UNIVERSIDADE FEDERAL DE LAVRAS

RAFAELA BASTOS PEREIRA

ECOLOGIA DE POPULAÇÕES E BIOLOGIA REPRODUTIVA EM *Hyalella* (CRUSTACEA, AMPHIPODA, HYALELLIDAE)

LAVRAS-MG
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Dissertação apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração em Ecologia e Conservação de Recursos em Paisagens Fragmentadas de Agrossistemas, para a obtenção do título de Mestre.

Orientadora
Dra. Alessandra Angélica de Pádua Bueno

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APROVADA em 3 de fevereiro de 2014.

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Dra. Daniela da Silva Castiglioni – UFSM

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Dra. Carla Rodrigues Ribas - UFLA

Dra. Alessandra Angélica de Pádua Bueno
Orientadora

LAVRAS – MG
2014

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"Até os jovens podem fatigar-se e esgotar-se, mas os que confiam no Senhor renovam suas forças, Ele dá-lhes asas de águia, correm sem se cansar, vão para frente sem se fatigar." (Isaías 40, 30-31)

RESUMO

O gênero *Hyalella* tem sido amplamente estudado nos últimos anos, especialmente quanto à sua taxonomia. Pouco se conhece da biologia e ecologia deste gênero, principalmente com relação às espécies brasileiras. Assim sendo, buscou-se s elucidar com o presente trabalho, a ecologia de populações e a biologia reprodutiva de *H. longistila* e *H. carstica*. Ambas são encontradas no estado de Minas Gerais, Sudeste do Brasil, em distintos córregos, porém, *H. carstica* habita um corpo d'água intermitente. Em ambos os córregos foram coletadas as macrófitas ou o substrato contido abaixo destas, durante vinte minutos, os quais foram minuciosamente triados ainda em campo. Todos os indivíduos foram preservados em etanol 70%, e fêmeas ovígeras e casais em pré-cópula foram individualizados em micro túbulos. No laboratório classificou-se estes crustáceos em machos, fêmeas, fêmeas ovígeras ou juvenis e cada indivíduo foi medido quanto ao comprimento do cefalotórax (CL), comprimento total (TL) e no caso de fêmeas ovígeras também a largura do marsúpio (MW). Adultos predominaram, na maioria dos meses, na população de *H. longistila*, enquanto os juvenis de *H. carstica* foram predominantes antes do período seco do córrego (Setembro a Novembro) e os adultos foram mais abundantes após. A razão média CL/TL de juvenis foi superior à dos adultos em ambas as espécies, sugerindo que esta razão seja utilizada como um indicativo da fase adulta junto de outros parâmetros. A razão sexual média total de ambas as populações foi de 1 macho : 0,75 fêmeas, sendo que esta razão favoreceu as fêmeas de *H. longistila* nos meses de verão e machos no restante do ano, e em *H. carstica* os machos predominaram o ano todo. A reprodução de *H. longistila* foi contínua e *H. carstica* reproduziu-se mais intensamente depois da seca, quando se sugeriu que ocorra a recolonização do local por adultos provenientes de centros de abundância populacional à montante. Em relação aos fatores ambientais, um efeito positivo e independente da temperatura sobre a abundância e de oxigênio dissolvido sobre a frequência de fêmeas ovígeras foi observada na população de *H. longistila*, enquanto a frequência de fêmeas ovígeras de *H. carstica* foi influenciada pela temperatura e condutividade. No geral, adultos pareados apresentaram maior CL que os não pareados em ambos os sexos e espécies. A fecundidade média de *H. longistila* foi 12,88 ± 2,00 ovos/fêmea e a de *H. carstica* foram 12,60 ± 7,20 ovos/fêmea. Diferenças foram observadas em relação ao número de ovos das fêmeas de *H. longistila* quando consideramos diferentes estágios de desenvolvimento, mas não para *H. carstica.* O estágio e número de ovos estavam correlacionados nas fêmeas de *H. longistila,* mas não em *H. carstica.* O volume médio dos ovos no primeiro estágio não diferiu entre espécies, mas nos estágios 2 e 3 sim. Uma relação linear foi observada entre o número de ovos no Estágio 1 e o tamanho da respectiva fêmea ovígera em ambas as espécies. O sucesso reprodutivo de *H. carstica* foi maior em classes de tamanho intermediárias, enquanto para fêmeas de *H. longistila* houve um aumento progressivo no sucesso à medida que aumentava o tamanho das fêmeas. Diferenças foram observadas entre as dinâmicas populacionais e a biologia reprodutiva das duas espécies. As distintas condições (especialmente do regime hídrico) sob as quais *H. longistila* e *H. carstica* foram encontradas podem ter ocasionado tais diferenças, no entanto, as peculiaridades da biologia de cada espécie também podem ter influenciado os resultados. Além disso, as coletas foram feitas em épocas diferentes, o que também pode ter sido um fator causal para as diferenças observadas. As informações geradas por este trabalho são importantes para subsidiar trabalhos futuros com estes anfípodos, assim como também para práticas conservacionistas para eles e para o ecossistema em que estão inseridos.

Palavras-chave: *Hyalella longistila; Hyalella carstica;* Dinâmica de populações; Período reprodutivo; Produção de ovos; Fecundidade; Sucesso de pareamento; Sucesso reprodutivo.

ABSTRACT

The genus *Hyalella* has been widely studied in the last years, especially their taxonomy. Little is known about the biology and ecology of this genus, particularly in respect to Brazilian species. Thus, we aimed to elucidate in the present work the population ecology and reproductive biology of *H. longistila* and *H. carstica*. Both are found on the state of Minas Gerais, Southeastern Brazil, in distincti streams, and *H. carstica* inhabits na intermittent waterbody. In both streams we collected macrophytes or the substrate above them during twenty minutes, and the animals were thoroughly separated from the sediment in the field. All individuals were preserved on etanol 70% and ovigeorus females and couples were individualized in microtubules. In the laboratory we classified such crustaceans in males, females, ovigerous females or juveniles and measured each individual in relation to the cephalothorax length (CL), total body length (TL) and in the case of ovigerous females the marsupium width (MW) also was measured. Adults predominated in most of the months on the *H. longistila* population, while juveniles of *H. carstica* predominated before the dry period of the stream (September to November) and adults were more abundant after it. The mean ratio CL/TL of juveniles was superior to the adults in both species, thus suggesting that such ration may be used as an indicative of the adult phase together with other parameters. The mean total sex ratio of both populations was 1 male : 0.75 females, and this ratio favored females of *H. longistila* on summer months and males in the rest of the year. In the *H. carstica* population males predominated along the year. The reproduction of *H. longistila* was continuous and *H carstica* reproduced more intensively after the dry, when we suggest that occur the local recolonization by adults from upstream centers of population abundance. In relation to the environmental factors, a positive and independent effect of temperature over abundance and dissolved oxygen over the frequency of ovigerous females was observed on the *H. longistila* population, while the frequency of ovigerous females of *H. carstica* was influenced by temperature and conductivity. In general paired adults presented larger CL than non-paired adults in both sexes and species. The mean fecundity of *H. longistila* was 12.88 ± 2.00 eggs/female and 12.60 ± 7,20 eggs/female for *H. carstica.* The stage and number of eggs was negatively correlated on females of *H. longistila* but not in *H. carstica.* The mean volume of eggs on the first stage did not differ between species, but had differed on stages 2 and 3. A linear relation was observed between the number of eggs on stage 1 and the size of the respective ovigerous female in both species. The reproductive success of *H. carstica* was higher on intermediate size classes, while for *H. longistila* there was a progressive increase in the success as the female size increased. Differences were observed between the population dynamics and reproductive biology of the two species. The distinct conditions (especially of the hydric regime) under which *H. longistila* and *H. carstica* were found may have caused such differences, however the peculiarities of the biology of each species also may have influenced the results. Furthermore, collections were made in distinct periods, what also may be a causal factor for the observed differences. The information generated by this work are important to subsidize future works with such amphipods, as well as for conservation practices for them and the ecosystem in which they are inserted.

Keywords: *Hyalella longistila; Hyalella carstica;* Population dynamics; Breeding period; Egg production; Fecundity; Pairing success; Reproductive success.

SUMÁRIO

CAPÍTULO 1

1 INTRODUÇÃO GERAL

O gênero *Hyalella* Smith (1874) compreende espécies de crustáceos exclusivamente de água doce, as quais são endêmicas do continente americano. Este gênero pertence à família Hyalellidae, segundo a classificação taxonômica mais recente da ordem Amphipoda (LOWRY; MYERS, 2013). As espécies de *Hyalella* podem ser encontradas em uma grande variedade de ambientes, como rios, lagos, áreas úmidas, córregos e adjacentes de ambientes lóticos e lênticos (KRUSCHWITZ, 1978; WELLBORN, 1995; GROSSO; PERALTA, 1999). A maioria das espécies é encontrada em ambientes epígeos, mas existem espécies que são encontradas tanto em ambientes epígeos quanto hipógeos, enquanto outras são troglóbias (PEREIRA, 1989; CARDOSO*;* BUENO; FERREIRA, 2011) ou hipotelminorreicas (RODRIGUES, BUENO, FERREIRA, 2012). Uma única espécie é conhecida por habitar solos úmidos em uma floresta de gimnospermas na Argentina, *H. rionegrina* (GROSSO; PERALTA, 1999).

Estes crustáceos podem viver aderidos à vegetação aquática ou em depósitos de detritos e substrato de fundo dos corpos d'água e podem alimentar-se de algas e bactérias associadas ao sedimento e às macrófitas aquáticas (HARGRAVE, 1970), além de detritos animais e vegetais em decomposição (COOPER, 1965). Dessa forma facilitam o fluxo de energia no ambiente aquático, atuando diretamente na conversão da energia de detritos em matéria orgânica fina particulada (WEN, 1992).

Além disso, anfípodos como *Gammarus pulex* (KAHN et al, 2011) e *G. roeseli* (GISMONDI; COSSU-LEGUILLE; BEISEL, 2013) e *H. azteca* (NELSON; BRUNSON, 1995) têm sido utilizados em testes de ecotoxicologia. Algumas características os tornam bons organismos experimentais, já que no geral são facilmente coletados, pois algumas espécies apresentam populações abundantes, são cultivados em laboratório com facilidade, tem curto ciclo de vida, mas principalmente destaca-se a sensibilidade que muitas espécies apresentam em relação à contaminantes (KRUSCHWITZ, 1978; PILGRIM; BURT, 1993; RINDERHAGEN et al., 2000).

O conhecimento acerca da Ecologia e Biologia das espécies de *Hyalella,* não somente subsidia práticas conservacionistas, mas gera informações que serão base para estudos posteriores, sendo estes mais aplicados. Isto, por conseguinte, amplia o conhecimento acerca do gênero, o qual ainda é incipiente especialmente para espécies brasileiras de *Hyalella*. Dentre estas, conhece-se aspectos ecológicos apenas para *H. castroi* e *H. pleoacuta,* espécies simpátricas encontradas na região sul do país (CASTIGLIONI; BOND-BUCKUP, 2008a).

Desse modo o presente trabalho comtém informações sobre a Ecologia de populações e Biologia reprodutiva de duas espécies brasileiras de *Hyalella,* sendo estas *H. longistila* e *H. carstica,* as quais foram coletadas no estado de Minas Gerais. O fato de as espécies estudadas viverem sob diferentes regimes de disponibilidade de água ao longo do ano, levou a distintas interpretações acerca das observações feitas em cada população, o que amplia o conhecimento acerca da ecologia do gênero, especialmente no que concerne às espécies brasileiras. No entanto, é necessário salientar que as coletas de cada espécie foram feitas em períodos distintos, e as condições do habitat podem ter alterado ao longo dos dois anos de intervalo entre as coletas de *H. carstica* e *H. longistila.*

Assim sendo, a presente dissertação está dividida da seguinte maneira: este primeiro capítulo contém informações gerais sobre o quê são os organismos estudados, o estado da arte em relação ao conhecimento da história de vida da ordem Amphipoda e do gênero *Hyalella*, mais especificamente. Neste capítulo constam também os objetivos e hipóteses da autora com o presente trabalho. O segundo capítulo está sob a forma de um artigo que trata da ecologia das populações estudadas de *H. longistila* e *H. carstica*, o qual contém uma abordagem sobre a dinâmica de cada população frente aos diferentes regimes hídricos a que são submetidas. E por fim, o terceiro capítulo que também está sob forma de artigo, aborda a biologia reprodutiva das duas espécies estudadas, contendo informações sobre seus períodos reprodutivos, bem como a produção de ovos e sua relação com as métricas das respectivas fêmeas ovígeras, sucesso de pareamento e sucesso reprodutivo.

2 Referencial teórico

2.1 Aspectos gerais

Ao longo do tempo os crustáceos desenvolveram uma grande diversidade morfológica, a maior dentre os habitantes da Terra (MARTIN; DAVIS, 2001). Além disso, estes animais apresentam diversas estratégias de história de vida e foram capazes de colonizar diversos tipos de habitat, ocupando desde a região bentônica até mares profundos, regiões costeiras e estuarinas, habitats terrestres e semi-terrestres, riachos, lagoas e rios de baixas a grandes latitudes. No entanto, a maioria das espécies é marinha, já que o subfilo originou-se há cerca de 600 milhões de anos nos oceanos e a conquista do ambiente de água doce data de aproximadamente 3,4 milhões de anos, no Terciário (RUPPERT; BARNES, 1996; SCHUBART; DIESEL; HEDGES, 1998).

De acordo com Brusca e Brusca (2007) a superordem Peracarida, juntamente com a ordem Decapoda, constituem a maioria dos crustáceos. Peracarida é formada por nove ordens, das quais Isopoda e Amphipoda são as mais representativas.

Aproximadamente 8000 espécies de Amphipoda são conhecidas, podendo estas apresentar de 1 mm a 25 cm, as quais conquistaram os ambientes marinhos, dulcícolas e terrestres (BRUSCA; BRUSCA, 2007). Caracterizam-se principalmente pela ausência de carapaça, corpo achatado lateralmente, sete pares de pereiópodos unirremes, sendo o primeiro e o segundo modificados em sub-quelas (os chamados gnatópodos).

O trabalho de Lowry & Myers (2013) representa a classificação mais recente da ordem Amphipoda. Os autores afirmam que este táxon é basicamente definido pela presença de uma sinapomorfia anteriormente não reconhecida, que se trata de robustas setas apicais nos ramos dos urópodos 1 e 2. Criou-se uma nova subordem nomeada Senticaudata que compreende quase todas as espécies de Amphipoda de água doce, além de algumas marinhas que antes faziam parte de Gammaridea, uma antiga subordem. A filogenia de Senticaudata foi determinada por meio de análise cladística de caracteres morfológicos e seus estados. Encontra-se entre as seis infraordens de Senticaudata a infraordem Talitrida, da qual faz parte Talitridira, uma parvordem recentemente criada, e nela se insere a superfamília Talitroidea. O gênero *Hyalella* Smith (1874) foi alocado nesta superfamília, mais especificamente na família Dogielinotidae, por Serejo (2004), que a subdividiu em três subfamílias na qual se encontrava Hyalellinae. No entanto,, de acordo com a classificação mais recente de Amphipoda, Hyalellidae foi elevado ao nível de família novamente e o gênero *Hyalella* atualmente pertence a esta família (LOWRY; MYERS, 2013)

Nos últimos anos o gênero *Hyalella* vem sendo amplamente estudado, principalmente com relação à taxonomia. Atualmente são conhecidas 61 espécies para este gênero, das quais 20 são encontradas no Brasil (GONZÁLEZ; WATLING, 2002a; GONZÁLEZ; WATLING, 2002b; GONZÁLEZ; WATLING, 2002c; GONZÁLEZ; WATLING, 2003a; GONZÁLEZ, WATLING, 2003b; BALDINGER, 2004; PEREIRA, 2004; GONZÁLEZ; BOND-BUCKUP; ARAUJO, 2006; CARDOSO; BUENO; FERREIRA, 2011; BASTOS-PEREIRA; BUENO, 2012; RODRIGUES; BUENO; FERREIRA, 2012; BASTOS-PEREIRA; BUENO, 2013; BUENO et al., 2013).

É um gênero endêmico das Américas, e apesar de ocorrer ao longo das regiões biogeográficas Neártica e Neotropical, a distribuição da maioria das espécies é restrita. Provavelmente a distribuição atual mais complexa se trata de *H. azteca* (Saussure, 1858), que ocorre na América do Norte e América Central. A América do Sul apresenta a maior diversidade para o gênero, contendo 51 espécies válidas, além de muitas que já foram coletadas e ainda estão sendo descritas (CARDOSO; BUENO; FERREIRA, 2011; BUENO et al., 2013). González e Watling (2003b) referem-se a, pelo menos, cem espécies que foram coletadas no Lago Titicaca (Bolívia) e cujas descrições ainda não foram publicadas.

No Brasil as espécies conhecidas restringem-se às regiões Sul e Sudeste, no entanto, é válido salientar que nem todas as regiões foram amostradas, portanto, essa realidade é passível de ser mudada. Na região Sul a maioria das espécies e éencontrada no estado do Rio Grande do Sul (*H. bonariensis, H. castroi, H. pleoacuta, H. cusvispina, H. montenegrinae, H. pseudoazteca, H. pampeana, H. imbya, H. kaingang, H. pampeana*) e apenas uma é encontrada no Paraná (*H. brasiliensis*). Para o estado de Santa Catarina há espécimes coletadas que representam espécies novas, embora ainda não tenham sido descritas. Na região Sudeste, cinco espécies ocorre no estado de São Paulo (*H meinerti, H. warmingi, H. dielaii, H. caeca* e *H. spelaea*), sendo estas duas últimas cavernícolas. Para o Rio de Janeiro duas espécies são conhecidas (*H. longistila, H. gracilicornis*) e em Minas Gerais ocorrem seis espécies (*H. warmingi, H. gracilicornis, H. longistila, H. carstica, H. minensis, H. xakriaba*) (BUENO; RODRIGUES; ARAUJO *in press*).

A espécie *H. longistila* foi originalmente descrita por Faxon em 1876, tendo recebido o nome de *Allorchestes longistilus.* Nomeada *Hyalella longistila* por Stebbing (1906), *Hyalella longistilus* por Barnard e Barnard (1983), *Hyalella (Mesohyalella) longistyla* por Bousfield (1996). Posteriormente esta espécie foi redescrita por González e Watling (2003^{a}) e renomeada *Hyalella longistila*, nome que perdura até o presente. Sua localidade-tipo situa-se próximo ao município de Campos, estado do Rio de Janeiro, e recentemente Bastos-Pereira e Bueno (2012) registraram uma nova ocorrência para a espécie em um córrego situado no município de Ijaci, sul de Minas Gerais. Juntamente com este novo registro de *H. longistila,* Bastos-Pereira (2012) descrevem *H. carstica*, uma espécie encontrada na região de Arcos e Pains, Minas Gerais.

2.2 História de vida

A compreensão da história de vida de um organismo está atrelada à compreensão de sua biologia evolutiva e ecologia. De forma geral, o conceito de história de vida postula que a seleção natural molda a ocorrência e duração de eventos chave ao longo do ciclo de vida de forma que a sobrevivência da prole seja otimizada. A idade e tamanho em que a maturidade sexual é atingida, o primeiro evento reprodutivo e posterior investimento ao longo da fase adulta, o sucesso reprodutivo (muitas vezes medido pelo tamanho e sobrevivência da prole), senescência e morte, entre outros aspectos da história de vida de um organismo, dependem das condições físicas e ecológicas às quais os organismos estão submetidos. Assim, os organismos evoluíram diversos tipos de histórias de vida, e encontrar padrões que se repetem no tempo e no espaço permite compreender melhor estes organismos (TOWNSEND; HARPER; BEGON 2010).

Considerando que há uma quantidade total limitada de energia e/ou outro recurso disponível para crescimento, sobrevivência e reprodução, relações negativas de compensação podem ser observadas, sendo estas conhecidas por *trade-offs*. Estes constituem os custos gerados para o *fitness* quando uma mudança benéfica em uma característica está relacionada a uma restrição ou mudança prejudicial em outra (STEARNS, 1989).

Dentre as teorias sobre estratégias de história de vida, a que encontrou maior respaldo por parte da comunidade acadêmica foram as estratégias r e K (MacARTHUR; WILSON, 1967; PIANKA, 1970; STEARNS, 1976).

Espécies capazes de se multiplicar rapidamente geralmente são bem sucedidas em ambientes efêmeros, ou periodicamente sujeitos à perturbações. O ambiente em que vivem permite um rápido crescimento, favorecendo assim a produção de indivíduos com grande número de descendentes pequenos para os quais se observa um *trade-off* entre tamanho e sobrevivência da prole. Neste caso, como não há competição expressiva entre os jovens, o tamanho da prole pode não ser importante. Estes são chamados r-estrategistas, pois na maior parte do ciclo de vida os organismos estão próximos da fase exponencial do crescimento populacional (r) (TOWNSEND; HARPER; BEGON 2010).

