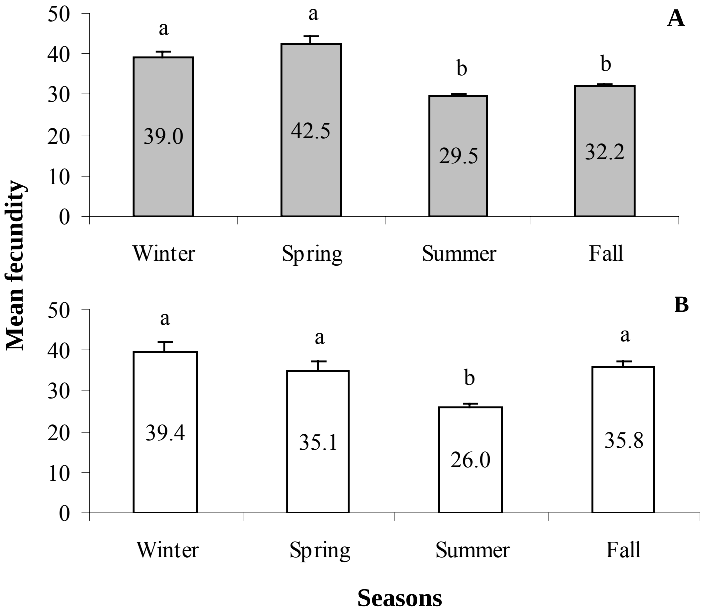

Fig. 3. Mean ± standard error of fecundity in each season. Only females carrying eggs (stage I, II and III) were considered in the analysis. Bars with at least one letter in common did not differ statistically (ANOVA and Bonferroni; α = 0.05). *Hyalella pleoacuta* (A) and *H. castroi* (B).

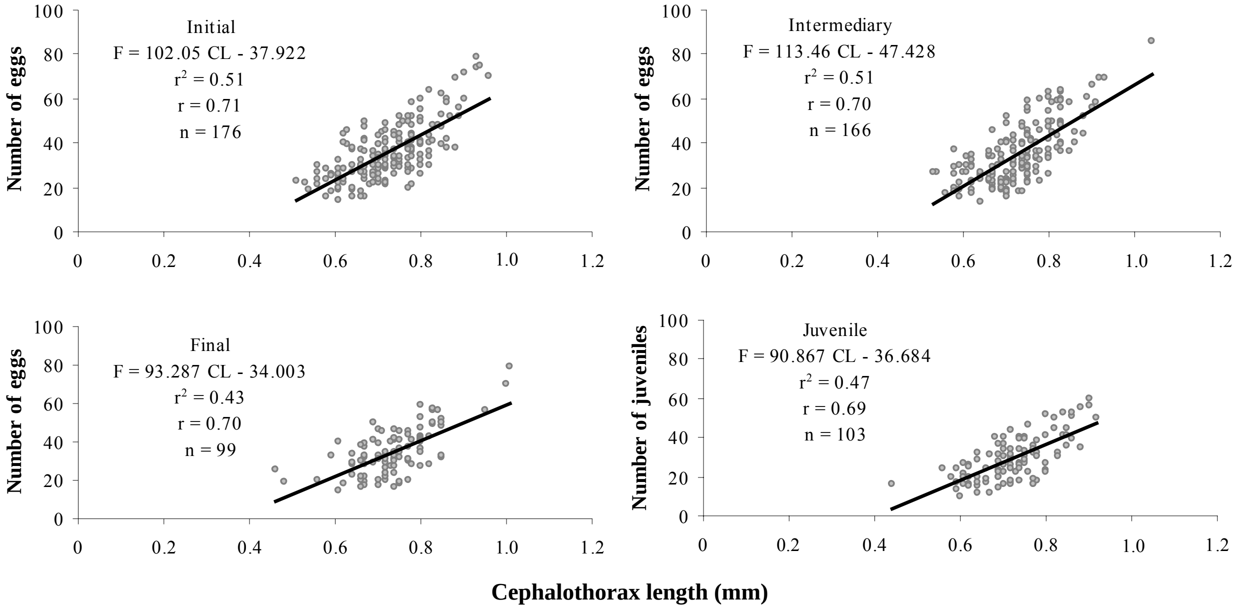

Fig. 4. *Hyalella pleoacuta*. Regression analyses between body size of ovigerous females (cephalothorax length - CL) and number of eggs at embryonic developmental stages and number of juveniles *H. pleoacuta*. F= fecundity.

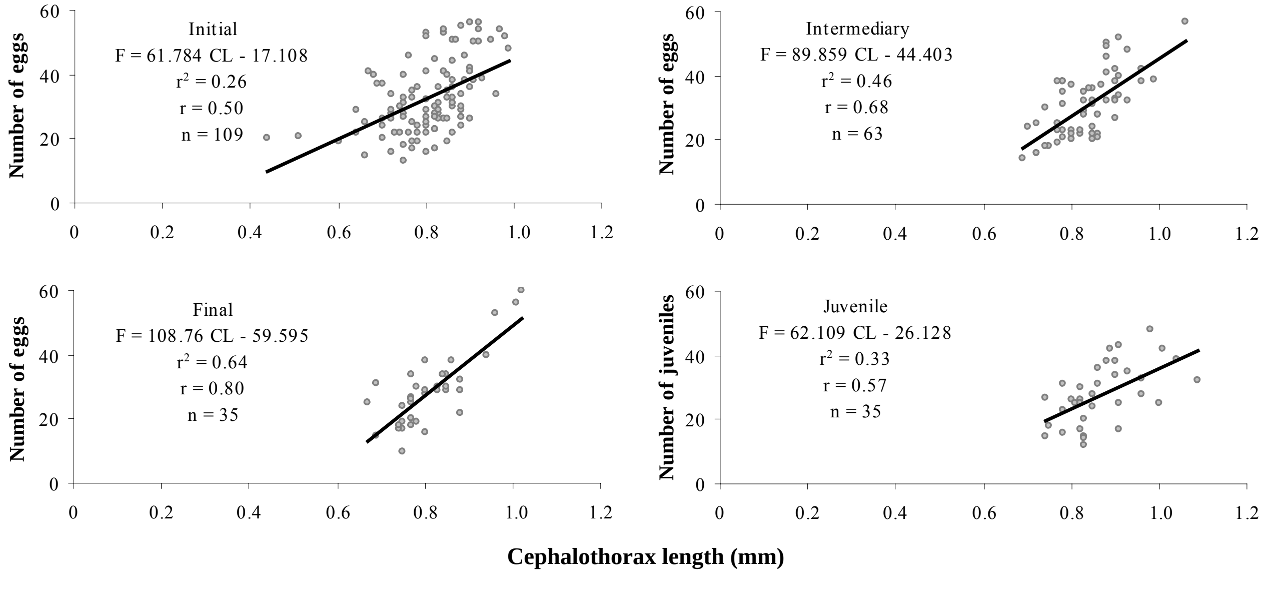

Fig. 5. *Hyalella castroi*. Regression analyses between body size of ovigerous females (cephalothorax length - CL) and number of eggs at embryonic developmental stages and number of juveniles *Hyalella pleoacuta*. F= fecundity.

Table 5. Fecundities of species of *Hyalella.*

	Locality	**NE**	**Authors**
Hyalella pleoacuta	São José dos Ausentes, southern Brazil	36.1*	Present study
Hyalella castroi	São José dos Ausentes, southern Brazil	31.4*	Present study
Hyalella azteca	U.S.A	18.0*	Pennak (1953)
Hyalella azteca	Washtenaw, Michigan, U.S.A.	1-50●	Cooper (1965)
Hyalella azteca	Suttle Lake, Oregon, U.S.A.	17.0*	Strong (1972)
Hyalella azteca	Siltcoos Lake, Orgeon, U.S.A.	18.0*	Strong (1972)
Hyalella azteca	Hunter Hot Springs, Oregon, U.S.A.	8.0*	Strong (1972)
Hyalella spp.	Southern Brazil	1-6 ●	Sampaio (1988)
Hyalella azteca		9.0*	Othman and Pascoe (2001)
Hyalella curvispina	River Luján, Província de Buenos Aires, Argentina	0* - 15.7*	Casset *et al.* (2001)
Hyalella sp.	River Luján, Província de Buenos Aires, Argentina	2.17* - 8.13*	Casset *et al.* (2001)
Hyalella azteca	Central Mexico	1-38●	Alcocer *et al.* (2002)
Hyalella azteca	Antelope Pond, Oklahoma, U.S.A.	5.2*	Wellborn *et al.* (2005)
Hyalella azteca	Blue river, Oklahoma, U.S.A.	5.3*	Wellborn *et al.* (2005)
Hyalella azteca	Briar Creek, Oklahoma, U.S.A.	6.2*	Wellborn *et al.* (2005)
Hyalella azteca	Washingtom Pond, Oklahoma, U.S.A.	5.2*	Wellborn *et al.* (2005)
Hyalella azteca	Cowan Creek, Oklahoma, U.S.A.	19.2*	Wellborn *et al.* (2005)
Hyalella azteca	Thunderbird Lake, Oklahoma, U.S.A.	14.7*	Wellborn *et al.* (2005)
Hyalella azteca	UOBS Creek, Oklahoma, U.S.A.	10.3*	Wellborn *et al.* (2005)
Hyalella azteca	Summings Spring, Oklahoma, U.S.A.	2.2*	Wellborn *et al.* (2005)

Note: NE= number of eggs; * = mean number of eggs; ● = number minimum and maximum of eggs.