Há organismos que apresentam um padrão diferente de história de vida, geralmente aqueles que sobrevivem em habitats onde há intensa competição por recursos. Nestes ambientes os indivíduos que conseguirem captar mais recursos serão bem sucedidos para gerar seus descendentes. Assim, os organismos despendem mais energia para o crescimento do que para reprodução, ou despendem energia de alguma forma que favoreça sua sobrevivência na disputa pelos recursos limitados. Estes são chamados K-estrategistas por passarem a maior parte do ciclo de vida em uma fase do crescimento populacional próxima da capacidade suporte (K) do ambiente onde vivem e produzem menos descendentes, no entanto, de maior tamanho corporal. Neste caso também se observa um *trade-off*, pois como a quantidade de recurso disponível para reprodução é limitada e as densidades populacionais são maiores, a seleção natural favorecerá a produção de menos descendentes de maior tamanho, os quais serão melhores competidores garantindo, portanto, uma maior sobrevivência. Embora haja organismos que não se enquadrem neste modelo r / K, podemos não assumi-lo integralmente, ou ainda considerá-lo como uma maneira de simplificar a compreensão da multiplicidade de histórias de vida existentes (TOWNSEND; HARPER; BEGON 2010).

2.3 Estratégias de história de vida em Amphipoda

Embora não tenha sido o pioneiro nos estudos de história de vida de crustáceos da ordem Amphipoda, o trabalho de Morino (1978) ofereceu uma importante contribuição para a compreensão dos padrões observados para o grupo. Por meio de uma revisão os padrões foram divididos em quatro categorias, estando estas relacionadas à latitude nas quais foram encontradas, e não à categoria taxonômica a qual pertence. O tipo 1 compreende organismos que se reproduzem ao longo de todo o ano e àqueles cuja reprodução se restringe à alguma estação; o tipo 2 engloba organismos que apresentam duas gerações completando um ciclo de vida anual; o tipo 3 trata dos anfípodos que apresentam uma única geração que completa o ciclo de vida em um ano; e o tipo 4 compreende organismos para os quais são necessários dois anos ou mais para que a reprodução aconteça. Constatou-se, por meio desta revisão, que de forma geral e em uma escala global as espécies de baixas altitudes tendem a reproduzir ao longo de todo o ano e tem curtos ciclos de vida, e espécies de altas altitudes tem longos ciclos de vida e reproduzem-se em estações restritas. O autor ressaltou que esta classificação foi apenas uma tentativa de compreender melhor os tipos de história de vida existentes entre Amphipoda, e certamente há espécies de tipos intermediários.

Posteriormente a esta revisão, os trabalhos que tratam dos aspectos de história de vida de Amphipoda passaram a ser mais frequentes. Van Dolah & Bird (1980) compararam os padrões reprodutivos entre espécies da epifauna e infauna de espécies marinhas de águas rasas do noroeste do Atlântico, tendo observado que as primeiras geralmente produzem mais ovos de menor tamanho que as últimas, mesmo que apresentassem o mesmo tamanho corporal e habitassem a mesma região. Fortes efeitos latitudinais e sazonais também foram evidentes já que para várias espécies o tamanho dos ovos aumenta e o número de ovos diminui nas populações de latitudes maiores e naquelas que se reproduzem em estações mais frias.

Nelson (1980) revisou os padrões reprodutivos de 65 espécies pertencentes a 89 populações de Amphipoda Gammaridea, e encontrou que de forma geral o tamanho das ninhadas nos meses de verão é menor que no inverno. Ele afirma ainda que os padrões são peculiares de cada família, devido à especificidade por habitat apresentada pelos organismos estudados.

Sainte-Maire (1991) revisou traços de história de vida de 302 populações representando 214 espécies de Amphipoda, e afirma que os gamarídeos apresentam uma grande diversidade de padrões de história de vida. Podem apresentar um único evento reprodutivo (semelparidade) ou diverso (iteroparidade), podendo ainda apresentar ciclos de vida semianuais, anuais, bianuais ou perenes. Os padrões são influenciados pela latitude, profundidade e salinidade. Espécies de baixas latitudes são caracterizadas por ciclos de vida semianuais ou anuais, pequeno tamanho corporal e alto potencial reprodutivo. Sainte – Marie (1991) afirma ainda que as espécies tendem a ser K-estrategistas em habitats altamente produtivos (favoráveis) e que raramente sofrem distúrbios.

Cunha et al. (2000) obteve conclusões semelhantes à Sainte-Maire (1991) estudando anfípodos em Portugal, e descreveu os anfípodos de baixas latitudes e águas quentes como semianuais, iteróparos, podendo atingir a maturidade sexual em até um mês e apresentam curtos ciclos de vida.

Johnson et al. (2001) revisou aspectos reprodutivos e relacionados ao desenvolvimento de peracáridos e observou que os gamarídeos tipicamente possuem uma geração que perpassa pelo inverno (*overwintering)* e uma ou mais gerações de primavera-verão. Johnson e colaboradores compilaram o comprimento de fêmeas, diâmetro de ovos e tamanho da prole de mais de 290 espécies de Amphipoda Gammaridea, e observaram que entre os Gammaroidea, *Gammarus* é o gênero mais estudado. No entanto, de forma geral os parâmetros reprodutivos e ciclos de vida de Amphipoda Gammaridea são mais conhecidos apenas para regiões litorâneas temperadas (JOHNSON et al., 2001).

Appadoo & Myers (2004) estudaram um sistema tropical nas Ilhas Maurícias quanto às estratégias reprodutivas apresentadas por crustáceos pertencentes a duas famílias (*Cymadusa filosa*, *Ampithoe laxipodus*, da família Corophiidae, e *Mallacoota schellenbergi*, da família Melitidae) tendo demonstrado multivoltinismo e reprodução contínua ao longo do ciclo de vida das três espécies. Cunha et al. (2000) fizeram observações similares e afirmam que os anfípodos de baixas latitudes de águas mais quentes são semianuais, iteróparos, multivoltinos, apresentam maturidade em tamanhos menores, assim como curtos ciclos de vida, o que também já havia sido sugerido por Sainte-Marie (1991). Steele & Steele (1991) atribuem a ocorrência de reprodução contínua à constante disponibilidade de alimento encontrada na região tropical, o que permite que juvenis sejam produzidos frequentemente.

Multivoltinismo e reprodução contínua também foram observadas para outras espécies encontradas em áreas tropicais, como *Pseudorchestia brasiliensis* no estado do Rio de Janeiro, Brasil (CARDOSO; VELOSO, 1996), *P. platensis* na Flórida (GARCES; MARSH, 1991).

Na verdade tratar das diferentes estratégias apresentadas por indivíduos filogeneticamente próximos que habitam diferentes regiões implica em considerar que sua história de vida pode ser influenciada pelas condições tanto abióticas quanto bióticas às quais está submetido. A densidade populacional, biomassa, as atividades reprodutivas, o desenvolvimento dos embriões, tamanho corporal, entre outros aspectos da biologia e ecologia dos organismos podem ser afetados por fatores abióticos como temperatura, salinidade, regime hídrico local, fotoperíodo, oxigênio dissolvido e condutividade da água (KRUSCHWITZ, 1978; PANOV & McQUEEN, 1998; WILHELM & SCHLINDER, 2000; MARANHÃO et al., 2001; SUBIDA et al., 2005; KESTRUP & RICCIARDI, 2010; SUYAN et al., 2013). Fatores bióticos também podem influenciar, tais como a biomassa de algas, presença de predação e competição intraespecífica (MARANHÃO et al., 2001; WELLBORN, 2002; COTHRAN, 2004; SUBIDA et al., 2005).

A disponibilidade de alimento também constitui um importante fator que influencia a evolução das estratégias reprodutivas e da história de vida, no geral, já que está diretamente relacionada com o desenvolvimento, crescimento e sobrevivência dos estágios larvais e/ou juvenis, assim como com a manutenção, crescimento e reprodução dos indivíduos adultos (SASTRY, 1983).

2.3 Ecologia de populações

Um grupo de organismos pertencentes à mesma espécie constitui uma população cuja dinâmica é regida pela interação de atributos do grupo e não dos organismos isoladamente, como densidade, natalidade, distribuição etária, potencial biótico, dispersão, recrutamento e forma de crescimento (ODUM, 1988). O tamanho das populações pode ser alterado por quatro processos: natalidade, mortalidade, imigrações e emigrações. A compreensão dos motivos que levam a modificações no tamanho e estrutura populacional é importante para os estudos em ecologia, já que esta ciência busca não somente compreender tais processos, mas também prevê-los e controlá-los (TOWNSEND; HARPER; BEGON 2010).

As taxas de natalidade, mortalidade, migração, densidade e abundância populacionais, assim como a distribuição de frequência em classes de tamanho constituem informações que levam ao conhecimento da dinâmica das populações (HUTCHINSON, 1981). Além destes processos inerentes de cada população, os indivíduos também interagem tanto com o ambiente, quanto entre si e com outros organismos, seja na competição por espaço, parceiro reprodutivo alimento, ou outros recursos (FONTELES-FILHO, 1989).

No caso de espécies cujas adaptações para dispersão não são muito desenvolvidas é pouco provável que sejam observados movimentos migratórios. Os eventos de colonização geralmente são raros em tais espécies, e provavelmente é por isso que a distribuição geográfica de *Hyalella* é de difícil compreensão, já que os anfípodos deste gênero não apresentam boa capacidade natatória, nem outras adaptações que permitem longos deslocamentos (WELLBORN; CAPPS, 2013). Na América do Norte, por exemplo, *Hyalella* forma um complexo de muitas espécies, sendo a maioria delas fenotipicamente crípticas e não descritas formalmente (WITT; HEBERT 2000; WITT et al., 2006; WELLBORN; BROUGHTON, 2008). As espécies deste complexo são comumente encontradas ao longo de todo o continente norte-americano em lagos permanente e riachos (BOUSFIELD 1996; WELLBORN; BARTHOLF, 2005; WITT et al., 2006). Por outro lado, muitas espécies de *Hyalella* ocorrem em habitats únicos e/ou isolados com substanciais divergências genéticas entre espécies (WELLBORN et al., 2005; WITT et al., 2006).

A única forma de dispersão de *Hyalella* por meio terrestre registrada até o presente trata de indivíduos encontrados agarrados em vertebrados, os quais agiram como vetores de dispersão (ROSINE 1956, 1962; DABORN, 1976; PECK, 1975; SWANSON, 1984). Wellborn e Capps (2013) sugerem que uma possível explicação para este paradoxo entre capacidade de dispersão e distribuição geográfica apresentada por *Hyalella* é que embora os eventos de dispersão no grupo sejam raros e envolvam pequenos propágulos, as espécies deste gênero tem uma grande probabilidade de estabelecimento bem sucedido de uma nova população, e esta, uma vez estabelecida, tem uma grande probabilidade de persistir ao longo do tempo sem que haja reposição por colonizadores subsequentes. Os autores inclusive relatam que uma única fêmea ovígera é capaz de estabelecer uma nova população cujos descentes persistem por várias gerações, ao menos em condições laboratoriais já que este processo ainda não foi elucidado em campo.

Nos estudos relacionados à estrutura e dinâmica populacional de Amphipoda frequentemente constam informações sobre o tamanho dos indivíduos, os quais são distribuídos em classes de tamanho. Além disso, a densidade populacional, taxas de natalidade e mortalidade, produtividade secundária em termos de abundância e biomassa, recrutamento, sobrevivência, entre outros aspectos, tem sido mensurados nos estudos deste cunho (*e. g.* EDWARD; COWELL, 1992; PARDAL et al., 2000; PICKARD; BENKE, 1996; MARANHÃO et al., 2001). Além disso, há trabalhos que analisaram populações de Amphipoda sob uma abordagem de metapopulações (MUNGUIA; MACKIE; LEVITAN, 2007), ou ainda por meio de modelagem (ANDERSON et al., 2013).

As espécies do gênero *Hyalella* foram estudadas quanto à dinâmica e estrutura populacional especialmente no hemisfério norte, onde principalmente a espécie *H. azteca* é bem conhecida em relação à ecologia de populações (COOPER, 1965; EDWARDS; COWELL, 1992; STRONG, 1972; KOKKOTIS; McLAUGHLIN, 2002).

No Brasil Castiglioni e Bond-Buckup (2008a) estudaram os ciclos de vida de *H. castroi* e *H. pleoacuta*¸ espécies simpátricas que habitam a região sul do país. As coletas foram feitas em tanques de cultivo de truta no município de São José dos Ausentes, Rio Grande do Sul. Em laboratório as duas espécies foram cultivadas permitindo identificar a duração do comportamento pré-copulatório, o tamanho de machos e fêmeas em pré-cópula, o tamanho corporal 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. Embora muitas semelhanças tenham sido observadas entre as duas espécies com relação ao recrutamento, proporção de adultos em relação a juvenis na população, assim também como a razão sexual tendendo para mais fêmeas. As autoras inferiram que a coexistência de *H. pleoacuta* e *H. castroi* é facilitada pelas diferenças observadas no ciclo de vida, especialmente no tamanho corporal, nas estratégias reprodutivas, e na ocupação de distintos micro-habitats.

Rodrigues (2011) estudou a distribuição e alguns aspectos populacionais como a estrutura demográfica e razão sexual de espécies de *Hyalella* coletadas no estado do Rio Grande do Sul, Brasil. Além das coletas em 146 áreas úmidas, características físicas do ambiente foram consideradas, como altitude e tamanho da área. Foram encontradas três espécies de *Hyalella* que já ocorriam no Rio Grande do Sul e uma conhecida apenas para a Argentina. Além destas, foram identificadas mais oito morfoespécies, sendo que uma possui características troglomórficas, e representa o primeiro crustáceo hipotelminorreico registrado para a América do Sul (RODRIGUES; BUENO; FERREIRA, 2012).

Em relação à *H. longistila*, até o presente foram publicados apenas trabalhos relativos à descrição, redescrição e nova ocorrência (GONZÁLEZ; WATLING, 2003a) sendo sua biologia e ecologia desconhecidas, e o mesmo é observado para a espécie *H. carstica* (BASTOS-PEREIRA; BUENO, 2012).

2.4 Biologia reprodutiva

Embora uma variedade de estratégias reprodutivas seja observada, há uma série de eventos comuns que constituem um ciclo reprodutivo básico entre os crustáceos que se reproduzem sexualmente. Neste ciclo ocorre inicialmente a proliferação das células gonadais seguida pela produção dos gametas, comportamentos reprodutivos associados ao acasalamento, liberação dos gametas por transferência de esperma durante a cópula pelo macho, e ovulação por parte das fêmeas. Posteriormente os embriões são incubados até a eclosão e liberação das larvas ou juvenis. As diferenças observadas residem no tempo entre certos eventos e sua duração em diferentes espécies (SASTRY, 1983).

Quando os indivíduos imaturos ou juvenis adquirem condições fisiológicas que os permitem produzir gametas os quais podem fertilizar ou serem fertilizados, estes indivíduos atingiram a maturidade sexual (MANTELATTO, 2003). No caso de crustáceos da ordem Amphipoda, embora para as fêmeas a presença de ovário desenvolvido visível por apresentar coloração verde-escuro, ou ainda, a presença de ovos ou juvenis no marsúpio seja indicativa da maturidade sexual, para os machos observa-se basicamente o alargamento completo do própodo do segundo gnatópodo (KRUSCHWITZ, 1978). Este indicativo para os machos pode conter certa subjetividade, já que frequentemente observam-se variações sutis na morfologia dos indivíduos da mesma espécie, o que poderia levar a uma conclusão errônea a respeito de maturidade sexual atingida. Assim sendo, o comportamento pré-copulatório é o indicativo mais preciso de que os machos estão aptos para reproduzir (BOROWSKY, 1991).

As fêmeas de Amphipoda produzem uma determinada quantidade de ovos a cada muda, no entanto, não apresentam estocagem de esperma. Assim, foi necessária uma estratégia que permitisse que macho e fêmea estivessem juntos quando a fêmea ovulasse, garantindo que a fecundação ocorreria (COOPER, 1965; STRONG, 1972). Durante a pré-cópula o macho agarra a fêmea dorsalmente com os segundos gnatópodos alguns dias antes da fêmea realizar a muda. Devido ao elevado custo energético deste comportamento

(CALOW, 1979) os machos apenas se juntam às fêmeas quando a ovulação está prestes a ocorrer (IRIBARNE et al., 1995), quando acredita-se que há comunicação por meio de ferormônios (BOROWSKY, 1991).

Embora o controle da fêmea no acasalamento ainda não esteja claro, Jormalainen (1994) afirma que a maioria dos autores acredita que a formação dos pares ocorre mediante controle de ambos os sexos, embora não necessariamente com os dois tendo influências iguais. Por outro lado outros autores defendem a ideia que este comportamento seja controlado estritamente pelo macho, embora a fêmea possa resistir as tentativas do macho (WARD; PORTER, 1993)

Há diversas hipóteses com o intuito de explicar a correlação comumente observada entre o tamanho de machos e fêmeas que acasalam, na qual machos grandes pareiam com fêmeas grandes e vice-versa (PLAISTOW; BOLLACHE; CÉZILLY, 2003; WELLBORN; BARTHOLF, 2005; CASTIGLIONI; BOND-BUCKUP, 2008b). Duas destas hipóteses foram mais amplamente aceitas: competição entre machos (*male-male competition*) (WARD, 1983) e restrições para o carregamento (*loading constraints*) (ADAMS; GREENWOOD, 1983). Ward (1983) afirma que machos maiores têm vantagem ao competir com machos de menores tamanhos, tanto por conseguirem tomar a fêmea que o outro macho já estava carregando quanto por evitar que outro macho tome a fêmea que já estiver em pré-cópula com ele. Já Adams e Greenwood (1983) consideram que os machos se limitam a selecionar fêmeas de tamanho muito inferior ao seu, pois somente assim serão capazes de transportá-las até que ocorra a cópula.

Na maioria dos gamarídeos maduros, a cópula ocorre poucos minutos após a muda da fêmea, chamada muda pré-puberal (HYNES 1955). Após esta muda a fêmea libera os ovos no marsúpio, onde ocorre a fertilização. O marsúpio é uma sinapomorfia de Peracarida e consiste de uma bolsa incubadora ventral formada por grandes processos em forma de placas em determinadas coxas torácicas, os oostegitos; estes se sobrepõem formando o piso do marsúpio, e o teto são formados pelos esternitos torácicos (RUPPERT & BARNES, 1996).

O desenvolvimento dos ovos acontece nesta bolsa incubadora e se divide em duas fases: embrionária, que compreende desde a ovulação até a eclosão dos ovos, e o período pós-embrionário, em que ocorre a emergência dos juvenis do marsúpio (BOROWSKY, 1980). Em crustáceos da ordem Amphipoda, assim como nos demais Peracarida, os juvenis permanecem no marsúpio até que se assemelhem aos adultos, ou seja, os anfípodos apresentam desenvolvimento direto (STRONG, 1972; BOROWSKY, 1991; STEELE & STEELE, 1991).

A produção de ovos pode ocorrer em um único evento reprodutivo ou em múltiplas desovas, sendo uma a cada estação no caso de Amphipoda Gammaridea. Em espécies semélparas os indivíduos tem somente um período de investimento reprodutivo ao longo da vida, até o qual já cessaram seu crescimento, durante o qual se investe pouco ou nada em sobrevivência para eventos reprodutivos futuros, e depois do qual eles morrem. Já em espécies iteróparas, um indivíduo normalmente passa por vários eventos reprodutivos, podendo apresentar um extenso período de atividade reprodutiva. Durante cada estação reprodutiva o indivíduo continua a investir na sobrevivência futura e possivelmente no crescimento e, portanto, tem uma chance razoável de sobreviver para reproduzir novamente (SASTRY, 1983; HARTNOLL, 1985; BEGON; TOWNSEND; HARPER, 2006).

Entende-se por período reprodutivo o período em que fêmeas ovígeras são encontradas na população, podendo estes eventos ser contínuos os sazonais. Os ciclos reprodutivos podem, então, ser compreendidos como uma resposta integrada dos indivíduos de uma população ao ambiente, tanto em um sentido funcional quanto temporal (SASTRY, 1983), podendo haver influência de fatores bióticos e abióticos, como disponibilidade de alimento, interações intra e interespecíficas como predação e competição, temperatura, condutividade, pH, oxigênio dissolvido, fotoperíodo, entre outros fatores (*e. g.* KRUSCHWITZ, 1978; PILGRIM; BURT, 1992; PICKARD; BENCKE, 1996; MARANHÃO et al., 2001; KESTRUP; RICCIARDI, 2010).

A intensidade e a taxa com que as condições ambientais se modificam variam principalmente com a latitude, no entanto, populações de espécies que ocorrem em diferentes latitudes podem apresentar padrões temporais semelhantes ou ainda espécies que se distribuem ao longo de uma pequena amplitude de variação de latitude podem apresentar diferenças nos padrões de seus ciclos reprodutivos. Sastry (1983) atribui estes cenários distintos como sendo respostas a características particulares do ambiente em que as espécies vivem. Principalmente para espécies de ciclo de vida curto, escalas de tempo reduzidas e mudanças de pequena amplitude podem ser importantes na determinação dos padrões dos ciclos reprodutivos.

Em relação aos ciclos reprodutivos é importante salientar ainda que a fecundidade pode variar de acordo com a latitude. A fecundidade é um importante parâmetro reprodutivo, amplamente avaliado nos trabalhos de ecologia, o qual corresponde ao número de ovos produzidos por uma fêmea durante uma desova ou período reprodutivo (SASTRY, 1983; HARTNOLL, 1985). Mensurar a fecundidade consiste tanto em uma ferramenta para medir o potencial reprodutivo dos indivíduos de uma dada população, quanto em um pré-requisito para a compreensão da evolução das estratégias reprodutivas.

Organismos de maior tamanho corporal normalmente apresentam maior fecundidade, além disso, a idade, fatores ambientais e presença de predadores podem ter influência sobre este parâmetro reprodutivo (MORINO 1978, STEELE, 1981). Assim, a energia investida para produção de gametas pode ser distribuída tanto entre muitos ovos de tamanho reduzido, quanto em menos ovos maiores, constituindo padrões populacionais ou próprios da espécie, no geral (SASTRY, 1983).

A produção de ovos maiores está diretamente relacionada a um tempo maior de desenvolvimento, e isto normalmente ocorre quando a fêmea maximiza o gasto de energia por ninhada a fim de aumentar o *fitness* reprodutivo quando o risco de mortalidade é baixo. Quando este risco é alto, as fêmeas geralmente investem na produção de mais ovos menores, os quais requerem menos tempo de incubação, já que no caso de invertebrados que incubam os ovos a sobrevivência do adulto contribui diretamente para o sucesso reprodutivo, pois o desenvolvimento dos ovos depende do adulto. (STEELE; STEELE, 1975).

Van Dolah e Bird (1980) consideram os anfípodos como organismos ideais para comparar padrões reprodutivos por diversas razões: (i) o desenvolvimento dos ovos é completado dentro da bolsa incubadora, ou marsúpio; (ii) a ninhada eclode como juvenis, sem fases larvais a sofrerem metamorfoses, ou seja, o desenvolvimento é direto; (iii) o tempo de desenvolvimento, história de vida e ecologia de muitas espécies já foi descrito na literatura; (iv) o tamanho e número de ovos são facilmente mensurados. No entanto,, é necessário reconsiderar o terceiro item anteriormente mencionado, já que este amplo conhecimento relatado por Van Dolah e Bird (1980) não se refere à espécies tropicais e subtropicais de Amphipoda.

Embora Castiglioni e Bond-Buckup (2008b, 2009) tenham estudado espécies simpátricas de *Hyalella* encontradas no sul da América do Sul e informações relevantes para o conhecimento da biologia reprodutiva do gênero tenham sido obtidas, pesquisas nesta área ainda são incipientes, especialmente se tratando das espécies brasileiras de Amphipoda de água doce.

3 OBJETIVOS

O objetivo geral com este trabalho foi caracterizar aspectos da história de vida de *H. longistila* e *H. carstica,* mais especificamente abordando a ecologia de populações e a biologia reprodutiva.

3.1 Objetivos específicos

- Avaliar a estrutura demográfica das populações mensalmente;
- Mensurar o comprimento mínimo, máximo e médio do cefalotórax de machos e fêmeas;
- Propor uma nova métrica para distinção de juvenis e adultos;
- Estimar a razão sexual operacional mensal, por classe de tamanho e total;
- Caracterizar o período reprodutivo e recrutamento;
- Caracterizar o tamanho em que a maturidade sexual morfológica é atingida em machos e fêmeas;
- Avaliar a fecundidade mensal e média;
- Calcular o volume dos ovos por estágio de desenvolvimento;
- Verificar a relação do número de ovos produzidos com o tamanho da respectiva fêmea ovígera;
- Estimar o sucesso de pareamento e sucesso reprodutivo de machos e fêmeas.

4 HIPÓTESES

a) *Hyalella longistila* se reproduz ao longo de todo o ano em virtude da estabilidade garantida pelo habitat em relação ao fluxo de água contínuo;

b) Para *Hyalella carstica* a reprodução é mais intensa após o período de seca, dada a necessidade de recolonização da população local;

c) A razão sexual em ambas as espécies é direcionada para um maior número de fêmeas devido aos padrões reprodutivos do gênero em que o macho após o período pré-copulatório e posterior fecundação já está disponível para parear com outra fêmea, enquanto ela ainda despenderá energia para a incubação dos ovos e desenvolvimento dos embriões;

d) A dinâmica populacional de *H. longistila* e *H. carstica* (considerando abundância, recrutamento e frequência de fêmeas ovígeras) é influenciada pelos fatores abióticos a que estão submetidos.

e) Há correlação positiva entre o tamanho de machos e fêmeas de ambas as espécies encontradas em comportamento pré-copulatório.

REFERÊNCIAS

ADAMS, J.; GREENWOOD, P. J. Why are males bigger than females in pre-copula pairs of *Gammarus pulex*? **Behavioral Ecology and Sociobiology**, New York, v. 13, p. 239-241, 1983.

ANDERSON, K. E.; HARRISON, L. R.; NISBET, R. M.; KOLPAS, A. Modeling the influence of flow on invertebrate drift across spatial scales using a 2D hydraulic model and a 1D population model**. Ecological Modelling,** Amsterdam, v. 265, p. 207–220, 2013.

APPADOO, C.; MYERS, A. A. Reproductive bionomics and life history traits of three gammaridean amphipods, *Cymadusa filosa* Savigny, *Ampithoe laxipodus* Appadoo and Myers and *Mallacoota schellenbergi* Ledoyer from tropical Indian Ocean (Mauritius). **Acta Oecologica,** Paris, v. 26, p. 227-238, 2004.

BALDINGER, A. J. A new species of *Hyalella* (Crustacea: Amphipoda: Hyalellidae) from Ash Springs, Lincoln County, USA, with a key to the species of the genus in North America and the Caribbean region. **Journal of Natural History**, London, v. 38, p.1087-1096, 2004.

BASTOS-PEREIRA, R.; BUENO, A. A. P. New species and new report of *Hyalella* S. I. Smith, 1874 (Crustacea: Amphipoda: Dogielinotidae) from Minas Gerais state, Southeastern Brazil. **Zootaxa**, v. 3350, p. 58–68, 2012.

BASTOS-PEREIRA, R.; BUENO, A. A. P. A new species of freshwater amphipod (Dogielinotidae, Hyalella) from Southeastern Brazil. **Nauplius,** Ribeirão Preto, v. 21**,** p. 79–87, 2013.

BOROWSKY, B. Reproductive patterns of three intertidal salt-marsh gammaridean amphipods. **Marine Biology**, Berlin, v. 55, p. 327-334, 1980.

BOROWKSY, B. Patterns of reproduction of some amphipod crustaceans and insights into the nature of their stimuli. In: BAUER, R. T.; MARTIN, J. W. (Ed.). **Crustacean sexual**. New York: Columbia University, 1991. p. 33-66.

BOUSFIELD, E. L. A contribution to the reclassification of neotropical freshwater hyalellid amphipods (Crustacea: Gammaridea: Talitroidea). **Bolletino del Museo Civico di Storia Naturale di Verona**, Verona, v. 20, p. 175-224, 1996.

BRUSCA, R. C.; BRUSCA, G. J. **Invertebrates**. Rio de Janeiro: Guanabara Koogan, 2003. 966 p.

BUENO, A. A. P.; ARAUJO, P. B.; CARDOSO, G. M.; GOMES, K. M.; BOND-BUCKUP, G. Two new species of *Hyalella* (Amphipoda, Dogielinotidae) from Brazil. **Crustaceana**, Leiden, v. 86, p. 802 – 819, 2013.

BUENO; RODRIGUES; ARAUJO (*in press*). O estado da arte do gênero *Hyalella* Smith, 1874 (Crustacea, Amphipoda, Senticaudata, Hyalellidae) no Brasil. In: HAYASHI, C. (Eds). **Ecologia aquática**.

CALOW, P. The cost of reproduction – a physiological approach. **Biological Review**, Cambridge, v. 54, p. 23-40, 1979.

CARDOSO, G. M.; BUENO, A. A. P.; FERREIRA, R. L. A new troglobiotic species of *Hyalella* (Crustacea, Amphipoda, Dogielinotidae) from Southeastern Brazil. **Nauplius**, Ribeirão Preto, v. 19, n. 1, p. 17-26, 2011.

CARDOSO, R.S.; VELOSO, V.G. Embryonic development and reproductive strategy of *Pseudorchestoidea brasiliensis* (Amphipoda: Talitridae) at Prainha Beach, Brazil. **Journal of Natural History,** London, v. 35, p. 201−211, 2001.

CASTIGLIONI, D. S.; BOND-BUCKUP, G. Reproductive strategies of two sympatric species of *Hyalella* Smith, 1874 (Amphipoda, Dogielinotidae) in laboratory conditions. **Journal of Natural History**, London, v. 41, n. 25/28, p. 1571-1584, 2007.

CASTIGLIONI, D. S.; BOND-BUCKUP, G. Ecological traits of two sympatric species of *Hyalella* Smith, 1874 (Crustacea, Amphipoda, Dogielinotidae) from southern Brazil. **Acta Oecologica**, Paris, v. 33, n. 1, p. 36-48, 2008a.

______. Pairing and reproductive success in two sympatric species of *Hyalella* (Crustacea, Amphipoda, Dogielinotidae) from southern Brazil. **Acta Oecologica**, Paris, v. 33, n. 1, p. 49-55, 2008b.

CASTIGLIONI, D. S.; GARCIA-SCHROEDER, D.; BARCELOS, D. F.; BOND-BUCKUP, G. Intermolt duration and postembryonic growth of two sympatric species of *Hyalella* (Amphipoda, Dogielinotidae) in laboratory conditions. **Nauplius**, Ribeirão Preto, v. 15, n. 2, p. 57-64, 2007.

COOPER, W. E. Dynamics and production of a natural population of a freshwater amphipod, *Hyalella azteca*. **Ecological Monographs**, Lawrence, v. 35, p. 377–394, 1965.

COTHRAN, R. D. Precopulatory mate guarding affects predation risk in two freshwater amphipod species. **Animal Behaviour,** London, v. 68, p. 1133–1138, 2004.

CUNHA, M. R.; SORBE, J. C.; MOREIRA, M. H. The amphipod *Corophium multisetosum* (Corophiidae) in Ria de Aveiro (NW Portugal). I. Life history and aspects of reproductive biology. **Marine Biology,** Berlin, v. 137, p. 637–650, 2000.

DABORN, G. R. Colonization of isolated aquatic habitats. **Canadian Field Naturalist,** Ottawa, v. 90, p. 56–57, 1976.

EDWARDS, T. D.; COWELL, B. C. Population Dynamics and Secondary Production of *Hyalella 35zteca* (Amphipoda) in *Typha* Stands of a Subtropical Florida Lake. **Journal of the North American Benthological Society,** Lawrence, v. 11, p. 69–79, 1992.

FONTELES-FILHO, A.A. **Recursos pesqueiros: biologia e dinâmica populacional.** Fortaleza, 1989. 296p.

GARCES, H.A.; MARSH, G.A. Studies on the distribution and ecology of *Platorchestia platensis* (Kroyer) at Lake Wyman, Florida. **Quaterly Journal of the Florida Academy of Sciences**, Gainesville, v. 54, p. 1-10, 1991.

GISMONDI, E.; COSSU-LEGUILLE, C.; BEISEL, J. N. Do male and female gammarids defend themselves differently during chemical stress?**Aquatic toxicology,** Amsterdam, v. 140-141, p. 432-438, 2013.

GONZÁLEZ, E. R.; WATLING, L. A new species of Hyalella from the Andes in Perú (Crustacea: Amphipoda: Hyalellidae). **Revista de Biología Tropical**, v. 50, n.2, p. 649-658, 2002a.

GONZÁLEZ, E. R.; WATLING, L. Redescription of Hyalella 35zteca from its type locality, Vera Cruz, Mexico (Amphipoda: Hyalellidae). **Journal of Crustacean Biology**, Woods Hole, v. 22, n. 1, p. 173-183, 2002b.

GONZÁLEZ, E. R.; WATLING, L. Redescription of the freshwater amphipod Hyalella faxoni from Costa Rica (Crustacea: Amphipoda: Hyalellidae). **Revista de Biología Tropical**, San José, v. 50, n. 27, p. 659-667, 2002c.

GONZÁLEZ, E. R.; WATLING, L. A new species of *Hyalella* from Brazil (Crustacea: Amphipoda: Hyalellidae), with redescription of three other species in the genus. **Journal of Natural History**, London, v. 37, n. 17, p. 2045-2076, 2003a.

GONZÁLEZ, E. R.; WATLING, L. A new species of *Hyalella* from Colombia, and the redescription of *H. meinerti* Stebbing, 1899 from Venezuela (Crustacea: Amphipoda). **Journal of Natural History,** London, v.37, n.17, p. 2095-2111, 2003b.

GONZÁLEZ, E. R.; BOND-BUCKUP, G.; ARAUJO, P. B. Two new species of *Hyalella* from southern Brazil (Amphipoda: Hyalellidae) with a taxonomic key. **Journal of Crustacean Biology**, Woods Hole, v.26, n.3, p. 355-365, 2006.

GROSSO, L.; PERALTA, M. Anfípodos de agua dulce sudamericanos. Revisión del género *Hyalella* Smith. **Acta Zoologica Lilloana**, Tucuman, v. 45, p. 79-98, 1999.

HARGRAVE, B. T. Distribution, growth, and seasonal abundance of *Hyalella azteca* (Amphipoda) in relation to sediment microflora. **Journal of the Fisheries Research Board Canada**, Ottawa, v. 27, p. 685-699, 1970.

HARTNOLL, R.G. Growth. In: BLISS, D.E. (Ed.). **The biology of Crustacea: embriology, morphology and genetics.** London Academic Press, London, 1982, p. 111-196.

HUTCHINSON, G.E**. Introducción a la ecologia de poblaciones**. Editora Blume, Barcelona. 1981. 492p.

HYNES, H. B. N. The reproductive cycle of some British freshwater Gammaridae. **Journal of Animal Ecology,** Oxford, v. 24, n. 2, p. 352 – 387, 1955.

IRIBARNE, O.; FERNANDEZ, M.; AMSTRONG, D. Male choice in the amphipod *Eogammarus ociani* Bousfield: the role of current velocity, random assortment, habitat heterogeneity and male's behavior. **Marine and Freshwater Behavioral Physiology**, Victoria, v. 27, p. 223-237, 1996.

JOHNSON, W.S.; STEVENS, M.; WATLING, L. Reproduction and development of marine peracarideans. **Advances in Marine Biology,** San Diego, v.39, p. 105 – 260, 2001.

JORMALAINEN, V.; TUOMI, J.; MERILAITA, S. Effect of female resistance on size-dependent precopula duration in mate-guarding Crustacea. **Animal Behaviour,** London, v. 47, p. 1471–1474, 1994.

KESTRUP, Å.; RICCIARDI, A. Influence of conductivity on life history traits of exotic and native amphipods in the St . Lawrence River. **Fundamental Applied Limnology,** Stuttgart, v. 176, p. 249–262, 2010.

KHAN, F. R.; IRVING , J. R.; BURY, N. R.; HOGSTRAND, C. Differential tolerance of two *Gammarus pulex* populations transplanted from different metallogenic regions to a polymetal gradiente. **Aquatic toxicology,** Amsterdam, v. 102, p. 95 – 103, 2011.

KOKKOTIS, A. T.; McLAUGHLIN, J. D. Instar-specific head and body lengths of *Hyalella* (Amphipoda): criteria for starting and endpoints in experimental studies. **Hydrobiologia**, Haia, v. 474, p. 223–227, 2002.

KRUSCHWITZ, L. G. Environmental factors controlling reproduction of the amphipod *Hyalella azteca*. **Proceedings of the Oklahoma Academy of Science**, Oklahoma, v. 21, p. 16–21, 1978.

LOWRY, J. K.; MYERS, A. A. A phylogeny and classification of the Senticaudata subord. Nov. (Crustacea: Amphipoda). **Zootaxa,** v. 3610, p. 1–80, 2013.

MacARTHUR, R. H.; WILSON, E. O. **The Theory of Island Biogeography**. Princeton, 1967. 224 pp.

MANTELATTO, F. L. M. **Biologia e ecologia dos crustáceos decápodes: uma análise na evolução da investigação.** 2003. Nº de pág. Tese de Livre-Docência, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo.

MARANHÃO, P., BENGALA, N., PARDAL, M. & MARQUES, J. C. The influence of environmental factors on the population dynamics, reproductive biology and productivity of *Echinogammarus marinus* Leach (Amphipoda, Gammaridae) in the Mondego estuary (Portugal). **Acta Oecologica,** Paris, v. 22, p. 139–152, 2001.

MARTIN, J. W.; DAVIS, G. E. **An updated classification of the recent Crustacea**. Los Angeles, 2001. 132 p.

MORINO, H. Studies on the Talitridae (Amphipoda, Crustacea) in Japan. III. Life history and breeding activity of *Orchestia platensis*. **Seto Marine Biological Laboratory,** v. 24, p. 245-267, 1978.

MUNGUIA, P.; MACKIE, C.; LEVITAN, D. R. The influence of stage-dependent dispersal on the population dynamics of three amphipod species. **Oecologia,** Berlin, v. 153, p. 533–541, 2007.

NELSON, M. K.; BRUNSON, E. L. Postembryonic growth and development of *Hyalella azteca* in laboratory cultures and contaminated sediments. **Chemosphere**, Oxford, v. 31, p. 3129 – 3140, 1995.

NELSON, W.G. Reproductive patterns of gammaridean amphipods. **Sarsia**, Oslo, v. 65, p. 61- 71, 1980.

ODUM, E.P. **Ecologia**. Editora Guanabara S. A., Rio de Janeiro, 1988. 434p.
PANOV, V. E.; McQUEEN, D. J. Effects of temperature on individual growth rate and body size of a freshwater amphipod. **Canadian Journal of Zoology**, Ottawa, v. 76, p. 1107–1116, 1998.

PARDAL, M. A., MARQUES, J. C., METELO, I., LILLEBO, A. I. & FLINDT, M. R. Impact of eutrophication on the life cycle, population dynamics and production of *Ampithoe valida* (Amphipoda) along an estuarine spatial gradient (Mondego estuary, Portugal). **Marine Ecology Progress Series,** Washington, v. 196, p. 207–219, 2000.

PECK, S. B. Amphipod dispersal in the fur of aquatic mammals. **Canadian Field Naturalist,** Ottawa, v. 89, p. 181–182, 1975.

PEREIRA, V. F. G. C. *Hyalella dielaii* sp. nov. from São Paulo, Brazil (Amphipoda, Hyalellidae). **Revista Brasileira de Zoologia**, Curitiba, v. 21, n. 2, p. 179-184, 2004.

PIANKA, E. R. On r- and K-selection. **American Naturalist**, Chicago, v. 104, p. 592-595, 1970.

PICKARD, D. P.; BENKE, A. C.; PICKARD, D. P.; BENKE, A. C. Production dynamics of *Hyalella azteca* (Amphipoda) among different habitats in a small wetland in the Southeastern USA. **Journal of the North American Benthological Society,** Lawrence, v. 15, p. 537–550, 1996.

PILGRIM, W.; BURT, M. D. B. Effect of acute pH depression on the survival of the freshwater amphipod *Hyalella azteca* at variable temperatures: field and laboratory studies. **Hydrobiologia**, Haia, v. 254, p. 91-98, 1993.

PLAISTOW, S. J.; BOLLACHE, L.; CÉZILLY, F. Energetically costly precopulatory mate guarding in the amphipod *Gammarus pulex*: causes and consequences. **Animal Behaviour,** London, v. 65, p. 683–691, 2003.

RINDERHAGEN, M.; RITTERHOFF, J.; ZAUKE, G. P. Crustaceans as bioindicators. Biomonitoring of Polluted Water – Reviews on Actual Topics. Trans Tech Publications – Scitech Publications, **Environmental Research Forum**, v. 9, p. 161−194, 2000.

RODRIGUES, S. G. ***Hyalella* Smith, 1874 (Crustacea, Amphipoda, Dogielinotidae) em áreas úmidas do Rio Grande do Sul, Brasil.** 2011. 140pp. Dissertação de mestrado, Universidade Federal de Lavras.

RODRIGUES, S. G.; BUENO, A. A. P.; FERREIRA, R. L. The first hypothelminorheic Crustacea (Amphipoda, Dogielinotidae, *Hyalella*) from South America. **Zookeys**, v. 236, p. 65 – 80, 2012.

ROSINE, W. N. On the transport of the common amphipod, *Hyalella azteca*, in South Dakota by the mallard duck. **Proceedings of the South Dakota Academy of Science**, Sawyer, v. 35, p. 203, 1956.

ROSINE, W. N. New evidence of the transport of freshwater amphipods by waterfowl. **Proceedings of the South Dakota Academy of Science**, Sawyer, v. 41, p. 198, 1962.

RUPPERT, E. E.; BARNES, R. D. **Zoologia dos invertebrados**. 6ª edição. Editora Rocca Ltda., São Paulo, 1996. 1097p.

SAINTE-MARIE, B. A review of the reproductive bionomics of aquatic gammaridean amphipods: variation of life history traits with latitude, depth, salinity and superfamily. **Hydrobiologia,** Haia, v. 223, p. 189–227, 1991.

SASTRY, A.N. Ecological aspects of reproduction. In: VERNBERG, F.J.; VERNBERG, W.B. (Eds.). **The Biology of Crustacea: Enviromental adaptations**, Academic Press, New York, 1983, v. 8, p. 179-270, 1983.

SCHUBART C. D., DIESEL R., HEDGES S. B. Rapid evolution to terrestrial life in jamaican crabs**. Nature,** v. 393, p. 363-365, 1998.

SEREJO, C. Cladistic revision of talitroidean amphipods (Crustacea, Gammaridea), with a proposal of a new classification. **Zoologia Scripta**, Stockholm, v. 33, p. 551-586, 2004.