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Ecological traits of two sympatric species of *Hyalella* Smith, 1874 (Crustacea, Amphipoda, Dogielinotidae) from southern Brazil.

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Abstract

Information about populations provides subsides for the knowledge of the ecological stability of the species in a determined habitat, besides to allow the comprehension of the biology of species. This work was performed with the aim to test theoretical predictions regarding that the sympatric species *Hyalella pleoacuta* González, Bond-Buckup and Araujo, 2006 and *H. castroi* González, Bond-Buckup and Araujo, 2006 might show distinct population biology to facilitate its coexistence. The specimens were collected monthly with nets from August 2003 through July 2004 in two trout ponds at Vale das Trutas locality, São José dos Ausentes County, southern Brazil. In the laboratory, the specimens were measured as cephalothorax length (mm), being the sex and ovigerous conditions checked. The species *H. pleoacuta* were 2.94 times more frequent than *H. castroi*. Males were significantly greater than females (*H. pleoacuta* - males: 0.74 ± 0.002 mm and females: 0.66 ± 0.001 mm; *H. castroi* - males: 0.84 ± 0.00 mm and females: 0.72 ± 0.003 mm). Males and females of *H. castroi* showed a greater mean size body than *H. pleoacuta.* Sexual maturity was attained with 0.53 mm in males and 0.48 mm in females of *H. pleoacuta* and 0.72 mm in males and 0.67 mm in females of *H. castroi.* The frequency distribution in size classes showed bimodal, being one group compound essentially by juveniles and other group of adult in both *Hyalella* species. Sex ratio was female-biased in either species of *Hyalella*. Ovigerous females (carrying eggs or juveniles in the marsupium) were collected along year in both *Hyalella* species, but *H. pleoacuta* and *H. castroi* were found with more frequency during the autumn and fall, respectively. The recruitment occurs in all months of sampling, being the juvenile frequency superior to 50% of the amphipods collected in almost months in both species.

*Key words***.** Frequency distribution; body size; sex ratio; breeding period; recruitment; *Hyalella.*

1. Introduction

The population dynamics of several gammaridean amphipods has been analyzed, mainly with reference to the distribution of the animals in size classes, population density, age distribution, sex ratio, breeding period, recruitment and growth (La France and Ruber, 1985; Wilson and Parker, 1996; Moore and Wong, 1996; Costa and Costa, 1999; Cunha et al., 2000; Leite, 2002; Gonçalves et al., 2003; Marques et al., 2003; Guerao, 2003; Appadoo and Myers, 2004; Dias and Sprung, 2004; Kevrekidis, 2004, 2005; Subida et al., 2005). Information about populations provides subsides for the knowledge of the ecological stability of the species in a determined habitat, besides to allow the comprehension of the biology of species.

The genus *Hyalella* Smith, 1874 occurs in freshwater environments of the America, being frequent at the State of Rio Grande do Sul, Brazil. Most of the *Hyalella* species usually found associate to macrophytes, being that others species are found swimming at the water column or burrowing at the sediment of permanent reservoir, lakes and streams (Kruschwitz, 1978; Wellborn, 1995; Grosso and Peralta, 1999). However, some species are sympatrics, such as *Hyalella pleoacuta* González, Bond-Buckup and Araujo, 2006 and *Hyalella castroi* González, Bond-Buckup and Araujo, 2006, others live in cavern as *H. caeca* Pereira, 1989 and only the species, *H. rionegrina* Grosso and Peralta, 1999 is found in wet soils (Grosso and Peralta, 1999). The species of *Hyalella* are an omnivorous deposit feeder, primarily feeding on algae and bacteria associated with the sediments and aquatic macrophytes (Hargrave, 1970) and also feeding on dead animal and plant materials (Cooper, 1965). It is thus likely that this amphipod play an important role in faciliting energy flow in aquatic environments through the direct conversion of epiphytic and detrital energy into production of fine particle organic matter and micro and-macro-consumer biomass (Wen, 1992).

In the last years the knowledge about new species of *Hyalella* is increase (Pereira, 2004; González et al., 2006), but due to incorrect use of natural resource by man and also to pollution caused by agricultural and industrial development, many species will become extinct before will be discover by scientific community. Due to the fact of species of *Hyalella* are used as bioindicators of environmental quality is extreme important the knowledge of the life cycle of these species, especially the population dynamics in natural habitat and it conservation at the freshwater environments, not only in Brazil as all countries of the world where these species occur.

Although be known 13 species of *Hyalella* described to Brazil (González and Watling, 2001; Pereira, 2004; González et al., 2006), any study about population dynamic of these species was performed until moment. *Hyalella* species are the only representative of the gammaridean that occur at the freshwater environment at the State of Rio Grande do Sul, as in all Brazil (Bento and Buckup, 1998), and the most of papers dealing with these species are about taxonomy (Pereira, 1985; Pereira, 1989; Bousfield, 1996; Bond-Buckup and Araujo, 1998; Grosso and Peralta, 1999; González and Watling, 2002, 2003; Pereira, 2004; González et al., 2006). In this sense, this work was developed with the aim to test theoretical predictions regarding that the sympatric species *H. pleoacuta* and *H. castroi* might show distinct population biology to facilitate its coexistence. For these, the size-frequency distribution in size classes, body size of males and females, body size at the sexual maturity, sex ratio, breeding period and recruitment were analyzed during a year period.

2. Material and methods

2.1. Site of study and sampling of the amphipods

For the characterization of population structure, the sympatric species *Hyalella pleoacuta* and *H. castroi* were collected monthly from August 2003 to July 2004 at the Vale das Trutas locality (28°47'00"S - 49°50'53"W), São José dos Ausentes County, southern Brazil. São José dos Ausentes is located in the South Riograndense Highlands, and is of special ecological interest because it contains headwaters of two rivers, Antas River and Pelotas River. The regional climate is medium mesotherm**,** with cold winters and mild summers because of the altitude (1,100 m) (Nimer, 1989). During the development of the present work the minimum and maximum temperatures at the Municipality of São José dos Ausentes was registered at the July 2004 (3.4 °C) and February 2004 (24.1 °C), respectively.

At the Vale das Trutas locality there are three artificial ponds used to raise trout (*Oncorhynchus mykiss*) (Walbaum, 1792), which ranged from 20 to 50 m of length and about 2 m of deep. The water utilized for the culture of trout provide of a deviation of the Antas River water. It was choose 2 tanks for the amphipods sampling, being that in each tank were choose 2 sampling sites (sites 1 and 2 at the pond 1 and sites 2 and 3 at the pond 2). These sampling sites differed how the presence of different genera of macrophytes, such as *Senecio* Linnaeus, *Ludwigia* Linnaeus, *Hydrocotile* Linnaeus and *Callitriche* Linnaeus: site 1, compound predominantly by *Senecio* and some macrophytes of the genera *Callitriche*; site 2, compound essentially by *Senecio*; site 3, compound predominantly by *Ludwigia* and some *Hydrocotile* and site 4, compound predominantly by *Hydrocotile* and little specimens of the *Callitriche*. The macrophytes *Ludwigia*, *Hydrocotile*, and *Callitriche rimosa* have floating roots, which have no contact with the substrate, and in *Senecio* its roots found in contact with the sediment of the ponds of raise trout (personal observation).

For the capture of amphipods, the macrophytes found in each site of sampling were collected with net and the same were placed in plastic bags and transported in a cooler to the laboratory. Afterwards, the macrophytes were washed in running water and the amphipods were retained in a sieve with 0.177 mm of mesh.

2.2. Laboratory analysis

In the laboratory, the amphipods were identified and separated how the species, being the sex and ovigerous condition registered. The animals were grouped into four categories: juvenile (individuals that no have secondary sexual character developed), males (individuals with enlarged second gnathopods), females (individuals with oostegites and small second gnathopods) and ovigerous females (females carrying eggs or juveniles at the marsupium).

The cephalothorax length (CL, in mm) of all specimens was measured using a micrometer eyepiece in a stereoscopic microscope (0.01 mm). According to Edwards and Coweel (1992) and Pickard and Benke (1996) the cephalothorax length has positive correlation with the total length of *Hyalella azteca* (Saussure, 1858), and so this dimension can be used to represent the animals size.

Amphipods were grouped into 22 CL size classes (0.05 mm amplitude) for characterization of the population structure. The number of size classes was determined by means of the value of ¼ of the standard deviation of the cephalothorax length of amphipods sampled all period study (Markus, 1971). The frequency distribution of size classes for males and females was analyzed monthly during a 1-year period, in order to follow temporary variations in population frequency distribution, and to analyze seasonal recruitment. The normality of the frequency distributions was compared by the Shapiro-Wilk test (α= 0.05; Zar, 1996).

It was determined the minimum, maximum and mean size of cephalothorax length of males and females at the two *Hyalella* species. The mean size (cephalothorax length) of animals was compared between sex and species using a *t* test (α= 0.05; Zar, 1996).

The sexual maturity in *Hyalella pleoacuta* and *H. castroi* was determined by means of size of smaller male and female found in precopulatory behavior (Borowsky, 1991). The size at maturity of females was also estimated as the cephalothorax length of the smallest ovigerous females in the population sampled (Wellborn and Cothran, 2004; Wellborn et al., 2005).

Sex ratio was expressed as the total number of adult males divided by the total number of adult females (except ovigerous females) per month, season and size class (CL – mm). This sex ratio is known as operational sex ratio (OSR), because is based on the rate of males and females that are apt to reproduce in a population at a given moment (Stearns, 1992). A chi-square test for goodness of fit (χ^2; α= 0.05) was used to evaluate the sex ratio, and to compare the percentages of males and females of each *Hyalella* species.

The ovigerous female’s frequency in relation to adult females it was monthly and seasonally analyzed for determination of breeding period of either species of *Hyalella*. The proportion of ovigerous females was compared among months and seasons using the multinomial proportions test (MANAP; α= 0.05; Curi and Moraes, 1981). It was considered adult females all females that showed the same size (cephalothorax length) or superior size to smaller ovigerous females sampling throughout period study.

To estimate recruitment, amphipods with cephalothorax length less than 0.40 mm in *Hyalella pleoacuta* and 0.45 mm in *H. castroi* were considered juveniles, which can not have the sex determined, because the secondary sexual characters not are developed, as for example, the oostegites at the females and the enlarged second gnathopods at the males. The amphipods smaller than smaller male and females found in precopulatory behavior were also considered as juveniles or immature (*H. pleoacuta* - males: 0.53 mm and females: 0.48 mm; *H. castroi* - males: 0.70 mm and females: 0.67 mm). The proportion of juveniles was compared among months and seasons using the multinomial proportions test (MANAP; $\alpha = 0.05$; Curi and Moraes, 1981).

3. Results

3.1. Population structure

A total of 23,033 individuals of *Hyalella pleoacuta* were obtained, being 4,066 males, 3,071 females, 1,526 ovigerous females (1,418 females carrying eggs at the marsupium and 108 females carrying juveniles at the marsupium) and 14,370 juveniles. However, *H. castroi* it was collected in less number, totalizing 7,831 individuals, being 1,167 males, 1,183 females, 304 ovigerous females (264 females carrying eggs at the marsupium and 40 females carrying juveniles at the marsupium) and 5,178 juveniles.

Hyalella pleoacuta was 2.94 times more frequent than *H. castroi* (χ^2=3743.38; p<0.05). It was observed that *H. pleoacuta* was more frequent than *H. castroi* in all months analyzed (p<0.05) (Figure 1).

Normality was rejected for the overall frequency distributions obtained for both species of *Hyalella* (p<0.05), considering all pooled data. Both *Hyalella* species have a multivoltine life cycle, in which the frequency distribution in size classes showed bimodality, being compound by one group of juveniles and other of adult animals (Figure 2). The small individuals mainly constituted the population in both *Hyalella* species, with the juveniles representing 62.4% in *Hyalella pleoacuta* and 66.1% in *H. castroi.* At the figures 3 and 4 are show the monthly frequency distribution in *H. pleoacuta* and *H. castroi*, respectively, which can be observe clearly two age groups in all year months of study.

Both *Hyalella* were found in all sampling sites during the period study (Figure 5). *Hyalella pleoacuta* was found with more frequency at the site 4, characterized by presence of macrophyte of the *Hydrocotile* genera, which found completely submerged in the water and its roots have no contact with the substrate (p<0.05). However, *H. castroi* was found in greater frequency at the site 2 where there was predominance of *Senecio*, being that its roots found in contact with the sediment (p<0.05).

3.2. Body size and size at sexual maturity

The cephalothorax length of males of *Hyalella pleoacuta* ranged from 0.42 to 1.12 mm and of females from 0.41 to 1.04 mm. The males are significantly greater than females (mean ± standard error = males: 0.74 ± 0.002 mm and females: 0.66 ± 0.001 mm) (t=29.369; p<0.05). In *H. castroi*, the males showed a cephalothorax length that ranged from 0.46 to 1.25 mm and the females from 0.67 to 1.12 mm, being that males showed a greater mean size than females (males: 0.84 ± 0.004 mm and females: 0.72 ± 0.003 mm) (t= 19.336; p<0.05). Males and females of *H. castroi* showed a greater mean cephalothorax length than males and females of *H. pleoacuta* (males: t= -19.402; females: t= -15.979; p<0.05).

The size at sexual maturity estimated as the cephalothorax length (CL) of the smaller individual found in precopulatory mate-guarding behavior was 0.53 mm in males and 0.48 mm in females of *H. pleoacuta* and 0.72 mm in males and 0.67 mm in females of *H. castroi*. Considering the size of smaller ovigerous female, as an indicative of sexual maturity, this size is 0.43 mm CL in *Hyalella pleoacuta* and 0.51 mm CL in *H. castroi.*

3.3. Sex ratio

The sex ratio of the total population (male: female) for both *Hyalella* species favored the females (*H. pleoacuta* – 0.88 male: 1female, χ^2= 16.27, p<0.05; *H. castroi*: 0.78 male: 1 female, χ^2= 19.18, p<0.05). There was a significant difference (p<0.05) in the frequency of amphipods in September and October 2003 and February, March, May and June 2004, when the proportion of females was higher than that of males in *H. pleoacuta* (Figure 6A). The females were more frequent than males in October and December 2003 and January, March, May and June 2004 (p<0.05) in *H. castroi* (Figure 6B).

In the seasonal sex ratio, was observed that females were more frequent than males at the winter, spring and autumn in *H. pleoacuta* (p<0.05; Figure 7A) and winter, spring and summer in *H. castroi* (p<0.05; Figure 7B).

Sex ratio were calculated for each size class of cephalothorax length, in which the proportions differed in all size classes (p<0.05), favoring one sex (females) in intermediate classes and opposite sex (males) in larger classes for both *Hyalella* species (Figure 8).

3.4. Breeding period

Ovigerous females of both *Hyalella* species (carrying eggs or juveniles at the marsupium) were collected throughout year, but were observed greater fluctuations at the frequency of ovigerous females in *H. castroi* than *H. pleoacuta* (Figure 9A). Ovigerous females of *H. pleoacuta* and *H. castroi* were collected with greater frequency during autumn and fall, respectively (p<0.05) (Figure 9B).

3.5. Recruitment

Recruitment occur in all sampling months, being that the juvenile frequency was superior to 50% of the total of amphipods collected in almost months in both *Hyalella* species. The ratio of juveniles was superior to adult's ratio in all months, except February, June and July 2004 in *H. pleoacuta* (Table I). However, the juveniles predominated in all months, with only exception of January 2004, when there was no significant difference at the ratio of juveniles and adults in *H. castroi* (Table I). It was observed a peak of juveniles at the spring in both species of *Hyalella* and a second peak of juveniles was observed at the winter in *H. pleoacuta* and summer in *H. castroi* (Figure 10).

Discussion

4.1. Population structure and life history patterns

According to Díaz and Conde (1989) bimodality or polymodality in size-frequency distributions may result from slow growth at the immature or mature phase, recruitment pulses and migration, mortality, or differential behavior and unimodality is usually characteristic of a stable population, which shows continuous recruitment and a constant mortality rate through different life phases. Besides, either species of *Hyalella* reproduce all year, and the size-frequency distribution showed bimodal in both species, probably as result a reproduction more intense in some time of the year (colder months) or differential mortality along year. The bimodality or polymodality it seems to be common among gammarideans amphipods, such as *Corophium multisetosum* Stock, 1952 (Cunha et al., 2000), *Echinogammarus longisetosus* Pinkster, 1973 (Guerao, 2003), *Talorchestia brito* Stebbing, 1891 (Gonçalves et al., 2003), *Talitrus saltator* (Montagu, 1808) (Marques et al., 2003), *Cymadusa filosa* Savigny, 1816, *Ampithoe laxipodus* Appadoo and Myers, 2004 and *Mallacoota schellenbergi* Ledoyer, 1984 (Appadoo and Myers, 2004) and *Gammarus chevreuxi* Sexton, 1913 (Subida et al., 2005).