STEARNS, S. C. Life-history tactics: a review of the ideas. **The Quarterly Review of Biology,** Chicago, v. 51, p. 3 – 47, 1976.

STEARNS, S. C. Trade-offs in life-history evolution. **Functional Ecology,** London, v. 3, p. 259–268, 1989.

STEELE, V.J. The effect of photoperiod on the reproductive cycle of *Gammarus lawrencianus* Bousfield. **Journal of Experimental Marine Biology and Ecology**, Groton, v. 53, p. 1−7, 1981.

STEELE, D.H.; STEELE, V. J. Morphological and environmental restraints on egg production in amphipods. In: WENNER, A.; KURIS, A. (Eds.). **Crustacean Egg production – Crustacean Issues,** v.7. A. A. Balkema, Rotterdam, 1991. P. 157-170.

STRONG, D. R. Life History variation among populations of an amphipod (*Hyalella azteca*). **Ecology**, Davis, v. 53, p. 1103–1111, 1972.

SUBIDA, M. D.; CUNHA, M, R.; MOREIRA, M. H. Life history, reproduction and production of *Gammarus chevreuxi* (Amphipoda: Gammaridae) in the Ria de Aveiro, northwestern Portugal. **Journal of the North American Benthological Society,** v. 24, p. 82–100, 2005.

SUYAN, X. U. E.; JIANGUANG, F.; JIHONG, Z. Effects of temperature and salinity on the development of the amphipod crustacean *Eogammarus sinensis*. **Chinese Journal of Oceanology and Limnology**, v. 31, p. 1010 –1017, 2013.

SWANSON, G. A. Dissemination of amphipods by waterfowl. **Journal of Wildlife Management,** v. 48, p. 988–991, 1984.

TOWNSEND, C. R.; BEGON, M.; HARPER, J. L. **Fundamentos em Ecologia.** Porto Alegre, 2010, 576 pp.

VAN DOLAH, R.F.; BIRD, E. A comparison of reproductive patterns in epifaunal and infaunal gammaridean amphipods. **Estuarine and Coastal Marine Science**, London, v. 2, p. 593-604, 1980.

WARD, P.I. Advantages and a disadvantages of large size for male *Gammarus pulex* (Crustacea: Amphipoda). **Behavioral Ecology and Sociobiology**, Boston, v. 14, p. 69-76, 1983.

WARD, P. I.; PORTER, A. H. The relative roles of habitat structure and male-male competition in the mating systems of *Gammarus pulex* (Crustacea, Amphipoda): a simulation study. **Animal Behavior,** v. 45, p. 119 – 133, 1993.

WELLBORN, G. A. Determinants of reproductive success in freshwater amphipod species that experience different mortality regimes. **Animal Behavior,** London, v. 50, p. 353−363, 1995.

WELLBORN, G. A. Trade-off between competitive ability and antipredator adaptation in a freshwater amphipod species complex. **Ecology**, Davis, v. 83, p. 129 – 136, 2002.

WELLBORN, G.A.; BARTHOLF, S.E. Ecological context and the importance of body and gnathopod size for pairing success in two amphipod. **Oecologia**, Berlin, v. 143, p. 308-316, 2005.

WELLBORN, G.A.; BROUGHTON, R. E. Diversification on an ecologically constrained adaptive landscape. **Molecular Ecology**, Malden, v. 17, p. 2927–2936, 2008.

WELLBORN, G. A.; CAPPS, E. Establishment of a new population by a single gravid colonist: implications for *Hyalella* biogeography and speciation. **Evolutionary Ecology,** Dordrecht, v. 27, p. 101–115, 2013.

WEN, Y. H. Life history and production of *Hyalella azteca* (Crustacea, Amphipoda) in a hypereutrophic prairie pond in southern Alberta. **Canadian Journal of Zoology**, Ottawa, v. 70, p. 1417-1424, 1992.

WILHELM, F. M.; SCHINDLER, D. W. Reproductive strategies of *Gammarus lacustris* (Crustacea: Amphipoda) along an elevation gradient. **Functional Ecology,** Davis, v. 14, p. 413-422, 2000.

WITT J.D.S.; HEBERT P.D.N. Cryptic species diversity and evolution in the amphipod genus *Hyalella* within central glaciated North America: a molecular phylogenetic approach. **Canadian Journal of Fisheries and Aquatic Sciences**, Edmonton, v. 57, p. 687−698, 2000.

WITT, J. D. S.; THRELOFF, D. L.; HEBERT, P. D. N. DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus : implications for desert spring conservation. **Molecular Ecology,** Malden, v. 15, p. 3073–3082, 2006.

CAPÍTULO 2

ARTIGO 1

On the population dynamics of two Brazilian freshwater amphipods

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ABSTRACT

Most of the works regarding *Hyalella* have a taxonomic nature. The knowledge regarding temporal variations on the population structure such as abundance, sex ratio and size distribution of individuals provide important information that enables the comprehension of the ecological stability of species. The present work aimed to elucidate some ecological traits of *H. longistila* and *H. carstica* on subtropical streams located on Southeastern Brazil, as well as provide a new approach for determining freshwater amphipods biometrics. Amphipods were collected on two distinct streams on Minas Gerais state during one year. Collections were made with the aid of hand net, amphipods were separated from macrophytes and sediments in the field and then brought to the laboratory. Individuals were categorized into males, females, ovigerous females and juveniles, and all of them were measured and divided into size classes. Adults predominated in most of the months on the *H. longistila* population, while for *H. carstica* juveniles predominated before the dry period and after it adults were more abundant. The mean ratio CL/TL of juveniles was superior to adults in both species, thus we suggest this ratio be used as an indicative of adult phase, together with other parameters. The smallest adults of *H. longistila* had 0.39 mm of CL, while *H. carstica* had 0.37 mm. Males were larger than females for both species. Growth models were determined for both species. Sex ratio of *H. longistila* favored females on summer months and males in the rest of the year, and for *H. carstica* males predominated along the year. Reproduction was continuous for *H. longistila* and presented a peak on July/12 and October/12. *Hyalella carstica* reproduced more intensively after the dry period, when we suggest that occur the recolonization of the local by adults from upstream centers of population abundance. Regarding environmental factors, an independent and positive effect of temperature of water on the abundance and dissolved oxygen on the frequency of ovigerous females was observed for the *H. longistila* population, and the frequency of *H. carstica* ovigerous females was influenced positively by temperature and conductivity of the water. The species adopted different life strategies and the different hydric regime seemed to influence greatly the population dynamics, especially for *H. carstica*. Studies like the present one may subsidize future works as well as conservations practices for the species and the ecosystem as a whole.

Keywords: Size classes. Recruitment. Breeding period. Growth curve. *Hyalella.*

1 INTRODUCTION

Inhabiting marine, freshwater or terrestrial environments, crustaceans of the order Amphipoda develop an important role in their ecosystems because of the food source that represents for birds, fishes and macroinvertebrates, and also because of their diverse food habits (STONER, 1979; BOCHER et al., 2001). Besides they intensively take part on the food webs, many species are considered good bioindicators of the habitat quality due to their sensibility to contaminants and other environmental changes (KRUSCHWITZ 1978), what can affect birth and survival rates, so that fluctuations may be observed on their populations.

The knowledge regarding natural temporal variations on the population structure such as abundance, sex ratio and size distribution of individuals provide important information that enables the comprehension of the ecological stability of species (HUTCHINSON, 1981; RICKLEFS, 1993). Studies concerning the population dynamics of Amphipoda usually verify the body size, which is distributed in classes, the population density, birth and mortality rates, secondary productivity in terms of biomass and abundance, recruitment, survival, breeding period and sex ratio (*e. g.* EDWARDS; COWELL, 1991; PARDAL et al., 2000; PICKARD; BENKE, 1996; MARANHÃO et al., 2001).

Despite six freshwater amphipod families occurs on South America, most of them are restricted to subterranean environments, except Hyalellidae. This family comprises the genus *Hyalella*, endemic of American continent that inhabits epigean, hipogean and hypothelminorheic habitats (GROSSO; PERALTA, 1999; RODRIGUES; BUENO; FERREIRA, 2012; LOWRY; MYERS, 2013).

At present, there are 61 described species for this genus, of which 20 occur in Brazil, however most of the works regarding them have a taxonomic nature (BASTOS-PEREIRA AND BUENO, 2013; BUENO et al., 2013).

Species of the genus *Hyalella* were studied in relation to the population dynamics and structure especially on the north hemisphere, where mainly the species *H. azteca* is well known in respect to the ecology of its distinct populations widespread on North and Central America (COOPER, 1965; STRONG, 1972; EDWARDS; COWELL, 1992; KOKKOTIS; McLAUGHLIN, 2002).

Castiglioni and Bond-Buckup (2008) studied ecological traits of two sympatric species of *Hyalella* from Southern Brazil, for which the authors found many similarities regarding their ecology. In fact their co-occurrence is possible due to slight differences on their life cycle, especially body size and reproductive strategies. This study constitutes the most recent and the information available about the ecology of Brazilian species of *Hyalella.*

Until the present, the only published works about *H. longistila* consist on its description, re-descriptions and a new occurrence on Southern Brazil, and the same occurs with *H. carstica*, a recently described Brazilian species (BASTOS-PEREIRA; BUENO, 2012). The present work aimed to elucidate some ecological traits of these two species by analyzing their population dynamics during one year, as well as provide a new approach for determining freshwater amphipods biometrics.

2 MATERIAL AND METHODS

2.1 Study site and sampling

The species *H. longistila* was taken monthly from a stream that belongs to the municipality of Ijaci (Figure 1) during 12 months (from March/12 to February/13). This county is located on the South of the state of Minas Gerais. The stream belongs to the watershed of Grande River and is characterized by the presence of abundant macrophytes of the genus *Typha* and some *Salvinia.* The mean depth of the stream is 1.4 m most of the year, and its mean width is 10 m. It was observed that although sparse individuals of *H. longistila* can be found throughout the stream, the great population concentration is associated to the *Typha* stand.

For *H. carstica,* collections were made monthly for 13 times (from April/10 to April/11) in a stream located in the municipality of Arcos, state of Minas Gerais (Figure 1). In a wider scale, the stream is among the watersheds of São Francisco River and Grande River in an area that works as an important karstic aquifer due to its potential of hydric recharge (TEIXEIRA; DIAS, 2003). Along the stream a small dam can be observed, which influences on the interruption of the water flow during the dry months (personal observation). Menegasse, Gonçalves and Fantinel (2002) relate a period of hydric deficiency in this region from May to September, and on October the flow normalizes. Specimens were found associated to macrophytes of the genus *Spirodela.*

On April/10 additional adults of *H. carstica* were taken from the same stream, but downstream to the point where the other individuals were collected for population analysis. These amphipods were taken alive to the laboratory in order to provide information about the life cycle of this species, like the size with which juveniles hatch, how much they grow at each molt, when the first sexual dimorphisms appear. Some macrophytes also were taken to laboratory to provide the amphipods similar conditions to their natural habitat.

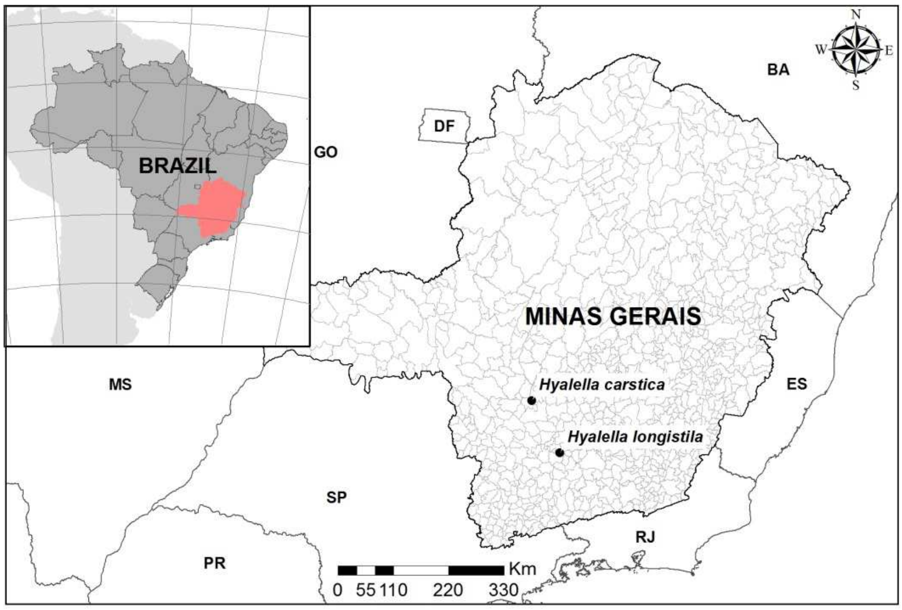

Figure 1 Map indicating the place where *Hyalella longistila* and *H. carstica* were found, both on the state of Minas Gerais, Southeastern Brazil. The SIGLAS indicate the other Brazilian states that surround Minas Gerais.

Since amphipods live associated to the macrophytes and substrate, macrophytes were collected in the case of *H. carstica*, and the bottom substrate and suspense organic material were collected where *H. longistila* inhabits, both during twenty minutes on each month. Collections were made with the aid of a hand net and put in plastic trays, when pairs in precopula and ovigerous females were recorded and individualized in microtubules. Such procedure avoided that eggs and juveniles were released from the marsupium. All amphipods collected for population analysis were separated from sediments in the field, preserved in ethanol 70% and brought to the laboratory.

2.2 Laboratory analysis

In the laboratory individuals were grouped into four demographic categories: males (those with enlarged propodus on the second pair of gnathopods), females (those with small propodus on the second gnathopod, besides the presence of developed ovary, which present dark green color), ovigerous females (those containing eggs or juveniles on the marsupium), and juveniles (those lacking developed secondary sexual characters).

Juveniles were identified considering those individuals with size inferior to the smallest ovigerous female and the smallest female and male in pre-copula (BOROWSKY, 1991). Males and ovigerous females are easily recognized, but large juveniles and small females may be confounded. Thus it is suggested that a ratio between cephalothorax and total body length must be obtained in order to properly differ juveniles from females. Thereunto, all amphipods had their cephalothorax (CL) and total body length (TL) measured with the aid of a stereomicroscope containing a milimetric lens and the mean ± standard deviation, maximum and minimum cephalothorax lengths were determined.

The additional adults of *H. carstica* brought alive to the laboratory were sexed, couples were formed and individualized in 500 mL beakers filled with water and containing some individuals of *Spirodella* sp, the macrophyte to which specimens live associated in the field. The water was changed at each two days, and when necessary the macrophytes were replaced. Individuals were observed once a day. When females got ovigerous, the male was removed from the beaker. The female was kept individualized until the juveniles hatch. Just after hatching, juveniles were measured and individualized in order to obtain information about their growth. Each day juveniles were observed, recording whether they were alive and the presence of molts. When they dead or molts were found, the CL of individuals was measured. Dead amphipods were removed from the experiment and preserved on ethanol 70%, while the justmolted juveniles return to the individual recipients, repeating this procedure at each molt until the amphipod death. Individuals were reared at 12 h/12 h light/dark regime, and fed with food for fish.

Physicochemical parameters such as temperature, dissolved oxygen, pH and conductivity were measured monthly in both streams in order to analyze a possible relationship among such parameters and the population dynamics.

2.3 Data analysis

The maximum and minimum cephalothorax lengths were determined and the means were compared between sexes through t test ($\alpha = 0.05$) for both species. The cephalothorax length of all individuals was grouped into size classes and their amplitude was determined according to one quarter of the standard deviation of the measurements (MARKUS, 1971). The frequency distribution of size classes was represented monthly on histograms, which were built including all demographic categories. Histograms were analyzed monthly in order to follow the temporal variations of the size distribution and recruitment.

The growth of individuals was estimated through the displacement of modes, which represented the frequency distribution of size classes (MACDONALD; PITCHER, 1979; MACDONALD, 1987). The model proposed by Bertalanffy (1938) was used to describe the growth of individuals:

$$C_t=C_\infty[1-e^{-k(t+to)}]$$

in which C_t represents the mean CL (mm) of individuals with age t (days); C_∞ corresponds to the theoretical maximum CL (mm); k is the parameter related to the growth rate (mm/day); e is the basis of the natural number; t is the age of individuals (days); and t_o is the parameter related to the CL when the juvenile hatches. For *H. longistila*, the data used to build this model was related to the individuals collected in the field, while for *H. carstica* data corresponded to the size of juveniles reared in laboratory.

The operational sex ratio (OSR) was calculated by dividing the total number of males by the number of non-ovigerous females per month and size class in the two studied populations. This ratio is based on the proportion of males and females that are able to reproduce in a given moment (STEARNS, 1992). The proportions obtained were evaluated through a chi-square test for goodness of fit (χ^2, α=0.05).

The frequency of ovigerous females in relation to the adult non-ovigerous was analyzed monthly for both species in order to infer about the breeding period of the studied populations. The proportion of juveniles in relation to adults also was analyzed among months to infer about the recruitment period.

Hierarchical partitioning (CHEVAN; SOUTHERLAND, 1991) was used to examine the independent effects of the four physico-chemical variables (temperature, pH, dissolved oxygen and conductivity) on three population traits: abundance, recruitment and frequency of ovigerous females – representing the reproductive activity of the population. This analysis considers all possible linear models independently and jointly in order to identify the most likely causal factors, thus providing a measure of the effect of each variable (CHEVAN; SUTHERLAND, 1991; WALSH et al., 2004). The significance of independent effects was calculated through the function rand.hp of the package hier.part using 500 iterations (MAC NALLY, 2002). Hierarchical partitioning was implemented using the previously mentioned package, which is freely available in the R program. All other statistical analyses were also performed through the software R (R DEVELOPMENT CORE TEAM, 2013).

3 RESULTS

Overall, 2442 individuals of *H. longistila* were collected along the year, from which 678 were males, 746 were non-ovigerous females (hereafter treated only as “females”), 335 ovigerous females and 683 juveniles. Although it seems to have occurred several discrete events of recruitment along the year, the highest number of juveniles was observed on October/12, the only month when they predominated on the population. On December/12 half of the population was adult and the other half was juvenile (Figure 2).

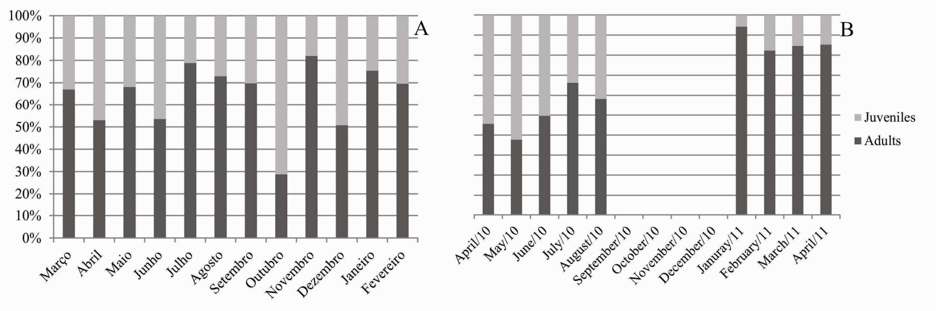

Figure 2 Relative frequency (%) of juveniles in relation to adults of *H. longistila* (A) and *H. carstica* (B) from streams on the state of Minas Gerais, Brazil.

For *H. carstica*, a total of 1735 individuals was collected, from which 381 were males, 265 females, 201 ovigerous females and 888 juveniles. During months of lower precipitation a gradual interruption of the water flow was observed, until the stream became completely dry from September/10 to November/10. Although in December/10 the dry period had already finished, the current was strong and there were not macrophytes in the sampled area, therefore amphipods could not be collected in this month. Generally, juveniles were more abundant before the dry period and adults predominated after the water flow had returned (Figure 2B).

Figure 3 presents frequency distribution of size classes of *H. longistila* for each studied month. The distribution was polimodal for most of the months. The abundance of collected individuals ranged from 120 to 190 throughout the months, except on August/12 when approximately half of the mean of amphipods collected by month were sampled, and on November/12 and December/12 when the abundance was more than twice higher than the mean.

The frequency distribution of size classes of *H. carstica* is shown on Figure 4A and B, which as well as for *H. longistila* was polimodal for most of the months. Individuals were more abundant in this population before the dry period, and as the water flow decreased, the abundance also diminished. After the dry period, the abundance of amphipods increased gradually but did not reach the size observed before the water flow interruption until the last collection of the present study.