The life history types of amphipods were distributed according to latitudinal gradients, considering the temperature as a factor that influence the longevity, breeding periods, body size at maturity, brood size, and size of embryos in gammaridean amphipods (Gable and Croker, 1977; Morino, 1978; Kolding and Fenchel, 1981; Wildish, 1982; Sainte-Marie, 1991). High-latitude gammaridean amphipods are characterized in general by univoltism, delayed maturity, large embryos, and few broods in a lifetime. By other hand, iteroparous or semiannual population types, with high reproductive potentials, many brood and small embryos are more characteristic of low latitude habitats (Sainte-Marie, 1991). At the freshwater gammaridean species there is a tendency towards an extended reproductive season with decreasing latitude (Sainte-Marie, 1991). However, either species of *Hyalella* analyzed in the present work do not square at the pattern described by Sainte-Marie (1991), although these species occur in high latitudes, they show characteristics of species that live in low latitudes: they are semiannual, iteroparous and multivoltines. Few studies regarding the life history and reproduction have been carried out on tropical and subtropical regions of the Brazil, being all developed with marine amphipods (Cardoso and Veloso, 1996; Leite, 1996; Leite, 2002; Lopes and Setuko, 2004). In this sense, more works should be performed to elucidate the life history pattern of the freshwater gammarideans that occur at the Neotropical region, especially at the Brazil.

Gammarideans are commonly distributed in all possible microhabitats of the freshwater environment. In sympatric populations of *H. pleoacuta* and *H. castroi*, however, a tendency of spatial separation could be detected. *Hyalella pleoacuta* was found with more frequency associate to macrophyte of the *Hydrocotile* genera, which found completely submerged in the water and its roots no have contact with the substrate. By other hand, specimens *H. castroi* was found in greater frequency associate to *Senecio* sp., being that its roots found in contact with the sediment ($p<0.05$). Similar spatial separation were observed in others freshwater gammarideans by Dick & Platvoet (1996) in two sympatric species, *Gammarus pulex* (Linnaeus, 1758) and *G. tigrinus* Sexton, 1939 and by Stürzbecher *et al.* (1998) in coexisting *Gammarus fossarum* Koch, 1835 types. This spatial separation could be an indication of differences in physiological preferences and/or mutual competitive exclusion.

4.2. Body size

Sexual size dimorphism (cephalothorax length) was observed in both *Hyalella* species, where the males attain greater sizes than females. Crustacean's growth usually is similar between sex until maturity and after that, the males and females show different ecological or reproductive demands, probably because of parental investment or mating effort, resulting in distinct growth rates between sexes (Low, 1978). The growth turn more slow at the females due to a prolongation of the intermolt period and a minor increment in size by occasion of the ecdysis, motivated by production and incubation of embryos (Hartnoll, 1982). Amphipods males, as like also either species of *Hyalella*, generally expend most of their reproductive effort in mating while females allocate relatively more time and energy to parental effort, especially the production of large gametes and during the incubation period (Wen, 1992). Besides, the females do not undergo ecdysis during the period of incubation, making it difficult for them to continue to grow at the same space as males (Cardoso and Veloso, 1996). It is probably this reproductive difference that makes males and females dimorphic in body size (Wen, 1993; Cardoso and Veloso, 1996). Thus the females attain lower size than males at the most of the crustaceans, as for example at the freshwater gammaridean amphipods

Gammarus pulex (Linnaeus, 1758) (Adams and Greenwood, 1983), *Hyalella azteca* (Geisler, 1944; Wen, 1993; Othman and Pascoe, 2001; Wellborn et al., 2005), *G. leopoliensis* Jadzewski and Konopacka, 1989 (Zielinski, 1998), *G. locusta* (Linnaeus, 1758) (Costa and Costa, 1999) and *Echinogammarus longisetosus* Pinkster, 1973 (Guerao, 2003).

Sexual dimorphism, as observed in species of *Hyalella* analyzed in the present study, is considered essential for efficient male performance during precopulatory mate-guarding behavior, because a larger male-to-female size ratio may allow the male to carry the female more easily during the precopulatory mate-guarding behavior (Sexton, 1924; Adams and Greenwood, 1983; Adams et al., 1985). The large males have two advantages over small males: they are better able to successfully take over a female in a competition with another male, and better able to resist such takeover attempts themselves while paired (Ward, 1983).

The sympatric species of *Hyalella* analyzed in the present work showed differences as to mean size of cephalothorax length, being *H. castroi* larger than *H. pleoacuta*. These species differed also as to size at sexual maturity, with *H. castroi* reaching the maturation with larger size that *H. pleoacuta*. The body size, perhaps the most ecologically significant trait for individual, is a determinant of ecological success in *Hyalella* species, being an important phenotypic axis for a tradeoff between resource exploitation and predation vulnerability (Wellborn, 1994, 2002). Fish, especially bluegill, are important predators of *Hyalella* in lakes of the U.S.A., and are strongly size selective. Vulnerability to predation increases with body size owing to size selective predation by bluegill and related fish in species of the *Hyalella azteca* complex, being that large-body species was more susceptibly to predation than small-body species (Wellborn, 1994). In the case of *Hyalella* species analyzed in the current work, the results probably suggested that *H. castroi* is more vulnerable to predation by trouts, due to its great size and this fact possibly explain the low number of individuals collected of this species in comparison of *H. pleoacuta*.

4.3. Sex ratio

Moore (1981) states that sex ratio in populations can be either 1:1, skewed towards females, or males (more rare) or may show seasonal variations, with male's dominance in coolers months and females dominance in warmer months. In amphipods, it is usual that the sex ratio shows seasonal fluctuations and the females are more numerous than males (Powell and Moore, 1991; Cardoso and Veloso, 1996; Mardsen, 2002; Appadoo and Myers, 2004; Kevrekidis, 2004, 2005), as observed in *H. pleoacuta* e *H. castroi.* According to Emmerson (1994), the reproductive potential increases with female's dominance at the population. However, with males dominance there is more intraspecific competition among male's conspecifics, can be result in a population more balance.

Sex ratio deviation of *H. pleoacuta* and *H. castroi* appear to be related to the reproduction system. These species, together with other gammaridean amphipods, show a reproductive strategy which male spend more time choosing, holding and carrying the females and thus the males stand more susceptibility to predators than females (Moore, 1981; Powell and Moore, 1991; Kevrekidis, 2005). Besides, different rates of growth and maturation, mortality and longevity, life history, environment pressure, habitat fragmentation, occupation of distinct microhabitats, food availability, predation and parasites can to cause deviation at the proportion of males and females in a population (Wilson and Pianka, 1963; Wenner, 1972; Moore, 1981; Sheader, 1983; Ali and Salman, 1986; Powell and Moore, 1991).

The sex ratio investigated in the size classes of cephalothorax length showed an anomalous pattern in both *Hyalella* species, similar to that described by Wenner (1972), in which the proportions differed in intermediate classes, favoring one sex (females), and in larger classes favoring the opposite sex (males). This may be related to the large energy investment in reproduction, because females expend part of energy and time at the production of large gametes (Cardoso and Veloso, 1996) and in some amphipod species the females can accomplish parental care behavior (Aoki and Kikuchi, 1991; Thiel, 1999, 2003). In some species, mainly at which the females carrying the juveniles hatching in the marsupium or adhered to body female (Aoki and Kikuchi, 1991; Borowsky, 1991; Thiel, 2003), the molt of female can be adjourn and this fact limit its growth, because the females invest at the care with the brood (Thiel, 2003), as observed in species of *Hyalella* analyzed in the present work.

4.4. Breeding period

Ovigerous females of *H. pleoacuta* and *H. castroi* were found along year, indicating that reproduction is continuous in these species, but it was observed a reproductive peak at the fall and winter in *H. pleoacuta* and *H. castroi*, respectively. Steele and Steele (1991) stated that in tropics there is continuous food availability and thus the species that live there can reproduce all year. The continuous reproductive strategies of both sympatric species of *Hyalella* analyzed in the current work can represent adaptations to compensate for the high mortality of their juveniles. Continuous reproduction it was recorded in some marine tropical amphipods, such as *Pseudorchestoidea brasiliensis* (Dana, 1853) at the Rio de Janeiro, Brazil (Cardoso and Veloso, 2001), *Platorchestia platensis* (Kroyer, 1845) in Guadeloupe (Ciavatti et al., 1993), *P. platensis* in Florida, USA (Garces and Marsh, 1991) and *Cymadusa filosa, Amphithoe laxipodus* and *Mallacoota shellenbergi* in Mauritius, Indian Ocean (Appadoo and Myers, 2004). A continuous reproduction it was also observed at the freshwater amphipods *Hyalella azteca* from Central México (Alcocer et al., 2002) and *Gammarus troglophilus*

Hubricht and Mackin, 1940 from southern Illinois (Jenio, 1980). Availability of food for adults maintain, grow, and reproduce, and for juveniles to develop, grow, and survive may be one of the more important factors in the synchronization and coordination of reproductive activities in a particular environment, and also in the evolution of life history and reproductive strategies (Sastry, 1983).

Although some species of amphipods reproduce continuously, others species show a very restrict reproductive period. The reproduction at the amphipod species can be related mainly with the photoperiod (Steele et al., 1977; Steele, 1981; Williams, 1985; Steele and Steele, 1986; Powell and Moore, 1991), the temperature (Strong, 1972; Borowsky, 1980; Moore, 1983; Morrit and Stevenson, 1993, Wilhelm and Schindler, 2000), a combination of photoperiod and temperature (De March, 1978), or with food availability (Kanneworff, 1965; Powell and Moore, 1991; Costa and Costa, 1999). The great reproductive intensity at the colder months observed in *H. pleoacuta* and *H. castroi* coincided with the months at which there was greater abundance of macrophytes on the edges of the ponds (personal observation). These macrophytes were utilized by species of *Hyalella* as food and shelter. According to Hargrave (1970), *H. azteca* primarily feeding on algae and bacteria associated with the sediments and aquatic macrophytes, but Cooper (1965), recorded that this species can food on dead animal and plant materials too. Costa and Costa (1999) observed that in *Gammarus locusta* (Linnaeus, 1758) from Sado Estuary, Portugal, the frequency of ovigerous females appears to be also coupled with the algal availability.

A reproductive peak at the colder months has been observed in others gammaridean amphipods, such as *Gammarus duebeni* Lilljeborg, 1951, *G. oceanicus* Segerstråle, 1947 and *G. salinus* Spooner, 1947 (Kolding and Fenchel, 1981), in *G. multisetosum* Stock, 1952 (Cunha et al., 2000). However, most of the amphipods reproduces with more intensity at the warmer months as observed in *Gammarus fasciatus* Say, 1818 (Hynes, 1955), *G. lacustris* Sars, 1863 (Hynes and Harper, 1972), *G. palustris* Bousfield, 1969 (Gable and Croker, 1977), *G. duebeni* (Naylor et al., 1988), *Corophium curvispinum* Sars, 1895 (Van den Brink et al., 1993), *G. balcanicus* Schäferna, 1922 (Zielinski, 1995), *G. leopoliensis* Jazdzewski and Konopacka, 1989 (Zielinski, 1998) and *C. orientale* Myers, 1982 (Kevrekidis, 2005).

Reproductive strategies, mainly reproductive period of *H. pleoacuta* and *H. castroi* are different to those demonstrated for *H. azteca*, which the reproductive period occurs from spring to autumn, and the ovigerous females disappear in the other months (Cooper, 1965). In others studies performed with *H. azteca* by Strong (1972) and Wen (1992), the reproduction was observed with more intensity during the summer and Kruschwitz (1978) found ovigerous females from spring to summer. On the other hand, in populations of *H. azteca* from Central Mexico ovigerous females are found all year around (Alcocer et al*.,* 2002), similar to results observed in either species of *Hyalella* analyzed in the present study. Its important also stands out that the *H. pleoacuta* and *H. castroi* were collected in ponds used to raise trout, where the food availability throughout year, once the trouts are food twice day with fish food (43% of protein). The results observed in the present work, suggest that the species of *Hyalella* can be using this food to complement their feeding or the continuous production of excrement by fishes can guarantee adequate nutrients to algae production, which can be also used as foof by both species of *Hyalella.*

4.5. Recruitment

The recruitment of *H. pleoacuta* and *H. castroi* occurs throughout the year, due to a continuous reproductive period. Juveniles were abundant in all samples (>50%) in comparison of adults, but the percentage of juveniles reached a maximum during spring, due to a greater reproductive activity in colder months. Guerao (2003) observed that juveniles of the freshwater gammaridean *Echinogammarus longisetosus* corresponded more of 40% of the sampled population, with greater percentages during winter (February) and spring (April and June). The continuous recruitment and the high frequency of juveniles in all months of the year in either species of *Hyalella* may indicate a reproductive strategy to compensate the high juvenile mortality, probably due to predation. In the sampled ponds there was a great density of potential predators, mainly raise trout (*Oncorhynchus mykiss*), anomuran crustacean (*Aegla serrana*, Buckup and Rossi, 1977) and naids of odonats (Castiglioni and Bond-Buckup, personal observation).

Continuous recruitment was also observed in other amphipods, such as *Corophium multisetosum* in the Ria Aveiro, northwestern Portugal (Cunha et al., 2000), *Echinogammarus marinus* (Leach, 1815) in the Mondego estuary, Portugal (Maranhão et al., 2001), *Echinogammarus longisetosus* in Catalonia, Spain (Guerao, 2003) and *Orchestia gammarellus* (Pallas, 1766) Ria Formosa Lagoon, southern Portugal (Dias and Sprung, 2004) and *Gammarus chevreuxi* in the Ria de Aveiro, northwestern Portugal (Subida et al., 2005).

5. Conclusion

According to Abrams (1983), the coexistence of co-generic species traditionally presume morphologic and behavioral differences between them and, the most of the theoric considerations about coexistence of species suggest the differentiation at the niche as a pre-requisite. The two sympatric species of *Hyalella* showed a very similar population structure, especially the frequency distribution, the greater body size of males, the pattern of sex ratio, and reproduction and recruitment continuous. However, these species differed as body size, size at sexual maturity, and number of specimens collected, being that *H. pleoacuta* was approximately 3 times more frequent than *H. castroi* and also by fact of *H. pleoacuta* shows a small size. Besides, *H. pleoacuta* reproduce with more intensity at the fall and *H. castroi* at the winter and *H. pleoacuta* were found associate mainly with macrophytes that have its floating roots and, *H. castroi* were found in macrophytes that have its imprisoned roots to the sediment. These ecological differences and microhabitat specialization might be facilitating the coexistence of *H. pleoacuta* and *H. castroi* at the artificial ponds of raise trout.

The most outstanding traits of *H. pleoacuta* and *H. castroi* allowing the recovery and maintenance of its population structure along year, is its high reproductive investment. This is noticeable by continuous reproduction and also by the high numbers of juveniles that hatching all months in either species of *Hyalella*.

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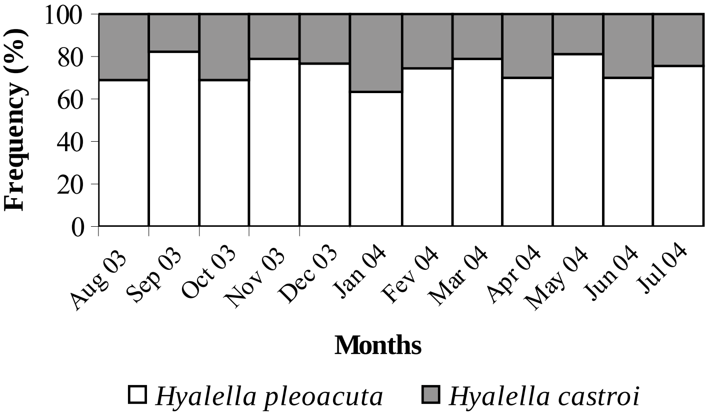

Figure 1. Proportion of *Hyalella pleoacuta* and *H. castroi* sampling along year of study.

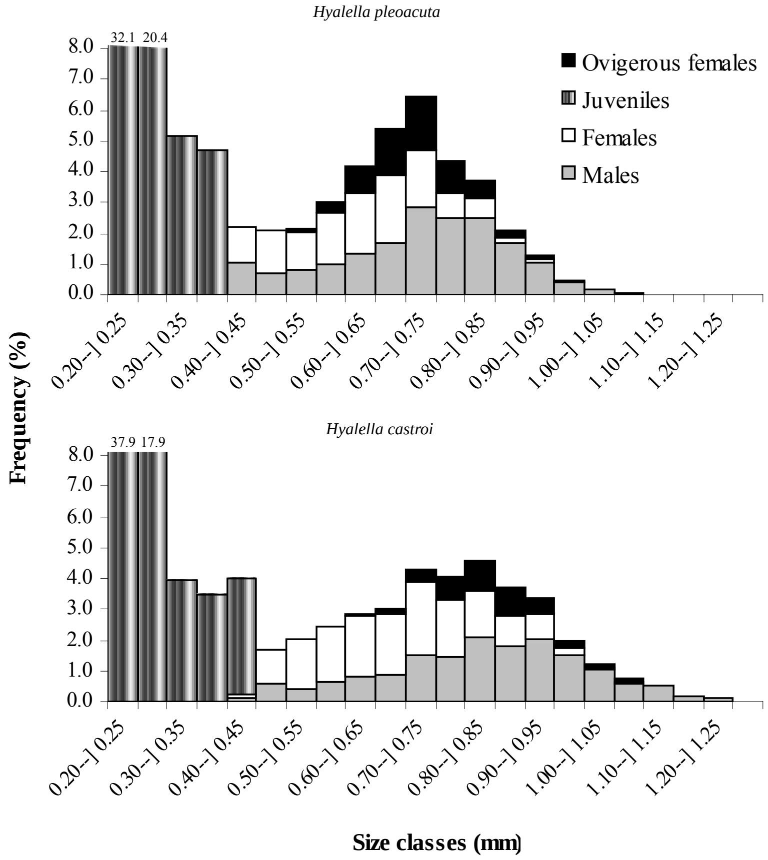

Figure 2. Monthly general bimodal size-distribution in *Hyalella pleoacuta* and *H. castroi*. Numbers above the columns indicate the relative frequency of juveniles.