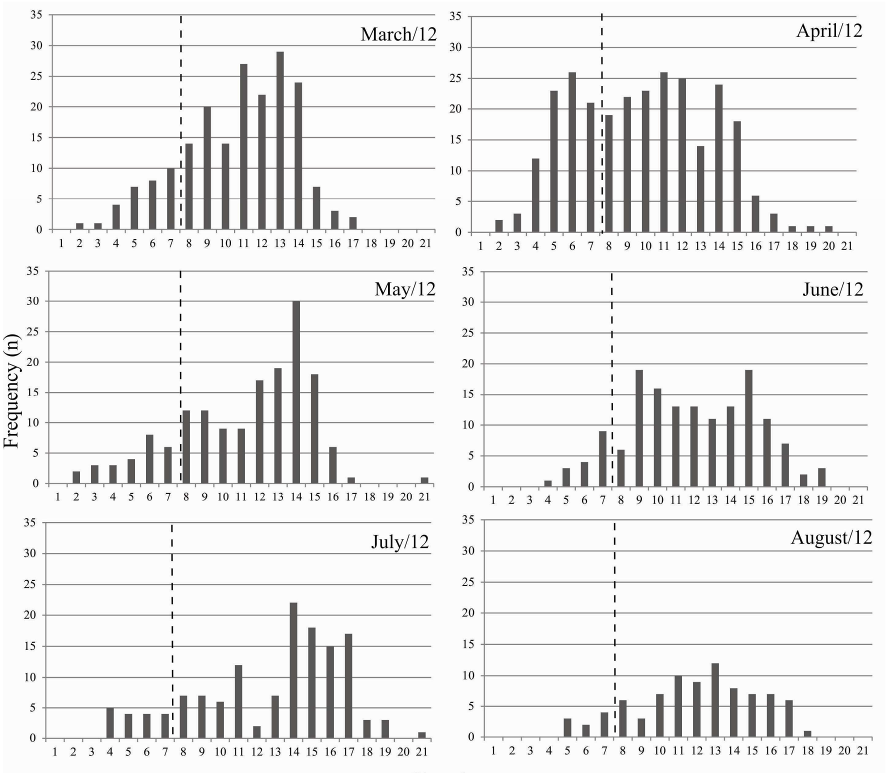

Figure 3 Absolute frequency distribution of size classes of *H. longistila*. Size class 1: 0.18-0.20 mm, 2:0.21-0.23 mm, 3: 0.24-0.26 mm, 4: 0.27-0.29 mm, 5: 0.30-0.32 mm, 6:

0.33-0.35 mm, 7: 0.36-0.38 mm, 8: 0.39-0.41 mm, 9: 0.42-0.44 mm, 10: 0.45-0.47 mm, 11: 0.48-0.50 mm, 12: 0.51-0.53 mm, 13: 0.54-0.56 mm, 14: 0.57-0.59 mm, 15: 0.60-0.62 mm, 16: 0.63-0.65 mm, 17: 0.66-0.68 mm, 18: 0.69-0.71 mm, 19: 0.72-0.74 mm, 20: 0.75-0.77 mm, 21: 0.78-0.80 mm. The dashed line separates juveniles from adults in the histogram.

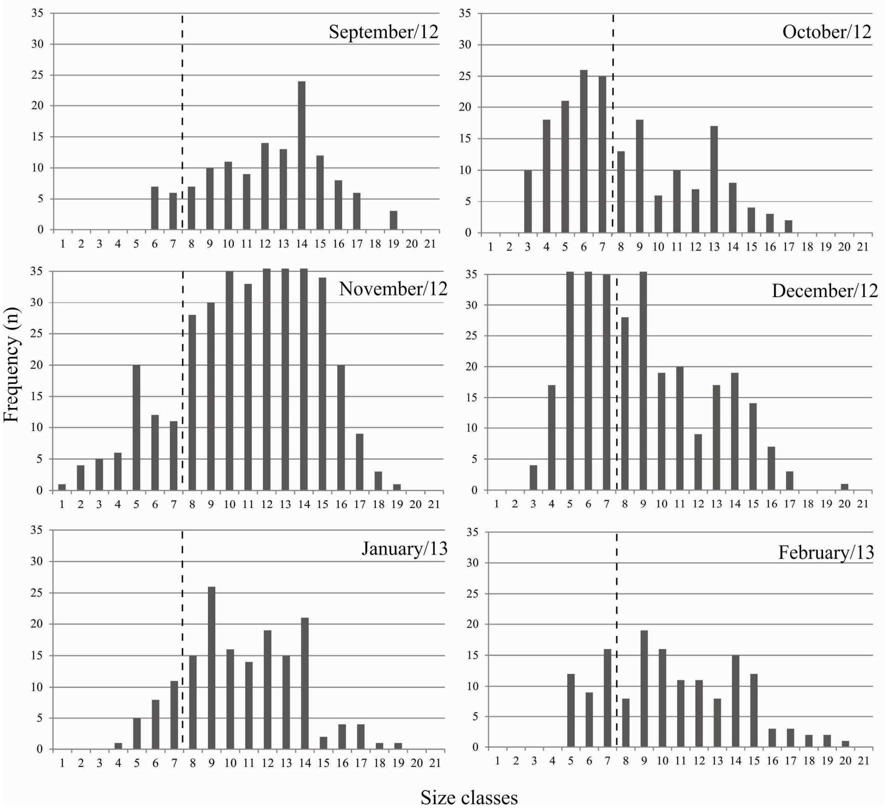

Figure 3 (continuation) Absolute frequency distribution of size classes of *H. longistila* (continuation). Size class 1: 0.18-0.20 mm, 2:0.21-0.2 3mm, 3: 0.24-0.26 mm, 4: 0.27-0.29 mm, 5: 0.30-0.32 mm, 6: 0.33-0.35 mm, 7: 0.36-0.38 mm, 8: 0.39-0.41mm, 9: 0.42-0.44 mm, 10: 0.45-0.47 mm, 11: 0.48-0.50 mm, 12: 0.51-0.53 mm, 13: 0.54-0.56 mm, 14: 0.57-0.59 mm, 15: 0.60-0.62 mm, 16: 0.63-0.65 mm, 17: 0.66-0.68 mm, 18: 0.69-0.71 mm, 19: 0.72-0.74 mm, 20: 0.75-0.77 mm, 21: 0.78-0.80 mm. The dashed line separates juveniles from adults in the histogram.

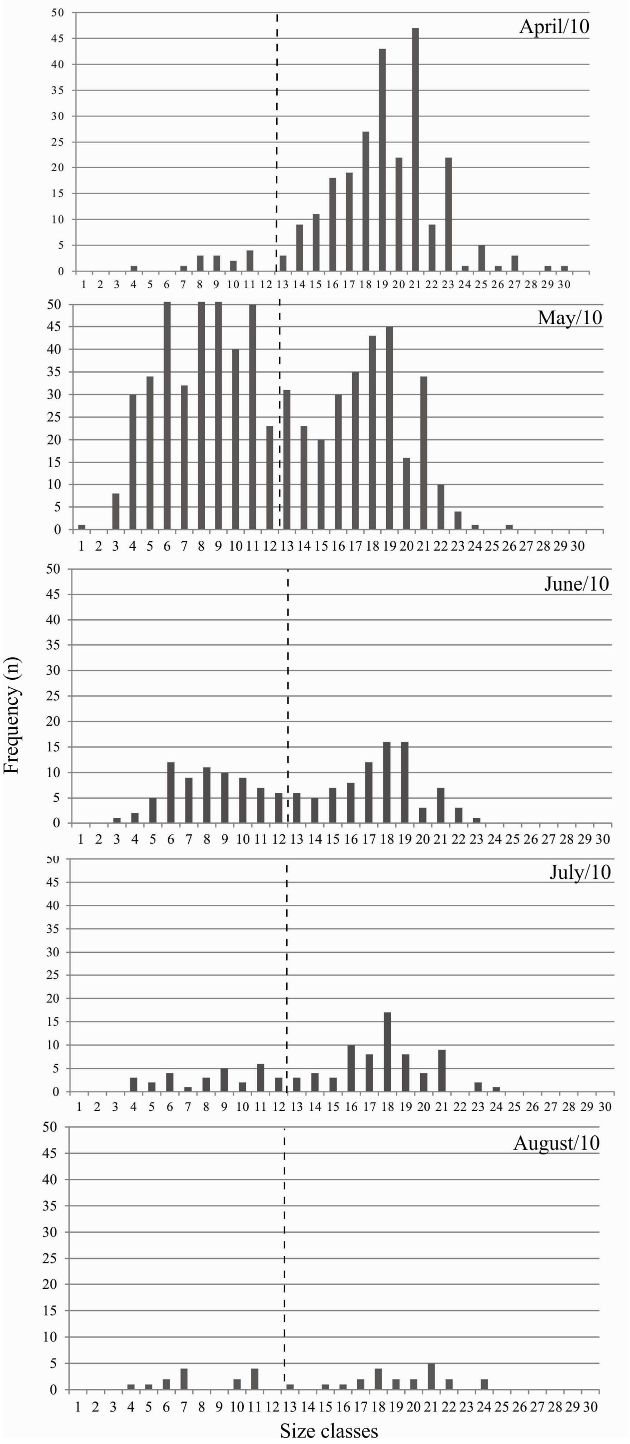
April/10
May/10
June/10
July/10
August/10
Frequency (n)
Size classes
0 5 10 15 20 25 30 35 40 45 50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Figure 4 Absolute frequency distribution of size classes of *H. carstica* before the dry period. Size class 1: 0.12-0.13 mm, 2:0.14-0.15 mm, 3: 0.16-0.17 mm, 4: 0.18-0.19 mm, 5: 0.20-0.21mm, 6: 0.22-0.23 mm, 7: 0.24-0.25 mm, 8: 0.26-0.27 mm, 9: 0.28-0.29 mm, 10: 0.30-0.31mm, 11: 0.32-0.33 mm, 12: 0.34-0.35 mm, 13: 0.36-0.37 mm, 14: 0.38-0.39 mm, 15: 0.40-0.41 mm, 16: 0.42-0.43 mm, 17: 0.44-0.45 mm, 18: 0.46-0.47mm, 19: 0.48-0.49 mm, 20: 0.50-0.51 mm, 21: 0.52-0.53 mm, 22: 0.54-0.55, 23: 0.56-0.57, 24: 0.58-0.59, 25: 0.60-0.61, 26: 0.62-0.63, 27: 0.64-0.65, 28: 0.66-0.67, 29: 0.68-0.69, 30: 0.70-0.71. The dashed line separates juveniles from adults in the histogram.

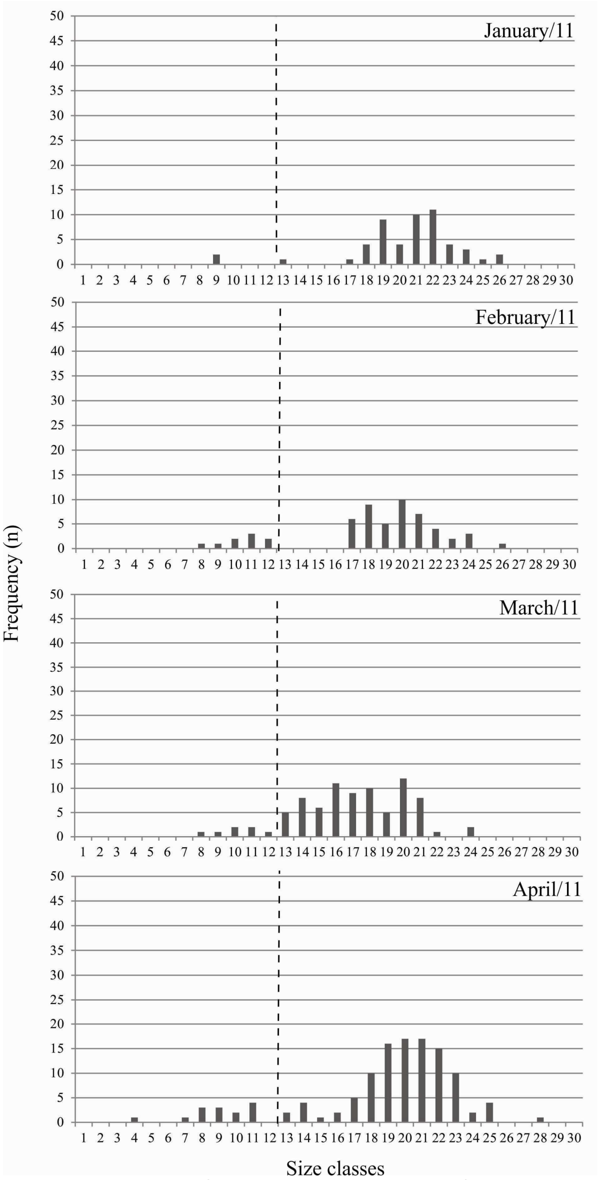

Figure 4 (continuation) Absolute frequency distribution of size classes of *H. carstica* after the dry period. Size class 1: 0.12-0.13 mm, 2:0.14-0.15 mm, 3: 0.16-0.17 mm, 4: 0.18-0.19 mm, 5: 0.20-0.21 mm, 6: 0.22-0.23 mm, 7: 0.24-0.25 mm, 8: 0.26-0.27 mm, 9: 0.28-0.29 mm, 10: 0.30-0.31 mm, 11: 0.32-0.33 mm, 12: 0.34-0.35 mm, 13: 0.36-0.37 mm, 14: 0.38-0.39 mm, 15: 0.40-0.41 mm, 16: 0.42-0.43 mm, 17: 0.44-0.45 mm, 18: 0.46-0.47 mm, 19: 0.48-0.49 mm, 20: 0.50-0.51 mm, 21: 0.52-0.53 mm, 22: 0.54-0.55, 23: 0.56-0.57, 24: 0.58-0.59, 25: 0.60-0.61, 26: 0.62-0.63, 27: 0.64-0.65, 28: 0.66-0.67, 29: 0.68-0.69, 30: 0.70-0.71. The dashed line separates juveniles from adults in the histogram.

The cephalothorax (CL) and total body length (TL) of both species were correlated in all the categories, what justifies the use of CL as a representative measurement of the individual size (Spearman correlation – *H. longistila:* rho= 0.92, p=2.2x10^{-16}; *H. carstica:* rho= 0.96, p=2.2x10^{-16}). Table 1 presents values of the ratio CL/TL for both species. Ratios were compared between species and categories and results are presented on this table. The mean ratio of juveniles was superior to adults, what allied to the size of the smallest ovigerous female and the smallest male and female in precopula may be used to determine the size in which juveniles become adult, it means, the size in which sexual dimorphism is obtained and individuals became sexually mature. Juveniles cephalothorax represents more than 10% of the total body length (it means, CL/TL > 0.10) while in adults the ratio is less or equal to 0.10.

Table 1 Ratio CL/TL (cephalothorax length/total body length) of adults and juveniles of *H. longistila* and *H. carstica*, an indicative of the size in which sexual dimorphism and sexual maturity are achieved. T and p values corresponding to the results of t test.

Category	***H. longistila***	***H. carstica***	**t test**	**p value**
Adults	0.0969	0.0966	1.84	0.03
Juveniles	0.1037	0.1104	9.02	<0.0001
t test	-10.62	22.06	-	-
p value	<0.0001	<0.0001	-	-

The smallest juvenile of *H. longistila* presented CL = 0.1800 mm, and for *H. carstica* the cephalothorax of the smallest individual was 0.1236 mm. Such measurements corresponds to the CL with which juveniles hatch from the female brood pouch (personal observation), what indicates that the sampling method was adequate since even the smallest individuals of the population could be sampled.

The mean CL of *H. longistila* males was 0.54 ± 0.07 mm (CL_{min} = 0.39 mm, CL_{max} = 0.75 mm). Females were smaller than males ($t = -18.75$, $p < 0.0001$), since their mean CL was 0.47 ± 0.07 mm (CL_{min} = 0.39 mm, CL_{max} = 0.80 mm), and ovigerous females mean was higher than non-ovigerous females (CL_{mean} = 0.57 ± 0.06 mm, CL_{min} = 0.39 mm, CL_{max} = 0.80 mm) ($t = 24.02$, $p < 0.0001$). Juveniles mean CL was 0.32 ± 0.04 mm (CL_{max} = 0.38 mm).

Males of *H. carstica* presented mean CL = 0.48 ± 0.05 mm (CL_{min} = 0.37 mm, CL_{max} = 0.66 mm). Females also were smaller in this species, their mean CL was 0.44 ± 0.05 mm (CL_{min} = 0.37 mm, CL_{max} = 0.62 mm), and they were smaller than the ovigerous females, which mean CL was 0.49 ± 0.04 (CL_{min} = 0.35 mm, CL_{max} = 0.62 mm) ($t = 6.30$, $p < 0.0001$). The mean of juveniles cephalothorax was 0.25 ± 0.06 mm (CL_{max} = 0.36 mm).

The growth models of *H. longistila* and *H. carstica* can be observed in the Figure 5. The studies species presented a very similar growth pattern, what can be observed through the curves and their respective confidence intervals, which were almost completely overlapped. An exponential growth is observed during juvenile stages of both species.

In general, there were more males than females of both *Hyalella* species, for which the mean ratio was 1 male: 0.75 female (*H. longistila* $\chi^2 = 10.92$; *H.carstica* $\chi^2 = 2.39$, $p<0.05$) (Figure 6). But there was a significant difference on the sex ratio of *H. longistila* during the summer months (December/12 – February/12), when females were more abundant (1 male: 1.47 females, $\chi^2 = 0.39$, $p<0.05$).

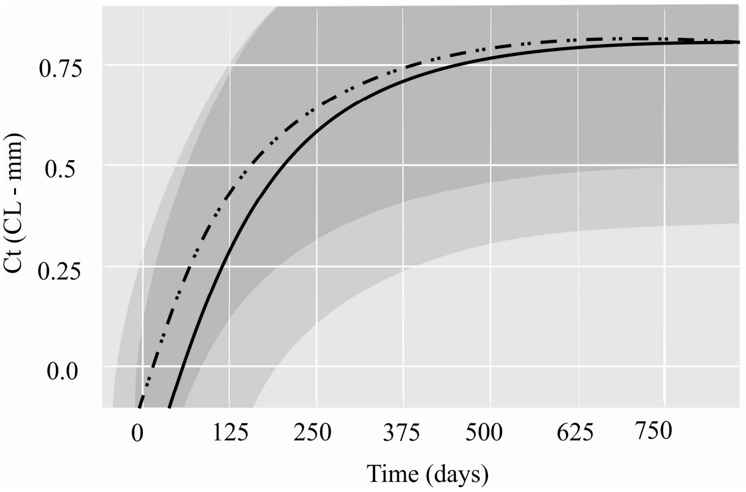

Figure 5 Growth curves of *Hyalella longistila* (▬▬▬) collected from March/12 to February/13 and *H. carstica* (·· ▬ ·· ▬) reared in laboratory from April/10 to April/11, both species from Minas Gerais state streams, Brazil. Ct: cephalothorax length (mm) of individuals in the time t (days); t: individuals age (days). *H. longistila* $Ct = 0.84[1 - e^{0.6127 - 0.0063t}]$, $r^2 = 0.63$; *H. carstica* $Ct = 0.71[1 - e^{0.3096 - 0.0066t}]$, $r^2 = 0.\ 35$.

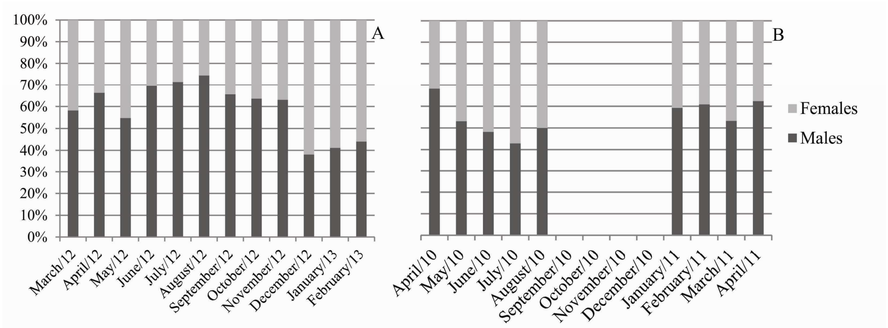

Figure 6 Operational sex ratio of populations of *H. longistila* (A) and *H. carstica* (B) from streams on the state of Minas Gerais, Brazil at each month.

Sex ratio differed among size classes for both species (*H. longistila* $\chi^2 = 61.42$; *H. carstica* $\chi^2 = 416.64$, $p<0.05$). On the population of *H. longistila* females were predominant in intermediate size classes and males in larger size classes. In the case of *H. carstica* males were more abundant in all size classes.

For this species, despite many females were collected, many of them were ovigerous and therefore were not included in this ratio (OSR).

Ovigerous females were collected throughout the year, but their frequency on the *H. longistila* population was higher in July/12 and October/12, as well as the number of adults in precopulatory behavior. For *H. carstica*, despite some ovigerous females were collected before the interruption of water flow, their higher frequency was observed on the months after the dry period, and the same occurred with couples (Figure 7).

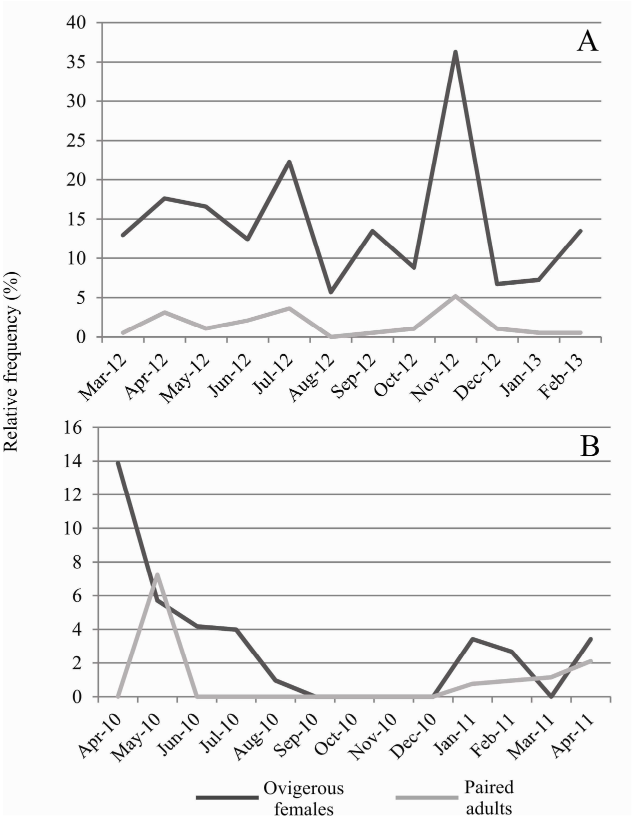

Figure 7 Relative frequency (%) of ovigerous females and paired adults among months of *H. longistila* (A) and *H. carstica* (B) from streams on Minas Gerais state, Brazil.