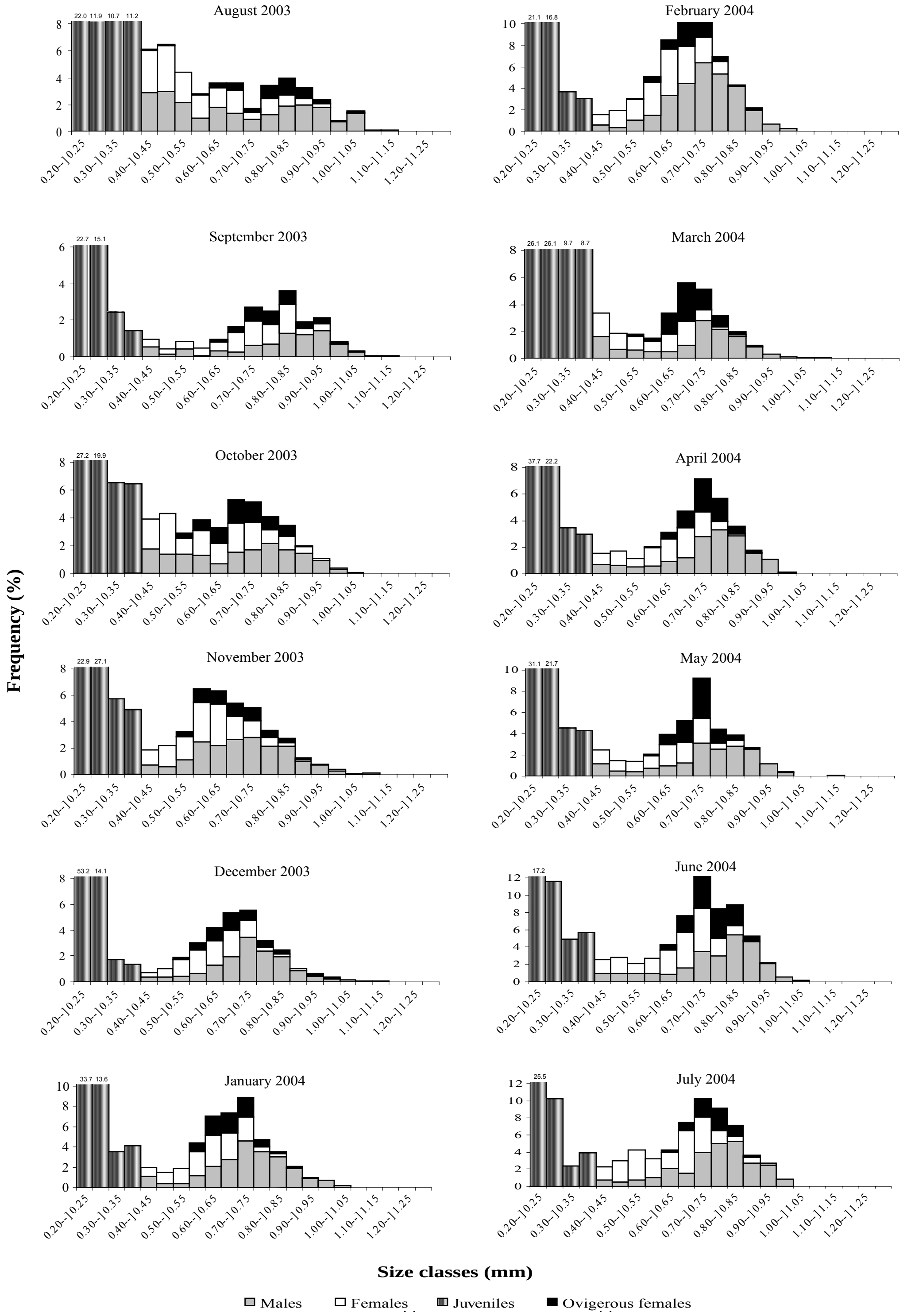

Figure 3. Monthly size–frequency distributions in *Hyalella pleoacuta*. Number above the columns indicate the relative frequency of juveniles.

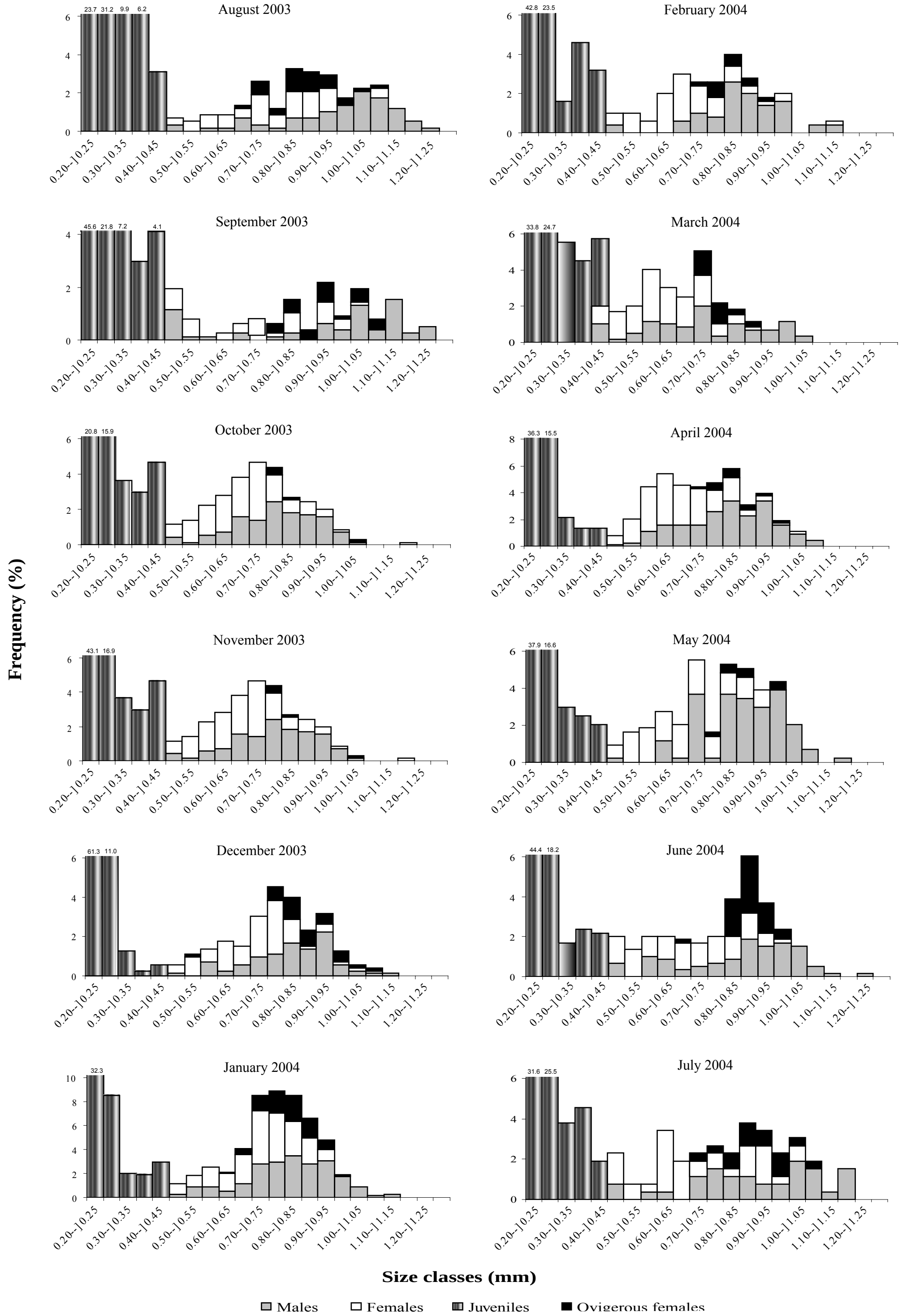

Figure 4. Monthly size–frequency distributions in *Hyalella castroi.* Number above the columns indicate the relative frequency of juveniles.

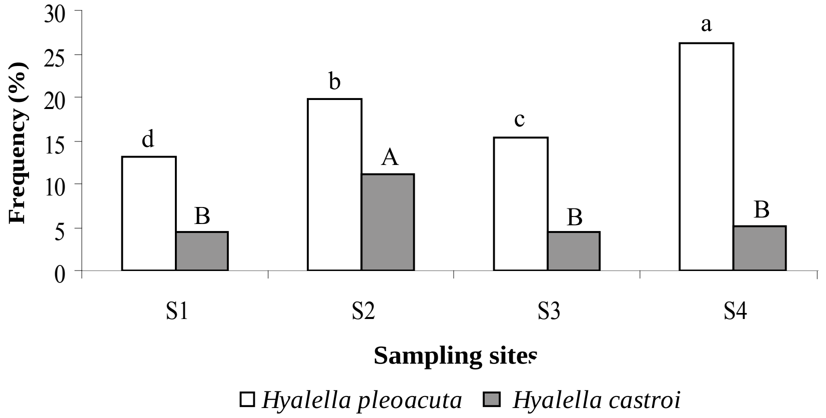

Figure 5. Frequency of specimens of *Hyalella pleoacuta* and *H. castroi* collected by sampling sites. Small letters correspond to the comparisons of the frequency of specimens of *H. pleoacuta* among the sampling sites and the capital letters correspond to the comparisons of the frequency of specimens of *H. castroi* among the sampling sites. Values with at least one letter in common did not differ statistically ($p>0.05$).