Regarding environmental factors, an independent and positive effect of temperature on the abundance and dissolved oxygen on the frequency of ovigerous females was observed for the *H. longistila* population, and the frequency of *H. carstica* ovigerous females was influenced positively by temperature and conductivity (Figure 8). pH was not significant for any variable in both populations. Data regarding such physicochemical parameters are presented on Table 2.

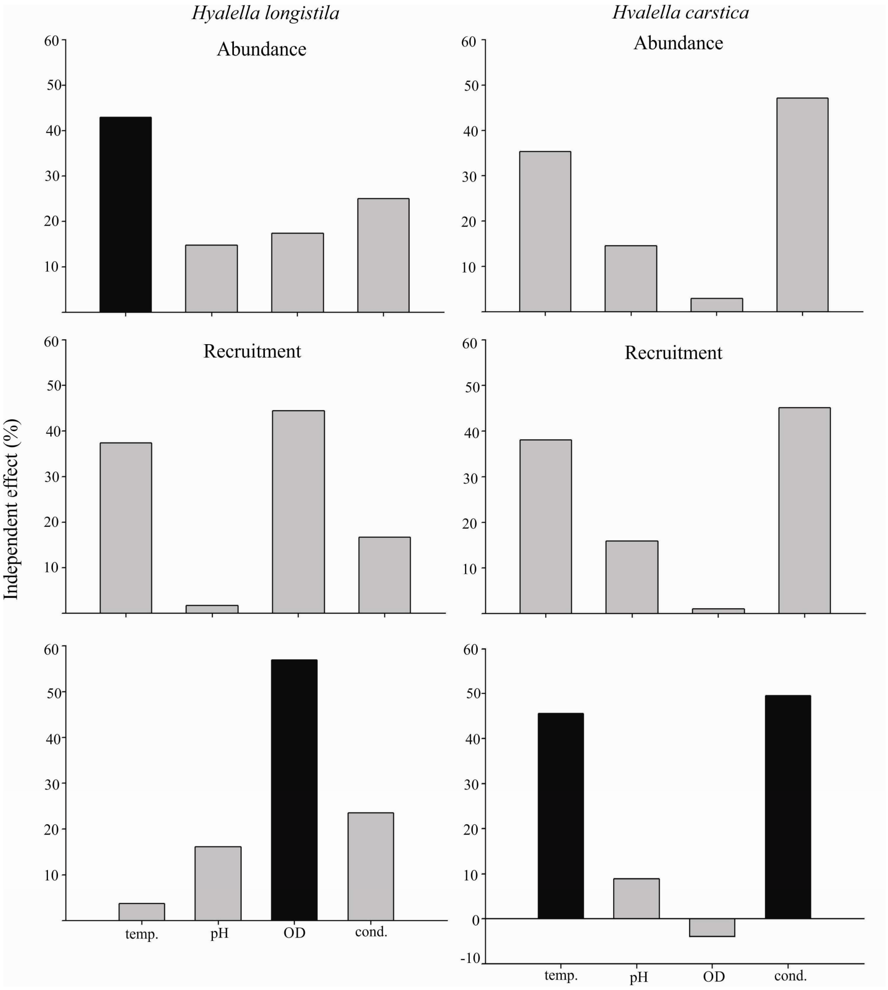

Figure 8 Percentage of independent effects of measured physic-chemical parameters of water on population parameters of *Hyalella longistila* and *H. carstica* as determined by hierarchical partitioning. Black bars represent significant effects ($p<0.05$) as determined by randomization tests. Positive or negative relations are shown by bars above or below the horizontal axis, respectively.

Table 2 Physico-chemical parameters of the streams in which *H. longistila* and *H. carstica* were found, Minas Gerais state, Brazil.

***Hyalella longistila* habitat**					***Hyalella carstica* habitat**				
Month	**Temp.**	**pH**	**OD**	**Cond.**	**Month**	**Temp.**	**pH**	**OD**	**Cond.**
Mar/12	24.3	5.4	65.2	100.3	Apr/10	24.1	7.3	6.6	56
Apr/12	20.3	6.4	68.2	73.0	May/10	19.8	7.5	5.9	63
May/12	18.3	6.8	72.2	68.7	Jun/10	19.1	7.5	5.5	64
Jun/12	16.0	7.8	67.2	70.6	Jul/10	15.8	7.4	4.7	75
Jul/12	16.8	6.2	82.0	84.0	Aug/10	20.1	7.8	8.2	56
Aug/12	18.0	6.7	58.0	72.0	Sep/10	*	*	*	*
Sep/12	20.2	6.8	54.7	109.0	Oct/10	*	*	*	*
Oct/12	24.5	7.5	40.7	113.0	Nov/10	*	*	*	*
Nov/12	25.0	7.9	68.0	116.0	Dec/10	23.6	6.69	6.01	48
Dec/12	25.5	6.6	49.0	116.0	Jan/11	25.9	7.4	5.37	45.1
Jan/13	22.2	6.8	71.6	73.0	Feb/11	25	7.4	8	43.7
Feb/13	23.4	6.8	62.0	92.0	Mar/11	24	7.4	10.8	41.1
					Apr/11	21.6	7.68	6.38	38.7

Temp. = temperature (ºC); OD = dissolved oxygen (%); Cond. = Electrical conductivity (µS.cm^{-2}); * dry period, unavailable data.

4 DISCUSSION

Recruitment pulses, differential or catastrophic mortality, or behavioral differences may reflect on bimodality or polymodality in the size frequency distribution of a population (DÍAZ; CONDE, 1989). In the present study juveniles of *H. longistila* were found in all months, but they only predominated over adults on October/12. Their frequency may be explained by the occurrence of several discrete recruitment events along the year, what may have reflected on the polymodal distribution observed most of the months for this species. Polymodality is common among amphipods, as already observed for *Mallacoota schelenbergi*, *Ampithoe valida* and *Cymadusa filosa* (APPADOO; MYERS, 2004), *H. castroi* (CASTIGLIONI; BOND-BUCKUP, 2008), *Corophium multisetosum* (CUNHA et al., 2000), *Echnogammarus marinus* (GUERAO, 2003) and *Gammarus chevreuxi* (SUBIDA et al., 2005).

Ovigerous females of *H. longistila* were observed in all months, thus suggesting a continuous reproductive activity, and juveniles observed frequently probably are associated to this activity. The dissolved oxygen has a positive significant effect on the variation of ovigerous females frequency in this species. The highest percentage of dissolved oxygen coincided with the highest observed frequency of females carrying eggs. Subida et al. (2005) found that dissolved oxygen, among other variables, influence abundance and biomass of amphipods. And Pilgrim and Burt (1993) suggested that decreases in oxygen content combined with increased metabolism at higher temperature contributed to higher mortality of *H. azteca*. Thus, reproducing in periods with higher dissolved oxygen percentage may represent an attempt of females to optimize their reproductive success.

Despite being continuous, the reproduction of *H. longistila* was more intense during some months (in this case, July/12 and October/12), as observed for *H.castroi* and *H. pleoacuta* (CASTIGLIONI; BOND-BUCKUP, 2008). This fits on the pattern suggested by Sainte-Maire (1991) that in freshwater gammaridean species there is a tendency towards an extended reproductive season with decreasing latitude. Thus, *H. longistila* seems to reproduce more intensively twice a year, on the winter and early spring. The recruitment followed this reproductive activity throughout the year. A scheme of the possible annual cycle of this population is presented on Figure 9.

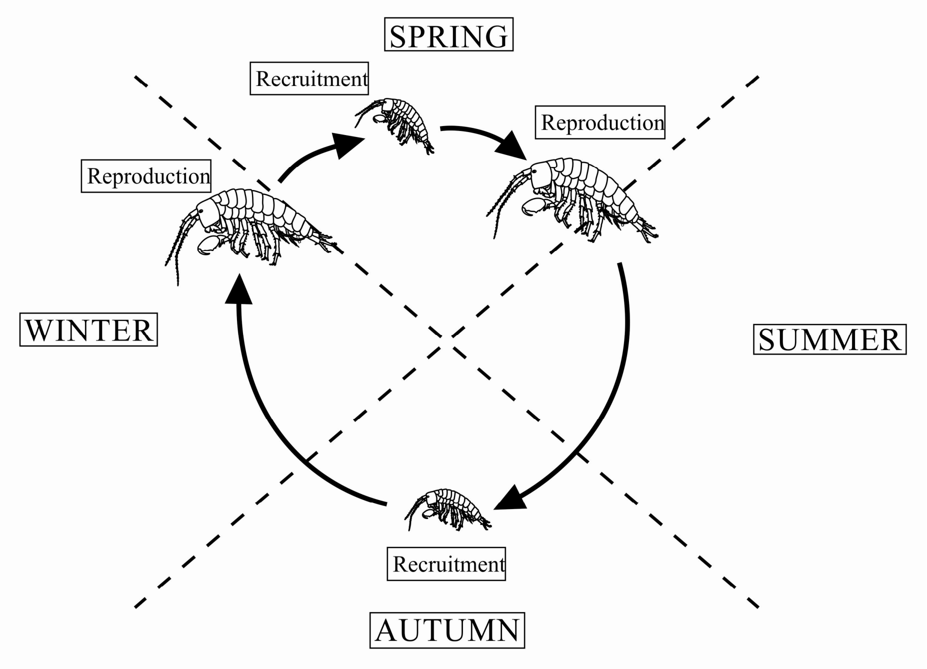

Figure 9 Scheme of annual cycle of *Hyalella longistila* found in a stream on South of the state of Minas Gerais, Brazil. Large individuals represent adults, and small individuals represent the juveniles.

Castiglioni et al. (2009) found that fluctuations in reproductive intensity in species of *Hyalella* from the state of Rio Grande of Sul, Brazil, appear to be related to the macrophyte cover since it represents food and shelter for breeding females and recruits, thus contributing for the reproductive success. In the present study, the *Typha* stand in which *H. longistila* was collected was large, with abundant macrophytes and apparently did not change during the study period. The availability of food and shelter seemed to be continuous, what may have provided suitable conditions for reproduction all year round.

Hyalella carstica presented a different pattern since in most of the months, since before the drought a bimodal distribution of size classes was observed, and after the drought the distribution was predominantly unimodal.

We observed that before the drought, especially on May/10, juveniles predominated at the population and when the water flow returned adults were more abundant. Thus, such pattern of distribution probably may be explained by the presence of both juveniles and adults before the drought and the predominance of adults after such period of water interruption. Then we suggest that a process of recolonization occurs in this population. Although the completely dry condition of the stream occurred on the spring, during winter months the water level decreased gradually, and consequently the number of individuals decrease, as well as the frequency of adults in precopulatory behavior and ovigerous females. As water level rises again on the early spring, adult individuals arrive in the studied place and reestablish the local population. These adults reproduce on spring and summer and originate the juveniles observed in the autumn and early winter, which grow and probably move downstream as water level decreases. Individuals responsible for recolonizing the study site probably came from upstream portions of the water body, which function as a center of population abundance.

A scheme of the annual cycle of *H. carstica* can be observed on Figure 10. According to the hierarchical partitioning the frequency of ovigerous females of *H. carstica* is influenced positively by the temperature and electrical conductivity. Higher temperatures may improve growth rates (COOPER, 1965), thus favoring females to achieve sexual maturity faster. Kestrup and Ricciardi (2010) found that conductivity affects the growth rate and mortality of amphipods. The electrical conductivity of the stream where *H. carstica* inhabits probably was favorable for them, since the stream belongs to a karstic province, where the availability of calcium carbonate is high.

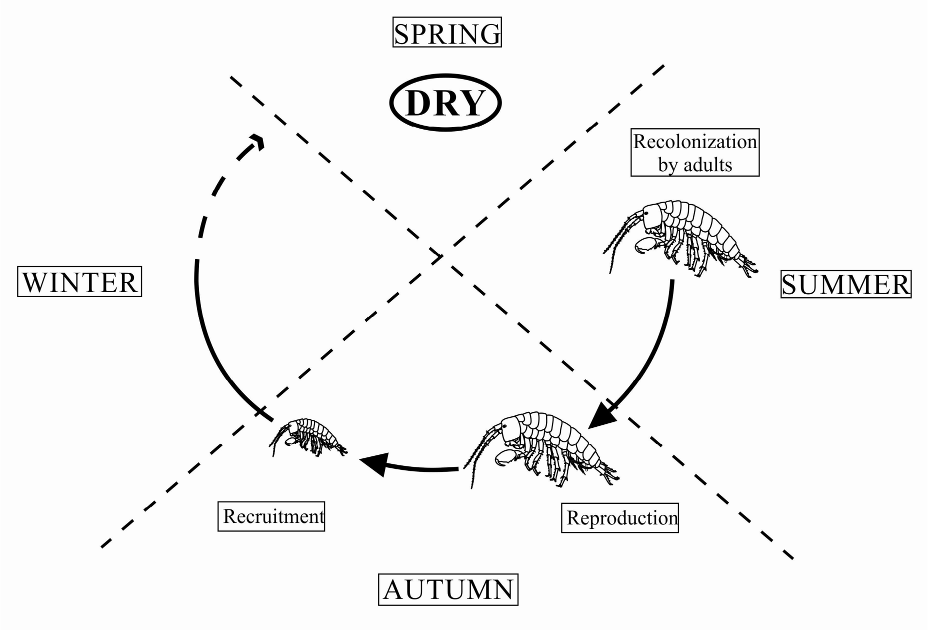

Figure 10 Scheme of annual cycle of *Hyalella carstica* found in an intermittent stream on Midwest Minas Gerais state, Brazil. Large individuals represent adults, and small individuals represent the juveniles.

Wellborn and Capps (2013) stated that even a single female may have a reasonably high probability of establishing a new population. Furthermore, Pöckl et al. (2003) suggested that the high potential reproductive capacity of amphipods in general characterized by rapid production of numerous broods probably is an adaptation to high mortality during growth until sexual maturity is achieved. This may provide an opportunistic strategy of emigration from centers of population abundance to colonize new habitats when conditions are favorable. This led us to believe that adults observed on the population of *H. carstica* on early spring probably were the great responsible for the re-establishment of the local population. Pöckl et al. (2003) still affirmed that this expansion of population is desirable to combat environmental catastrophes, both frequent and short-term floods and droughts, and more long-term climatic changes.

Seasonal variations on the amphipod abundance generally are related to changes of temperature and food availability, as observed by Cunha et al. (2000), Prato and Biandolino (2003), Galassi et al. (2006) and Xinqing et al. (2013). In the current study, the effect of temperature over the observed variance on abundance was significant according to the hierarchical partitioning. Panov and McQueen (1998) recorded a negative relationship between water temperature and maximum size attained by the amphipods. Smaller individuals attain sexual maturity faster and then may reproduce earlier than larger individuals, what may provide greater population sizes.

Hyalella carstica was found only on part of the year, before and after the drought, and the population abundance was higher before the dry. After this period of flow interruption the population size increased gradually but did not reach the previous maximum until the last sampling month. It is possible that if more collections were made the observed abundance would continue increasing, but further samplings are necessary to confirm this supposition.

Both *H. longistila* and *H. carstica* presented a very similar growth, although data regarding the first species was collected in natural conditions, and for the second under laboratory conditions. Individuals of *Hyalella*, as well as other amphipods, grow continuously, but during juvenile stages higher rates are generally observed characterizing an exponential growth (WELTON; CLARKE, 1980). Such pattern was observed both for the individuals of *H. longistila* brought from the field and those of *H. carstica* reared in laboratory. Othman and Pascoe (2001) also observed this type of growth for *H. azteca* and Castiglioni et al. (2007) observed for *H. castroi* and *H. pleoacuta.*

Some authors have observed that temperature influences the growth rate positively, accelerating the growth (COOPER, 1965; STRONG, 1972;

KRUSCHWITZ, 1978). Despite lower body sizes are attained under higher temperatures (PANOV; MACQUEEN, 1998), the exponential pattern continues being observed on the juvenile stages. In the current study temperature did not vary significantly, but highest temperatures coincided with the highest frequencies of juveniles on the population, when individuals probably grew faster.

In works regarding the relation of amphipods size and ecological traits, some authors measure the total body length of amphipods (WELLBORN, 2005; XINQING et al., 2013) while other considers cephalothorax length as an indicative of the amphipod total body length (EDWARD; COWELL, 1992; CASTIGLIONI; BOND-BUCKP, 2008). But when trying to differ juveniles from adults considering only cephalothorax length may be complex, since limit sizes proposed for a certain species may not be valid for others, besides young females may be confounded with large juveniles. Body sizes may vary according to latitude and environmental conditions like temperature (PANOV; MACQUEEN, 1998; XINQING et al., 2013), but also may be shaped by ecological interactions, like predation and competition (SCHLUTER; MCPHAIL, 1992; WELLBORN, 1994; WELLBORN, 2002). Thus it was necessary a method that was independent of the taxa and environment, but that reflected a property of the individual on a given population, like the here proposed ratio between cephalothorax and total body lengths. These measurements are easily taken from amphipods and may ensure a more secure determination of the size in which juveniles become adults, together with data of the smallest ovigerous female and the smallest adults found in precopulatory behavior, as already proposed by Borowsky (1991).

According to Low (1978), crustaceans of both sexes grow similarly until sexual maturity and from this moment on different ecological and/or reproductive demands result on distinct growth rates. Body size perhaps is the most ecologically significant trait for an individual and is considered a determinant of ecological success in *Hyalella* species (WELLBORN, 1994, 2002). In the present study *H. longistila* and *H. carstica* presented sexual dimorphisms with males attaining greater sizes than females in both species. Females usually grow slowly due to the prolongation of intermolt period because of the production and incubation of eggs, since they do not molt during this period (HARTNOLL, 1982; CARDOSO; VELOSO, 1996). On the other hand, males invest their reproductive effort in mating, during which they keep growing and having ecdysis, what leads to greater body sizes (WEN, 1992).

This sexual dimorphism is common among most crustaceans, including amphipods, e. g. *Gammarus pulex* (ADAMS AND GREENWOOD, 1983), *H. azteca* (GEISLER, 1944; WEN, 1993; OTHMAN AND PASCOE, 2001; WELLBORN; BARTHOLF, 2005) and *G. locusta* (Costa and Costa, 1999). This may be essential for these crustaceans that present the reproductive behavior of precopulatory mating guarding, in which males carry females during some days before it molts, and this difference in size may allow the male to carry females more easily (ADAMS; GREENWOOD, 1983; ADAMS et al.., 1985).

In amphipods it is usual that the sex ratio fluctuates seasonally and females are more numerous than males (POWELL; MOORE, 1991; CARDOSO; VELOSO, 1996; APPADOO; MYERS, 2004; KEVREKIDIS, 2004, 2005). But for both *H. longistila* and *H carstica* populations there were more males than females when considering the sex ratio of the total population (1 male : 0.75 female).

According to Moore (1981), sex ratio may present seasonal variations with male dominance in cooler months and female dominance in warmer months. This was observed for *H. longistila* since despite males predominated during most of the months, on summer the ratio was skewed towards females (1 male : 1.47 females). For *H. carstica* males also were more abundant during all the study period (mean ratio 1 male : 0.84 females). Emmerson (1994) stated that the male dominance provides more intraspecific competition among males, which can result in a more balanced population, because the males ensure fertilization of all females.

Wenner (1972) and Castiglioni and Bond-Buckup (2008) recorded an anomalous pattern in which the proportions differed in intermediate classes, favoring females, and in larger classes favoring males, and this pattern was also observed for *H. longistila*. This may related to the same causes previously explained for sexual dimorphisms in relation to body size. Females spend part of their energy and time in the production and incubation of eggs and during this period they do not molt, thus assuming a different growth rate when compared to males (CARDOSO; VELOSO, 1996).

The subtropical temperature regimen, continuous reproduction and recruitment of amphipods and the abundance of *Typha* appeared to be the factors responsible for high densities and production in a population of *H. azteca* on macrophyte stands of a Florida Lake (EDWARDS; COWELL, 1992) and for *H. longistila* a very similar scenario was observed.

Studying organisms which biology facilitate laboratory estimates of critical rates functions, for which all life stages can be easily sampled and reared, and researching on populations that are discrete entities in space and/or time insure a greater degree of success (COOPER, 1965). Amphipods generally are considered convenient experimental organisms since they are adapted to both laboratory and field studies, and the present work had corroborated it. The dynamics of *H. carstica* population represented how part of the recolonization process occurs after the dry period, this species pattern of growth was determined under laboratory conditions, *H. longistila* represented the dynamics of a subtropical species that deals with constant and high availability of resources, and its growth pattern also was determined.

Despite belonging to the same genus and inhabiting subtropical streams, the studied species presented dissimilarities on their life history traits, what probably is associated to the different conditions provided by their habitats. Thus, the knowledge regarding biological and physical properties of both the environment and the population are of extreme importance in determining the success of a population.