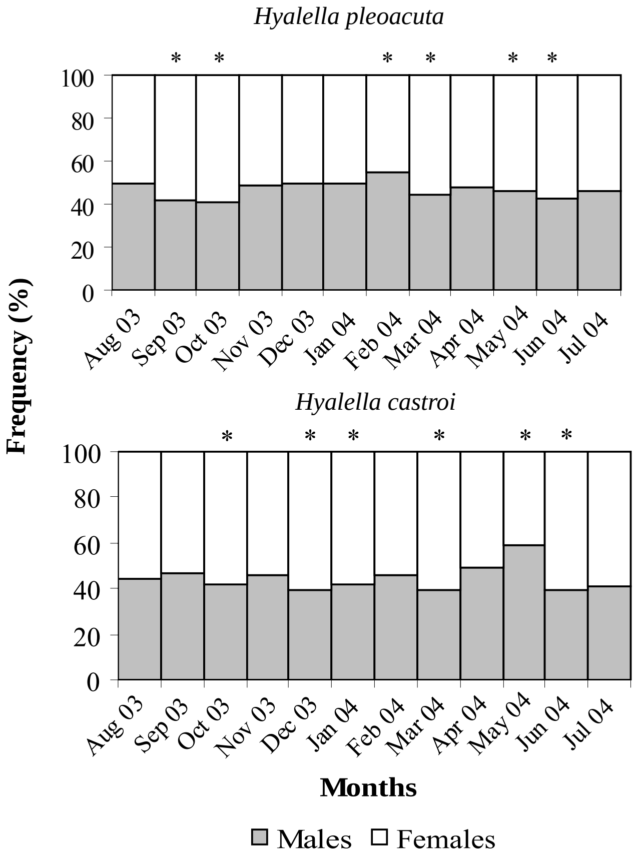

Figure 6. Sex ratio by month in *Hyalella pleoacuta* and *H. castroi*. Asterisks above the columns indicate significant differences between the proportions of males and females (p<0.05).

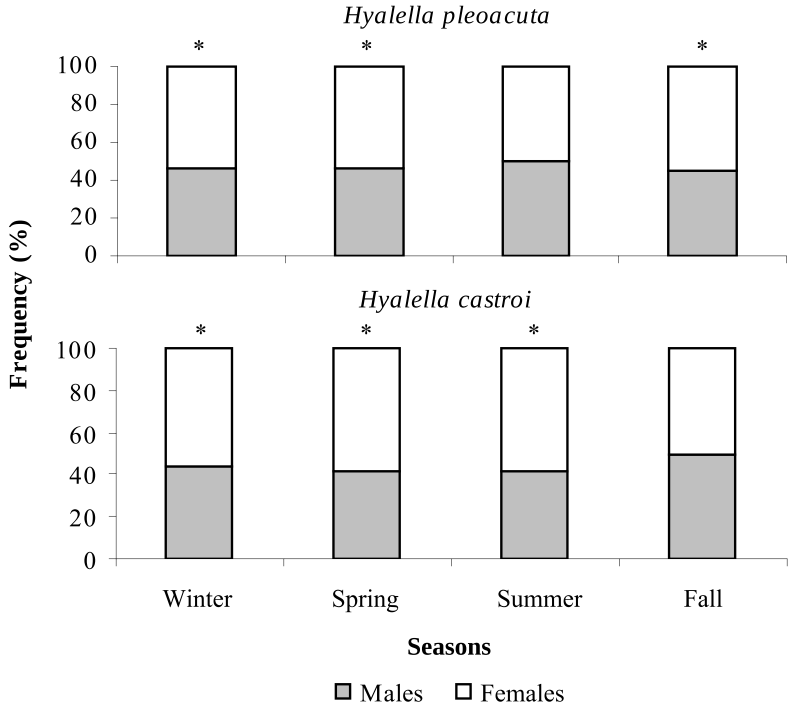

Figure 7. Sex ratio seasonal in *Hyalella pleoacuta* and *H. castroi*. Asterisks above the columns indicate significant differences between the proportions of males and females ($p<0.05$).

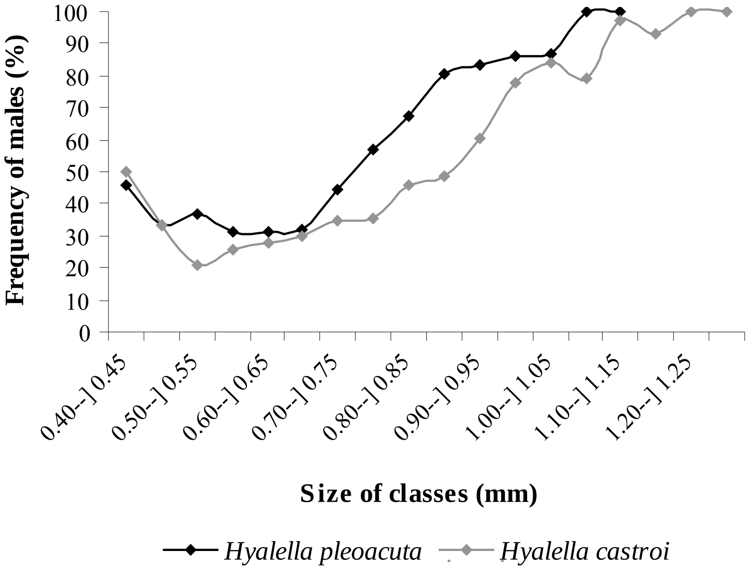

Figure 8. Frequency of males (%) by size classes of cephalothorax length (mm) in *Hyalella pleoacuta* and *H. castroi.*

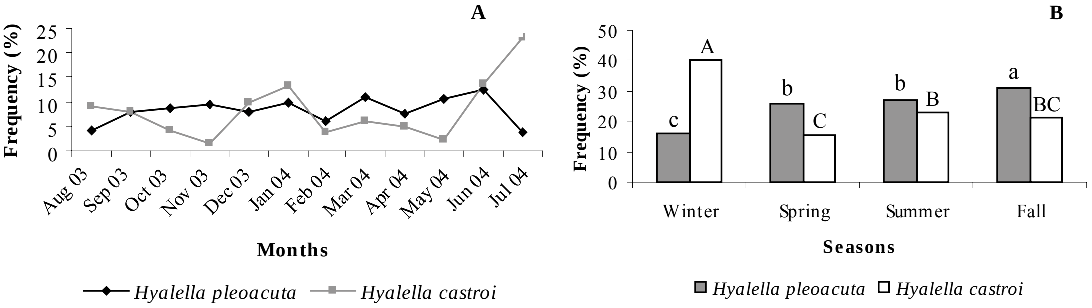

Figure 9. Frequency of females of *Hyalella pleoacuta* and *H. castroi* carrying eggs or juveniles in the marsupium along year of study (A) and along season of the year (B). Small letters correspond to the comparisons of the frequency of ovigerous females of *H. pleoacuta* among the seasons of the year and the capital letters correspond to the comparisons of the frequency of ovigerous females of *H. castroi* among the seasons of the year. Values with at least one letter in common did not differ statistically (p>0.05).

Table I. Proportion of juveniles and adults of *Hyalella pleoacuta* and *H. castroi* by month of the year.

	Hyalella pleoacuta		*Hyalella castroi*	
	Juveniles	**Adults**	**Juveniles**	**Adults**
Aug 03	0.62 a	0.38 b	0.75 a	0.25 b
Sep 03	0.82 a	0.18 b	0.86 a	0.14 b
Oct 03	0.63 a	0.37 b	0.56 a	0.44 b
Nov 03	0.62 a	0.38 b	0.72 a	0.28 b
Dec 03	0.71 a	0.29 b	0.74 a	0.26 b
Jan 04	0.57 a	0.43 b	0.48 a	0.52 a
Feb 04	0.46 b	0.54 a	0.76 a	0.24 b
Mar 04	0.74 a	0.26 b	0.74 a	0.26 b
Apr 04	0.68 a	0.32 b	0.57 a	0.43 b
May 04	0.65 a	0.35 b	0.63 a	0.37 b
Jun 04	0.42 a	0.58 a	0.70 a	0.30 b
Jul 04	0.44 a	0.56 b	0.67 a	0.33 b

Note: small letters correspond to the comparisons of frequency of juveniles and adults in each month of the year for each species of *Hyalella*. Values with at least one letter in common did not differ statistically ($p>0.05$).

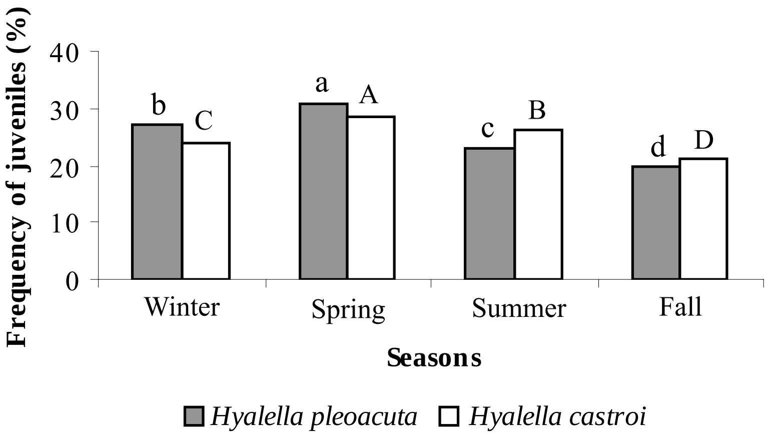

Figure 10. Recruitment of *Hyalella pleoacuta* and *H. castroi* by season of the year. Small letters correspond to the comparisons of the frequency of juveniles of *H. pleoacuta* among the seasons of the year and the capital letters correspond to the comparisons of the frequency of juveniles of *H. castroi* among the seasons of the year. Values with at least one letter in common did not differ statistically (p>0.05).