The conservation of the stream biodiversity depends on studies that provide information about the species requirements and that elucidate the relationship between organisms and their habitat. Thus, the knowledge regarding the ecology and biology of species is crucial for the elaboration and practice of conservation activities.

REFERENCES

ADAMS, J.; GREENWOOD, P. J. Why are males bigger than females in pre-copula pairs of *Gammarus pulex*? **Behavioral Ecology and Sociobiology**, New York, v. 13, p.239-241, 1983.

ADAMS, J.; GREENWOOD P. J.; POLLITT, P. R.; YONOW, T. Loading constraints and sexual size dimorphism in *Asellus aquaticus*. **Behaviour**, v. 92, p. 277−287, 1985.

APPADOO, C.; MYERS, A. A. Reproductive bionomics and life history traits of three gammaridean amphipods, *Cymadusa filosa* Savigny, *Ampithoe laxipodus* Appadoo and Myers and *Mallacoota schellenbergi* Ledoyer from tropical Indian Ocean (Mauritius). **Acta Oecologica,** Paris, v. 26, p. 227-238, 2004.

BASTOS-PEREIRA, R.; BUENO, A. A. P. New species and new report of *Hyalella* S. I. Smith, 1874 (Crustacea: Amphipoda: Dogielinotidae) from Minas Gerais state, Southeastern Brazil. **Zootaxa**, v. 3350, p. 58–68, 2012.

BASTOS-PEREIRA, R.; BUENO, A. A. P. A new species of freshwater amphipod (Dogielinotidae, Hyalella) from Southeastern Brazil. **Nauplius,** Ribeirão Preto, v. 21**,** p. 79–87, 2013.

BERTALANFFY, L. A quantitative theory of organic growth (inquiries on growth laws II). **Human Biology**, Washington, v. 10, n. 1, p. 181-213, 1938.

BOCHER, P.; CHEREL, Y.; LABAT, J.; MAYZAUD, P.; RAZOULS, S.; JOUVENTIN, P. Amphipod-based food web: *Themisto gaudichaudii* caught in nets and by seabirds in Kerguelen waters, southern Indian Ocean. **Marine Ecology Progress Series**, v. 223, p. 261–276, 2001.

BOROWSKY, B. Patterns of reproduction of some amphipod crustaceans and insights into the nature of their stimuli,. In: BAUER, R.T. & W. MARTIN (Eds). **Journal of Crustacean Sexual Biology**. New York, 1991. P. 33-66.

BUENO, A. A. P.; ARAUJO (DESCONSIDERAR NOTA CASO O NOME TENHA SIDO REGISTRADO SEM ACENTO GRÁFICO), P. B.; CARDOSO, G. M.; GOMES, K. M.; BOND-BUCKUP, G. Two new species of *Hyalella* (Amphipoda, Dogielinotidae) from Brazil. **Crustaceana**, Leiden, v. 86, p. 802 – 819, 2013.

CARDOSO, R.S.; VELOSO, V.G. Population biology and secondary production of the sandhopper *Pseudorchestoidea brasiliensis* (Amphipoda: Talitridae) at Prainha Beach, Brazil. **Marine Ecology Progress Series**, Amelinghausen, v. 142, p. 111-119, 1996.

CASTIGLIONI, D. S.; BOND-BUCKUP, G. Ecological traits of two sympatric species of *Hyalella* Smith, 1874 (Crustacea, Amphipoda, Dogielinotidae) from southern Brazil. **Acta Oecologica**, Paris, v. 33, n. 1, p. 36-48, 2008.

CASTIGLIONI, D. S.; BOND-BUCKUP, G. Egg production of two sympatric species of *Hyalella* Smith, 1874 (Crustacea, Amphipoda, Dogielinotidae in aquaculture ponds in southern Brazil.) **Journal of Natural History,** London, v. 43, p. 1273–1289, 2009.

CHEVAN, A.; SUTHERLAND, M. Hierarchical partitioning. **The American Statistician,** v. 45, p. 90-96, 1991.

COOPER, W. E. Dynamics and production of a natural population of a freshwater amphipod, *Hyalella azteca*. **Ecological Monographs**, Lawrence, v. 35, p. 377–394, 1965.

COSTA, F. O.; COSTA, M. H. Life history of the amphipod *Gammarus locusta* in the Sado estuary. **Acta Oecologica,** Paris, v. 20, p. 305 – 314, 1999.

CUNHA, M. R.; SORBE, J. C.; MOREIRA, M. H. The amphipod *Corophium multisetosum* (Corophiidae) in Ria de Aveiro (NW Portugal). I. Life history and aspects of reproductive biology. **Marine Biology,** Berlin, v. 137, p. 637–650, 2000.

DÍAZ, H.; CONDE, J.E. Population dynamics and life of mangrove crab *Aratus pisonii* (Brachyura, Grapsidae) in a marine environment. **Bulletin of Marine Science**, Miami, v. 45, p. 148-163, 1989.

EDWARDS, T. D.; COWELL, B. C. Population dynamics and secondary production of *Hyalella azteca* (Amphipoda) in *Typha* stands of a subtropical Florida lake. **Journal of the North American Benthological Society,** Lawrence, v. 11, p. 69–79, 1992.

EMMERSON, W.D. Seasonal breeding cycles and sex ratios of eight species of crabs from Mgazana, a mangrove estuary in Transkei, southern Africa. **Journal of Crustacean Biology**, Langley, v. 14, n.3, p. 568-578, 1994.

GALASSI, M. E.; FRANCESCHINI, M.; C.; NEIFF, A. P. Population estimates of *Hyalella curvispina* Shoemaker (Amphipoda) in aquatic vegetation of Northeastern Argentinian ponds. **Acta Limnologica Brasiliensia,** Rio Claro, v. 18, p. 101–108, 2006.

GEISLER, S. S. J. Studies on the postembryonic development of *Hyalella azteca* (Saussure). **Biological Bulletin**, Woods Hole, v. 86, p. 6-22, 1944.

GROSSO, L.; PERALTA, M. Anfípodos de agua dulce sudamericanos. Revisión del género *Hyalella* Smith. **Acta Zoologica Lilloana**, Tucuman, v. 45, p. 79-98, 1999.

GUERAO, G. 2003. Some observations on the life history of the freshwater amphipod *Echinogammarus longisetosus* Pinkster, 1973 (Gammaridea) from Catalonia (Spain, N Iberia peninsula). **Animal Biodiversity and Conservation**, Barcelona, v. 26, n. 1, p. 31-39.

HARTNOLL, R.G. Growth. In: BLISS, D.E. (Ed.). **The biology of Crustacea: embryology, morphology and genetics.** London Academic Press, London, 1982, p. 111-196.

HUTCHINSON, G. E. **Introducción a la ecologia de pobliaciones**. Editora Blume, Barcelona, 1981. 492p.

KESTRUP, Å.; RICCIARDI, A. Influence of conductivity on life history traits of exotic and native amphipods in the St . Lawrence River. **Fundamental Applied Limnology,** Stuttgart, v. 176, p. 249–262, 2010.

KEVREKIDIS, T. Population dynamics, growth and reproduction of *Corophium insidiosum* (Crustacea: Amphipoda) at low salinities in Monolimni lagoon (Evros Delta, north Aegean Sea). **Hydrobiologia**, v. 522, p. 117-132, 2004.

KEVREKIDIS, T. Life history, aspects of reproductive biology and production of *Corophium orientale* (Crustacea: Amphipoda) in Monolimni lagoon (Evros Delta, North Aegean Sea). **Hydrobiologia**, v. 537, p. 53–70, 2005.

KOKKOTIS, A. T.; McLAUGHLIN, J. D. Instar-specific head and body lengths of *Hyalella* (Amphipoda): criteria for starting and endpoints in experimental studies. **Hydrobiologia**, v. 474, p. 223–227, 2002.

KRUSCHWITZ, L. G. Environmental factors controlling reproduction of the amphipod *Hyalella 78zteca*. **Proceedings of the Oklahoma Academy of Science**, v. 21, p. 16–21, 1978.

LOW, B.S. Environmental uncertainty and parental strategies of marsupials and placentals. **American Naturalist,** v. 112, p. 319-335, 1978.

LOWRY, J. K.; MYERS, A. A. A phylogeny and classification of the Senticaudata subord. Nov. (Crustacea: Amphipoda). **Zootaxa,** v. 3610, p. 1–80, 2013.

MacDONALD, P. D. M. The analysis of length-frequency distributions, In*:* SUMMERFELT, R. C.; HALL, G. **Age and growth of fish.** Ames, Iowa State University Press, 1987. P. 371 – 384.

MacDONALD, P. D. M.; PITCHER, T. J. Age-groups from size-frequency data: a versatile and efficient method on analyzing distribution mixtures. Journal of the Fisheries Research Board of Canada, v. 36, p. 987-1001, 1979.

MacNALLY, R. Multiple regression and inference in ecology and conservation biology: further comments on identifying important predictor variables. Biodiversity Conservation, v. 11, p. 1397-1401, 2002.

MARANHÃO, P., BENGALA, N., PARDAL, M. & MARQUES, J. C. The influence of environmental factors on the population dynamics, reproductive biology and productivity of *Echinogammarus marinus* Leach (Amphipoda, Gammaridae) in the Mondego estuary (Portugal). **Acta Oecologica,** Paris, v. 22, p. 139–152, 2001.

MARKUS, R. **Elementos de estatística aplicada**. Faculdade de Agronomia e Veterinária da UFRGS: Centro Acadêmico Leopoldo Cortez, Porto Alegre, 1971. 329 p.

MENEGASSE, L. N.; GONÇALVES, J. M.; FANTINEL, L. M. Disponibilidade hídrica na província cárstica de Arcos-Pains-Doresópolis, Alto São Francisco, Minas Gerais, Brasil. **Águas Subterrâneas**, Rio Claro, v. 16, n. 1, p. 1-19, 2002.

MOORE, P.G. The life histories of the amphipods *Lembos websteri* Bate and *Corophium bonnellii* Milne Edwards in kelp holdfasts. **Journal of Experimental Marine Biology and Ecology**, v. 49, p. 1–50, 1981.

OTHMAN, M. S.; PASCOE, D. Growth, development and reproduction of *Hyalella 79zteca* (Saussure, 1858) in laboratory culture. **Crustaceana,** v. 74, n. 2, p. 171−181, 2001.

PANOV, V. E.; McQUEEN, D. J. Effects of temperature on individual growth rate and body size of a freshwater amphipod. **Canadian Journal of Zoology**, v. 76, p. 1107–1116, 1998.

PARDAL, M. A., MARQUES, J. C., METELO, I., LILLEBO, A. I. & FLINDT, M. R. Impact of eutrophication on the life cycle, population dynamics and production of *Ampithoe valida* (Amphipoda) along an estuarine spatial gradient (Mondego estuary, Portugal). **Marine Ecology Progress Series,** v. 196, p. 207–219, 2000.

PICKARD, D. P.; BENKE, A. C.; PICKARD, D. P.; BENKE, A. C. Production Dynamics of *Hyalella 79zteca* (Amphipoda) among different habitats in a small wetland in the Southeastern USA. **Journal of the North American Benthological Society,** v. 15, p. 537–550, 1996.

PILGRIM, W.; BURT, M. D. B. Effect of acute pH depression on the survival of the freshwater amphipod *Hyalella 79zteca* at variable temperatures: field and laboratory studies. **Hydrobiologia**, v. 254, p. 91-98, 1993.

PÖCKL, M.; WEBB, B. W.; SUTCLIFFE, D. W. Life history and reproductive capacity of *Gammarus fossarum* and *G. roeseli* (Crustacea□: Amphipoda) under naturally fluctuating water temperatures: a simulation study. **Freshwater Biology,** v. 48, p. 53–66, 2003.

POWELL, R.; MOORE, P. G. The breeding cycles of females of seven species of amphipod (Crustacea) from the Clyde Sea area. **Journal of Natural History,** v. 25, p. 435-479, 1991.

PRATO, E.; BIANDOLINO, F.; SCARDICCHIO, C. Postembryonic growth, development and reproduction of *Gammarus aequicauda* (Martynov, 1931) (Gammaridae) in laboratory culture. **Zoological Studies,** v. 45, p. 503–509, 2006.

R CORE TEAM R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/. 2013.

RICKLEFS, R. E. A **Economia da Natureza**. 3ª Edição. Editora Guanabara Koogan, Rio de Janeiro, 1993. 470p.

RODRIGUES, S. G.; BUENO, A. A. P.; FERREIRA, R. L. The first hypothelminorheic Crustacea (Amphipoda, Dogielinotidae, *Hyalella*) from South America. **Zookeys**, v. 236, p. 65 – 80, 2012.

SAINTE-MARIE, B. A review of the reproductive bionomics of aquatic gammaridean amphipods: variation of life history traits with latitude, depth, salinity and superfamily. **Hydrobiologia,** v. 223, p. 189–227, 1991.

SCHLUTER, D.; MCPHAIL, J. D. Ecological character displacement and speciation in sticklebacks. **American Naturalist,** v. 140, p. 85–108, 1992.

STEARNS, S. C. **The evolution of life histories.** Oxford University Press, Oxford, 1992. 249p.

STONER, A. W. Species-specific predation on amphipod Crustacea by the Pinfish *Lagodon rhomboides*□: mediation by macrophyte standing crop. **Marine Biology,** v. 55, p. 201–207, 1979.

STRONG, D. R. Life History Variation Among Populations of an Amphipod (*Hyalella azteca*). **Ecology**, v. 53, p. 1103–1111, 1972.

SUBIDA, M. D.; CUNHA, M, R.; MOREIRA, M. H. Life history, reproduction and production of *Gammarus chevreuxi* (Amphipoda: Gammaridae) in the Ria de Aveiro, northwestern Portugal. **Journal of the North American Benthological Society,** v. 24, p. 82–100, 2005.

TEIXEIRA, P. S. D.; DIAS, M. S. Levantamento espeleológico da região cárstica de Arcos, Pains, Doresópolis, Córrego Fundo e Iguatama frente às atividades degradadoras. In: CONGRESSO BRASILEIRO DE ESPELEOLOGIA, 27., 2003, Januária. **Anais...** Januária: SBE, 2003. P.7.

WALSH, C. J.; PAPAS, P. J.; CROWTHER, D.; SIM, T. A. I.;YOO, J. Stormwater drainage pipes as a threat to a stream- dwelling amphipod of conservation significance, *Austrogammarus australis*, in southeastern Australia**. Biodiversity and Conservation,** v. 13, p. 781–793, 2004.

WELLBORN, G. A. Size-biased predation and prey life histories: a comparative study of freshwater amphipod populations. **Ecology**, v. 75, n.7, p. 2104 – 2117, 1994.

WELLBORN, G. A. Trade-off between competitive ability and antipredator adaptation in a freshwater amphipod species complex. **Ecology**, v. 83, p. 129 – 136, 2002.

WELLBORN, G.A.; BARTHOLF, S.E.; Ecological context and the importance of body and gnathopod size for pairing success in two amphipod 81zteca81phs. **Oecologia**, v. 143, p. 308-316, 2005.

WELLBORN, G. A.; CAPPS, E. Establishment of a new population by a single gravid colonist: implications for *Hyalella* biogeography and speciation. **Evolutionary Ecology,** v. 27, p. 101–115, 2013.

WELTON, J. S.; CLARKE, R. T. Laboratory studies on the reproduction and growth of the amphipod, *Gammarus pulex*. **Journal of Animal Ecology** v. 49, p. 581–592, 1980.

WEN, Y. H. Life history and production of *Hyalella azteca* (Crustacea, Amphipoda) in a hypereutrophic prairie pond in southern Alberta. **Canadian Journal of Zoology**, Ottawa, v. 70, p. 1417-1424, 1992.

WENNER, A. M. Sex-ratio as a function of size in marine Crustacea. **American Naturalist,** v. 106, p. 321-350, 1972.

XINQING, Z.; LINGFENG, H.; BANGQIN, H.; YONGQING, L. I. N. Factors regulating population dynamics of the amphipod *Ampithoe valida* in a eutrophic subtropical coastal lagoon. **Acta Oceanologica Sinica,** v. 32, p. 56–65, 2013.

CAPÍTULO 3

ARTIGO 2

Reproductive biology and egg production of two freshwater dwelling amphipods from Brazil

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ABSTRACT

Researches on the reproductive biology of freshwater amphipods are still incipient, especially related to Brazilian species. The present work aimed to elucidate the reproductive biology of two subtropical species of *Hyalella* found on Southeastern Brazil. *Hyalella carstica* lives on an intermittent stream and have to recolonize the local population after the dry period, while *H. longistila* inhabits a perennial stream. In general, paired adult were larger than non-paired for both sexes and species. The mean fecundity of *H. longistila* was 12.88 eggs per female, with peaks on August/12 and September/12, with 16.82 and 15.08 eggs per female, respectively. The mean fecundity of *H. carstica* was similar (12.6 eggs per female), and considering monthly, fecundity was higher on months after the dry period. Significant differences were observed in relation to the number of eggs found on the marsupium of *H. longistila* females when considering different developmental stages, but not for *H. carstica*. The developmental stage and the number of eggs found on the brood pouch were correlated on females of *H. longistila*, but no correlations were found for *H. carstica*. The stage of eggs was positively correlated with their volume on eggs of both studied species, with increasing volume as they developed. The mean volume of eggs on the first stage did not present significant differences among females of the two studied species, but stages 2 and 3 differed significantly. This results on larger juveniles and consequently adults of *H. longistila*. There a linear relation between female size and fecundity for both species. For males of both species and females of *H. carstica* pairing and reproductive success was higher at intermediate size classes, while for females of *H. longistila* there was a progressive increase on success as female increases body sizes. Differences on reproductive strategies of such species probably are related to the differences on the conditions to which they are submitted, especially regarding the hydric regime.

Keywords: Reproductive strategies. Intermittent stream. *Hyalella*. Fecundity.

1 INTRODUCTION

Amphipods are crustaceans that have colonized marine, freshwater and terrestrian habitats and are frequently used as bioindicators and biomonitors in a variety of aquatic ecosystems (BORGMANN et al., 1989; NELSON; BRUNSON, 1995; RINDERHAGEN et al., 2000). Their success on colonizing distinct environments, the ease to collect them, the high density of most of the populations, besides the ease on rearing them on laboratory and the short life cycle makes them good experimental organisms (KRUSCHWITZ, 1978; PILGRIM; BURT, 1993). The sensibility of amphipods to contaminants or environmental impacts generates responses which interpretations may subsidize conservations practices. The knowledge about reproductive strategies and other life history traits may be highly important to interpret data regarding bioindication and ecotoxicology (RINDERHAGEN et al., 2000).

Studying the reproductive biology in crustacean comprises both events related to court, including processes before and after copulation, and the reproductive cycle of the species, like sexual maturity and incubation of eggs (GONZÁLEZ-GURRIARÁN, 1985). The precopulatory mating behavior is the most precise indicative that males are apt to reproduce (BOROWSKY, 1991), while developed ovaries (dark green color and elongate) can be easily identified on mature females due to the transparency of their exoskeleton.

Females of amphipods produce a determined amount of eggs at each molt, however they do not present sperm storage. Then, it was necessary a strategy to allow that females and males were together when females ovulate, thus guaranteeing that fecundation occurred (COOPER, 1965; STRONG, 1972). During the precopulatory behavior, the male carries females dorsally with its second gnathopods during some days before female molts. Due to the high energetic costs of such behavior (CALOW, 1979), males only attach on females when ovulation is about to occur (CALOW, 1979), when probably there is communication mediated by pheromones (BOROWSKY, 1991).

In most of the mature gamarideans the copula occurs few minutes after the female molts (HYNES 1955). After such molt, the female releases eggs on the marsupium, where fertilization occurs. Eggs are incubated on the marsupium until juveniles hatch and the female release them after some hours or days.

The time spent on precopulatory mating behavior, copula, incubation of eggs and fecundity may vary among species or even among populations, depending on attributes of the taxon and/or on the influence from external factors (SASTRY, 1983).

Fecundity is an important reproductive parameter, widely evaluated on ecological researches, which corresponds to the number of eggs produced by a female during one spawning or reproductive period (SASTRY, 1983; HARTNOLL, 1985). Studying the fecundity consists both on a tool to measure the reproductive potential of individuals of a given population, and a pre-requisite for understanding the evolution of reproductive strategies. The energy invested on production of gametes may be distributed either for many small or less larger eggs, thus constituting patterns proper of the population or even of the species, in general (SASTRY, 1983).

Although Castiglioni and Bond-Buckup (2008b, 2009) have studied sympatric species of amphipods found on Southern Brazil, obtaining important information regarding the reproductive biology and ecology of the genus *Hyalella*, researches in this area are still incipient, especially related to Brazilian species of freshwater amphipods.

In this sense, the present work aimed to elucidate the reproductive biology and egg production of two subtropical species of *Hyalella* found on Brazil which are exposed to different conditions in their environments regarding the availability of water along the year.

2 MATERIAL AND METHODS

2.1 Study site and sampling

Two species of *Hyalella* were collected in distinct sites, but using the same methodology and on different periods. These amphipods use to live associated with macrophytes, which were sampled during twenty minutes with the aid of a hand net monthly during one year. *Hyalella longistila* was found in a *Typha* stand in a stream located at 21°10’26”S – 44°56’26”W, on the municipality of Ijaci, Southern Minas Gerais state, Brazil. This species was collected from March 2012 to February 2013. *Hyalella carstica* was found associated to *Spirodella* in an intermittent stream located at 20°19′59″S – 45°36′25″W, on the municipality of Arcos, Midwest Minas Gerais state, Brazil, and was collected from April 2010 to April 2011.