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Considerações finais

Os crustáceos são freqüentemente utilizados como bioindicadores e biomonitores em vários sistemas aquáticos (RINDERHAGEN *et al.*, 2000). Uma razão para isto, é que eles são um grupo de animais bem sucedidos, distribuídos em diferentes habitats, incluindo os ambientes marinho, terrestre e dulcícola.

Alguns aspectos dos crustáceos, principalmente as estratégias reprodutivas, podem ser altamente importantes para a interpretação de dados sobre estudos da bioindicação e para o desenvolvimento de estudos ecotoxicológicos. Não somente os aspectos reprodutivos, mas também outras respostas comportamentais dos crustáceos, como as mudanças na alimentação, locomoção ou comportamento de pré-cópula podem ter respostas sensitivas com respeito a bioindicação de toxicidade em determinado ambiente (RINDERHAGEN *et. al.* 2000).

Os anfípodos possuem grande importância nas comunidades das quais fazem parte, pois possuem hábitos detritívoros e herbívoros e servem de alimento para vários organismos. Desta maneira, estes crustáceos constituem-se importantes elos nas cadeias alimentares dos ecossistemas onde ocorrem, pois possibilitam a transferência de energia produzida pelas algas e vegetais superiores para os consumidores de níveis tróficos mais elevados (MOORE 1975, MATTHEWS *et al.* 1992, MUSKÓ 1993, PILGRIM & BURT 1993). Além disso, os anfípodos freqüentemente são utilizados como organismos testes em estudos ecotoxicológicos, devido a sua distribuição cosmopolita, a sensibilidade a distúrbios e a sua fácil adaptação a cultivos e experimentos laboratoriais (NEUPARTH *et al.* 2002). O anfípodos dulcícolas mais utilizados em testes de toxicidade tem sido a espécie norte-americana *Hyalella azteca* (Saussure, 1858) e *Gammarus lacustris* Sars, 1863 (BUYLE 1989, NELSON & BRUNSON 1995, DUAN *et al.* 1997).

O Centro Tecnológico de Saneamento Básico (CETESB), órgão responsável pelo controle e prevenção da poluição em São Paulo juntamente com o Laboratório de Ecotoxicologia da Universidade Federal do Rio Grande do Sul cultivam e utilizam a espécie exótica norte-americana *H. azteca* em testes de toxicidade. Entretanto em estudos moleculares desenvolvidos recentemente por DUAN *et al.* (1997), MCPEEK & WELLBORN (1998) e WITT & HEBERT (2000), verificou-se que esta espécie é um complexo de espécies distintas, tornando inviável o uso desta em estudos toxicológicos. O uso de *H. azteca* em testes de toxicidade realizados no Brasil, deve-se provavelmente a ausência de conhecimento sobre a bioecologia das espécies nativas e de padronização das mesmas em cultivos de laboratório. De acordo com o EPA (Environmental Protection Agency) (1989), os critérios para a utilização de um organismo como bioindicador são: um amplo conhecimento da distribuição da espécie, localização dentro da estrutura trófica, conhecimento da biologia, hábitos alimentares e fisiologia, manutenção e cultivo em laboratório.

De acordo com ARENZON (1996) A necessidade de criação de programas de monitoramento ambiental fazendo uso de espécies aquáticas autóctones reside principalmente no impacto, direto ou indireto, que a introdução intencional ou não de uma espécie exótica teria sobre a dinâmica ambiental. Além disso, a qualidade da resposta que uma espécie autóctone poderia fornecer seria mais próxima da realidade, já que em geral, sua condição ideal de cultivo, bem como as condições de realização dos testes, estão próximas das características ambientais. Neste sentido, o uso de espécies autóctones, adaptadas às características ambientais, certamente poderia prover resultados muito mais condizentes com a realidade do que os resultados obtidos com espécies exóticas.

Pesquisas sobre inventarimento da carcinofauna límnica no sul do Brasil tem apontado para um número bastante expressivo de novas espécies de *Hyalella* (BOND-BUCKUP *et al.*

2006). No entanto, nada se conhecia sobre a biologia e ecologia destas espécies. Nesse sentido, essa pesquisa visou preencher a lacuna sobre dados biológicos que possam subsidiar o uso destas espécies em estudos ecotoxicológicos.

Os resultados obtidos nas análises dos aspectos do ciclo de vida de *H. pleoacuta* e *H. castroi* expostos nos capítulos anteriores permitem as seguintes conclusões, as quais encontram-se sumarizadas no final desta seção e também listadas a seguir:

* A idade da primeira muda, a freqüência de mudas, a idade da determinação do sexo e a maturidade sexual foram muito similares entre as espécies simpátricas *H. pleoacuta* e *H. castroi*. Entretanto, estas espécies diferiram quanto ao período de intermuda total, tamanho corpóreo máximo e longevidade em condições laboratoriais.

* Foi observada correlação entre o tamanho dos machos e fêmeas nos casais em pré-copula, ou seja, machos grandes carregam fêmeas grandes em ambas espécies de *Hyalella*.

* O sucesso de pareamento e sucesso reprodutivo dos machos aumenta a medida que o seu tamanho corpóreo também aumenta em ambas espécies de *Hyalella*. Fêmeas de tamanho corpóreo intermediário apresentaram maior sucesso reprodutivo em ambas espécies de *Hyalella*.

* Ambas espécies de *Hyalella* mostraram as mesmas estratégias reprodutivas em condições de laboratório, especialmente quanto à duração do comportamento de pré-copula, a duração do período embrionário, fecundidade e viabilidade (número de juvenis liberados do marsúpio).

* Com relação à produção de ovos, *H. pleoacuta* produziu mais ovos do que *H. castroi,* mas nesta última os ovos são maiores. Em ambas espécies foi observada diminuição no número de ovos ao longo do seu desenvolvimento.

* Tanto *H. pleoacuta* como *H. castro* apresentaram uma estrutura populacional muito similar, especialmente quanto à distribuição de freqüência em classes de tamanho, o maior tamanho corpóreo dos machos em relação as fêmeas, o padrão de proporção sexual com predominância de fêmeas e a reprodução e recrutamento contínuos.

* As espécies diferiram quanto ao número de exemplares amostrados, sendo *H. pleoacuta* aproximadamente 3 vezes mais freqüente do que *H. castroi* e também pelo fato de *H. pleoacuta* apresentar um tamanho corpóreo menor do que *H. castroi*. Além disso, *H. pleoacuta* reproduz-se com maior intensidade no outono e *H. castroi* no inverno.

De acordo com Abrams (1983) a coexistência de espécies pertencentes ao mesmo gênero é tradicionalmente atribuída a diferenças morfológicas e comportamentais. No caso das espécies de *Hyalella* analisadas no presente trabalho pode-se inferir que a coexistência das mesmas é facilitada especialmente por diferenças no tamanho corpóreo e nas estratégias reprodutivas, como por exemplo, na duração do período embrionário total, no número e tamanho dos ovos produzidos, na estação reprodutiva, entre outros.

O potencial reprodutivo elevado, a reprodução e o recrutamento contínuos e também o fácil manejo de cultivo, mostram que *H. pleoacuta* e *H. castroi* possuem potencial para serem utilizadas em futuros estudos ecotoxicológicos no Brasil. Entretanto, necessita-se ainda conhecer o comportamento reprodutivo, o hábito alimentar, a sensibilidade das mesmas a determinados contaminantes e também fazer com que as duas espécies reproduzam-se continuamente em laboratório, para posteriormente serem utilizadas como bioindicadoras de qualidade ambiental.

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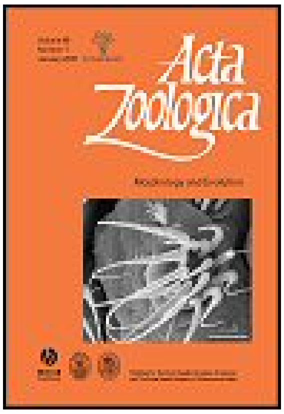

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Cloney, R. A. 1978. Ascidian metamorphosis: review and analysis. In F.-S. Chia and M. E. Rice (Eds): *Settlement and Metamorphosis of Marine Invertebrate Larvae*, pp. 255-282. Elsevier, New York.

Ruppert, E. E. 1997. Cephalochordata (Acrania). In Harrison, F. W. (Ed.): *Microscopic Anatomy of Invertebrates*, vol. 11, pp. 349-504. Wiley-Liss, New York.

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