For both species the collected material was put on plastic trays where amphipods were separated from debris. Ovigerous females and pairs in pre-copula were recorded and individualized in microtubules in the field. This procedure was adopted in order to avoid the egg loss and to provide conditions to analyze the characteristics of each couple, individually. All the collected amphipods were carefully separated from sediments, preserved in ethanol 70% and brought to the laboratory.

2.2 Laboratory analysis

All individuals were measured considering their cephalothorax length (CL) and total body length (TL), with the aid of a microscope containing a micrometric lens. The width of each ovigerous female brood pouch also was measured, considering the ventral distance between the pereiopods 4 or 5, which represents the greatest width of the body, where embryos are incubated in the marsupium.

Measurements of paired and non-paired adults were compared by means of ANOVA complemented by Bonferroni test (α = 0.05). The CL of paired male and females was compared in order to verify if there was a correlation between such measurements.

Analyses were performed in the software R (R DEVELOPMENTAL CORE TEAM, 2013).

2.2.1 Egg production

Eggs and juveniles were removed from the marsupium and counted with the aid of an stereomicroscope in order to evaluate the fecundity. The content of this brood pouch was characterized in relation to the three embryonic developmental stages, according to Hynes (1955) and Subida, Cunha and Moreira (2005), and one post-embryonic stage (juveniles that remained on the brood pouch). Such stages were recognized as the following: stage 1 – with little or any clivage, orange color, yolk occupying the entire space of the egg; stage 2 – the beginning of cell cleavage is visible, but body parts are not defined yet; stage 3 – yolk was consumed, reduced to a small spot on the embryo, and in this stage the eyes can be easily seen; stage 4 – juveniles that hatched and remained on the brood pouch.

The mean fecundity was calculated for each month. The number of eggs in each embryonic stage was analyzed and compared through ANOVA complemented by the Bonferroni test (α = 0.05). A Spearman correlation was performed between the developmental stage and the number of eggs in order to observe whether there is a relation between these two variables.

Since the eggs become ellipsoid as they develop, the measurements of the maximum and minimum radius were took (MARANHÃO et al., 2001), and this metrics were obtained through stereomicroscope with milimetric lens. The volume of eggs was calculated through the following formula (SUBIDA; CUNHA, MOREIRA, 2005):

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

in which R_{max} and R_{min} corresponds to the maximum and minimum radius of the egg, respectively. The mean volume of eggs was calculated for each developmental stage and was compared through ANOVA complemented by the Bonferroni test in order to verify differences among stages ($\alpha = 0.05$). The volume of eggs also was compared between the two species at each stage by t test. A correlation test also was performed between volume and developmental stage.

The relationship among the number of eggs on each developmental stage or the number of juveniles that remained on the brood pouch was analyzed in relation to the size of the female that carried, considering their cephalothorax and total body length, as well as their marsupium width. General linear models with Poisson error distribution were used to test the effect of female size on the egg production of both species, considering only eggs on the first stage in order to avoid underestimations caused by egg loss during development.

2.2.2 Pairing and reproductive success

The determination of reproductive period was made through the monthly analysis of the frequency of ovigerous females in relation to the number of adult non-ovigerous females.

In order to evaluate the pairing, the cephalothorax of males and females found in pre-copula was measured to verify a possible correlation. On each month the percentage of adults in precopulatory behavior was calculated in relation to non-paired adults.

An index of relative pairing success adapted from an index of feeding tendency described by Manly (1974) was used to evaluate the relation between the pairing success and body size for males (CASTIGLIONI; BOND-BUCKUP, 2008b).

$$\beta_i = \frac{r_i/n_i}{\sum_{j=1}^{m}(r_j/n_j)}, \quad i = 1, \dots m$$

in which β_i represents the relative pairing success of the size class i, and r_i and n_i are the proportion of paired in relation to the non-paired males in the size class i, respectively. The denominator is the sum of ratios throughout the m size classes of adults. This index was modified by Wellborn (1995), and according to him the reproductive success depends not only on the pairing success, but also on the number of eggs fertilized during the pairing. Therefore, reproductive success of males was defined as:

$$R_{M,I} = \frac{\beta_i e_i}{\sum_{j=1}^{m}(\beta_i e_i)}, \quad i = 1, \dots m$$

in which $R_{M,I}$ is the relative reproductive success of males of the size class i. The expected number of eggs fertilized by males paired in the size class i (e_i) was determined through the calculus of expected fecundity of each paired female using the regression equation of the female fecundity in relation to its CL. The denominator corresponds to the sum of such values throughout the size m classes.

Since the reproductive success does not depend on the body size of females (CASTIGLIONI; BOND-BUCKUP, 2008b), their success depend only on the egg production:

$$R_{F,I} = \frac{f_i}{\sum_{j=1}^{m}(f_i)}$$

in which $R_{F,I}$ is the relative reproductive success of females in the size class i, f_i is the mean observed fecundity (number of eggs on the brood pouch) of non-paired females in the size class i, and the denominator é the sum of fecundity values throughout the m size classes.

3 RESULTS

The minimum CL of paired *H. longistila* females was 0.44 mm, and the maximum was 0.67 mm, while paired males presented minimum CL of 0.46 mm and maximum equal to 0.66 mm. A positive correlation of Spearman was observed between the CL of paired males and females of this species (r = 0.97, t=22.97, p<0.0001). For *H. carstica* the smallest CL of paired females was 0.4120 mm, and the maximum CL recorded was 0.56 mm. The minimum CL of paired males was 0.43 mm, and the maximum was 0.60 mm. For this species a Spearman correlation between the CL of paired males and females was not significant (r = 0.10, p = 0.27).

In general, there were significant differences (t test, p<0.0001) between the mean CL of paired and non-paired adults, for both sexes and species as can be observed on Figure 1 and 2 (*H. longistila* paired *versus* non-paired males*:* t=22.20, females: t=6.80; *H. carstica* males: t=4.28, females: t = 3.09).

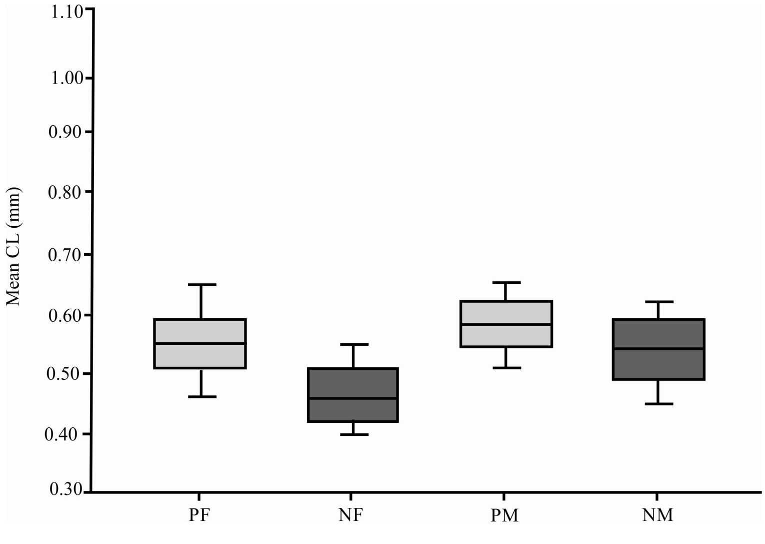


Figure 1 Body size of paired and non-paired females and males of *Hyalella longistila* from a stream on southeast of the state of Minas Gerais, Brazil. Vertical bars indicate the standard deviation. PF: paired females; NF: non-paired females; PM: paired males; NM: non-paired males.

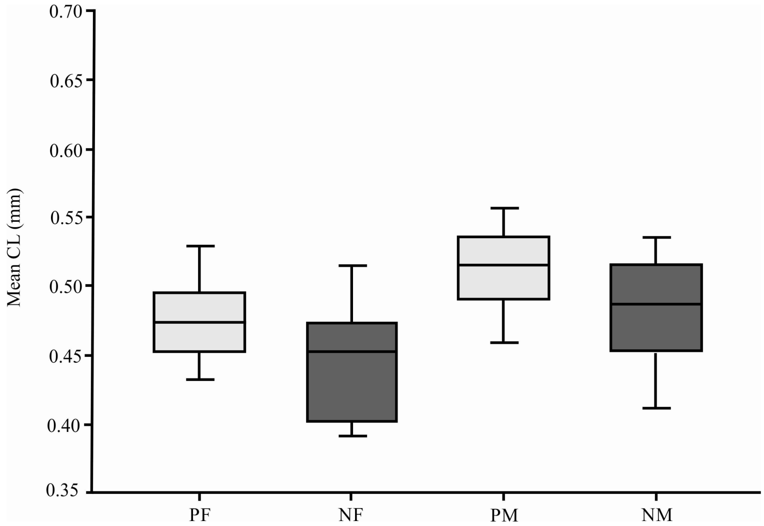


Figure 2 Body size of paired and non-paired females and males of *Hyalella carstica* from an intermittent stream on Midwest of the state of Minas Gerais, Brazil. Vertical bars indicate the standard deviation. PF: paired females; NF: non-paired females; PM: paired males; NM: non-paired males.

The mean fecundity of *H. longistila* was 12.88 ± 2.00 eggs per female, with peaks on August/12 and September/12, with 16.82 and 15.08 eggs per female, respectively. The mean fecundity of *H. carstica* was similar (12.60 ± 7.20 eggs per female), and considering monthly, fecundity was higher on months after the dry period.

Eggs in different stages were found in the same month for both species, indicating that reproduction is not synchronized among females. Significant differences were observed in relation to the number of eggs found on the marsupium of *H. longistila* females when considering different developmental stages (ANOVA + Bonferroni, F = 7.19, p = 0.0003). Significant differences were not found when comparing the number of eggs of *H. carstica* on different stages.

The developmental stage and the number of eggs found on the brood pouch were correlated on females of *H. longistila* (Spearman correlation, r= - 0.27, t = - 4.99, p<0.001), indicating that as the embryo developed, less eggs remained on the marsupium. Contrarily, the developmental stage and number of eggs per *H. carstica* female were not correlated (Spearman correlation, r = -0.22, t = - 1.55, p = 0.13), suggesting that there not a clear relationship between the number of embryos and their stage.

There were significant differences also among the volume of eggs in different stages for *H. longistila* (ANOVA + Bonferroni, F = 7828.92, p<0.0001). The stage of eggs was positively correlated with their volume on eggs this species (Pearson correlation; r = 0.79, p<0.0001, t=40.91, what suggest that eggs increase in volume as they develop. This correlation also was observed for eggs of *H. carstica* females (Pearson correlation; r = 0.24, p<0.0001, t=5.77). In this species there were also significant differences among the volume of eggs in different stages (ANOVA + Bonferroni, F = 18.11, p<0.0001).

The mean volume of eggs on the first stage did not present significant differences among females of the two studied species, but stages 2 and 3 differed significantly. Detailed data are presented on Table 1.

Table1 Mean volume of eggs on different development stages of *H. longistila* and *H. carstica* and results of t test comparing the two studied species.

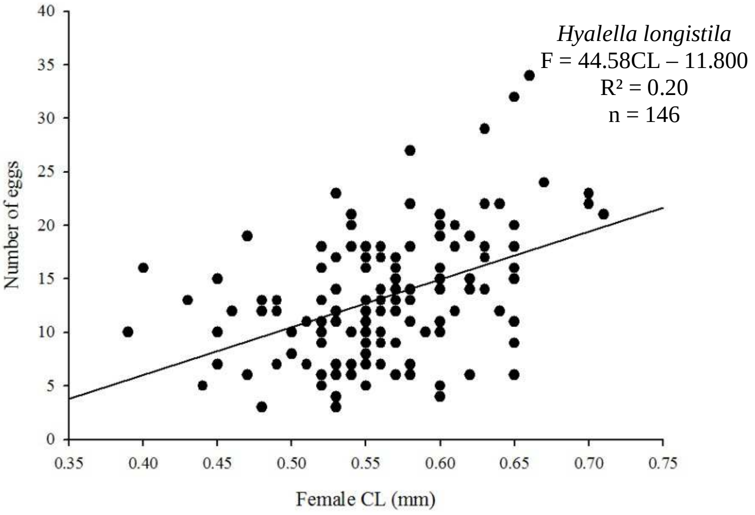
	Mean volume		**t test**	
	H. longistila	***H. carstica***	**t value**	**p value**
Stage 1	0.09	0.09	0.15	0.4416
Stage 2	0.12	0.10	-6.60	0.0001
Stage 3	0.17	0.14	-3.88	0.0001

The width of marsupium did not have a significant effect on their fecundity, while CL and TL was positively related to the increase of eggs produced per female. Since CL and TL were correlated, these two variables could not be included together on the model and both could be chosen as the predictive variable. Thus, CL was considered a representative metric of the female size for estimating fecundity for the greater facility with which such measurement can be obtained. Detailed data are presented on Table 2, and the relation between such variables can be observed on Figure 3.

Table 2 Results of the general linear models for predicting the number of eggs in relation to the female body size of *Hyalella longistila* and *H. carstica.*

	Estimate		**SE**		**t value**		**p value**	
	Hl	***Hc***	***Hl***	***Hc***	***Hl***	***Hc***	***Hl***	***Hc***
Intercept	-11.80	-14.67	4.15	10.51	-2.85	-1.40	0.005	0.01
CL	44.58	42.57	7.35	21.35	6.07	1.99	1.09×10^{-8}	0.04

CL: cephalothorax length; *Hl*: *Hyalella longistila; Hc*: *H.carstica*



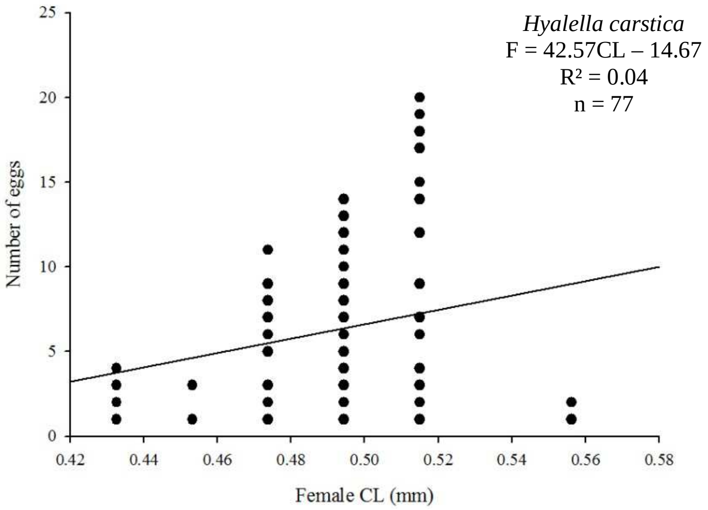


Figure 3 Relationship between fecundity (F) and the female size (cephalothorax length - CL in mm). The egg production was estimated based only on eggs on the first developmental stage of *H. longistila* and *H. carstica.*

The frequency of ovigerous females of *H. longistila* was higher from June/12 to October/12, and adults found in precopulatory behavior were more frequent from July/12 to November/12. On the *H. carstica* population, ovigerous females were more frequent on January/11 and February/11, and the higher frequency of couples occurred on February/11 and April/11, both after the dry period.

Regarding pairing success of *H. longistila* males, higher values were observed for size classes between 0.60 and 0.65 mm. Both sexes increased reproductive success as body size increased, but males presented a peak between 0.60 to 0.65 mm of CL, while females presented a progressive increase.

The reproductive success of largest females was two times higher when comparing with the success of the smallest paired females. The higher pairing success of *H. carstica* was observed for males with CL ranging from 0.48 – 0.49 and 0.56 – 0.57. The reproductive success of both sexes on this species presented a peak on intermediate body sizes, in which reproductive success was until fivefold the success of the smallest and the largest paired adults (Figure 4).

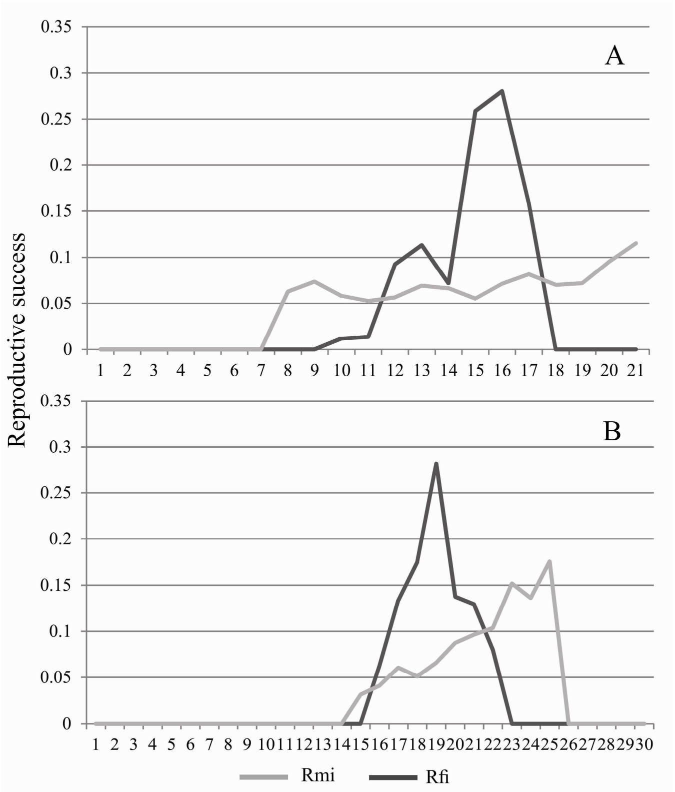


Figure 4 Reproductive success of paired males (Rmi) and females (Rfi) of *Hyalella longistila* (A) and *H. carstica* (B) expressed as a function of body size (represented by cephalothorax length – CL – divided in size classes – i). Numbers on horizontal axis represent the size classes, which in (A) ranged from 0.18 to 0.78 mm (with class amplitude of 0.3 mm), and in (B) ranged from 0.12 to 0.70 mm (with amplitude of 0.2 mm).

4 DISCUSSION

We have observed that both for *H. longistila* and *H. carstica* the mean and maximum CL of paired males and females did not correspond to the minimum and maximum sizes of adults of these populations, what can be confirmed observing such values on the previous chapter. It suggests that adults of intermediate sizes are reproducing more intensively, while smaller individuals also reproduce, since ovigerous females smaller than the smallest females found on precopulatory behavior were collected. Subida, Cunha and Moreira (2005) observed that brood size decreased drastically for the largest females of *Gammarus chevreuxi* due to senescence consequences. The low frequency of even absence of paired adults on the larger size classes of the present study probably has the same cause.

Bacela, Konopacka and Grabowski (2009) suggested the calculus of a maturity index given by the ratio between the minimum and the mean size of ovigerous females to evaluate when they reach maturity effectively. According to this ratio, both *H. longistila* (0.68) and *H. carstica* (0.76) reached maturity at young age, it means, most of females reproduce as soon as they became adults. Subida, Cunha and Moreira (2005) also observed that females of *Gammarus chevreuxi* were mature at small sizes, and such authors stated that young (small) females usually do not present optimized reproductive potential, their embryos use to be small irrespective of the female physiological condition. Reproducing at small sizes may be important especially if there is size-selective predation or specific parasites. If larger individuals are more susceptible, the presence of small adults reproducing will ensure the persistence of the population (APPADOO; MYERS, 2004).

Increases on fecundity as body size improves is ubiquitous among crustaceans (HARTNOLL, 1985), thus suggesting that such reproductive parameter may be constrained by allometry of physiological relationships (WELLBORN, 1995). The mean fecundity observed for *H. longistila* and *H. carstica* was similar, although they presented different body sizes. *Hyalella longistila* ovigeorus females were larger than *H. carstica*, then it was expected that the former presented higher fecundity. But instead, as eggs of *H. longistila* develop they attain larger volumes than *H. carstica*, what probably justifies the similarity between the fecundity of the studied species despite their differences in size.

Variations on embryo volume have been reported for several amphipods (VAN DOLAH; BIRD, 1980; KOLDING; FENCHEL, 1981; SHEADER, 1983). Non–significant differences were found between the volume of eggs on stage 1 between the currently studied species, but significant differences were found when comparing the volume of eggs on stage 2 and 3. The larger size of *H. longistila* eggs culminates on larger juveniles at hatching (personal observation) and adults grow until larger sizes, what corroborated observing the maximum total body length attained by both species (detailed information are available on the previous chapter).

Steele and Steele (1991) affirmed that tropical and subtropical species in general present small body sizes and produce small eggs, eventually presenting low fecundity as a consequence of their small body size. But the low fecundity is compensated by the short intervals among broods and their multivoltine life cycle, what allows a geometric increase on population size. Cunha et al. (2000) suggest that if the minimum size of incubating females is half the maximum size of females, this culminates on the occurrence of several molts during adult phase, what enables several consecutive broods. It consists on an indicative of iteroparous species, as well as Appadoo and Myers (2004) observed for *Cymadusa filosa* and *Ampithoe valida* and we have observed for *H. longistila and H. carstica.*

Ovigerous females of *H. longistila* were collected during all months, as well as *H. carstica*, except during dry months and on March/11. Furthermore, it was observed that reproduction was asynchronous among females of the same population for both species. Subida, Cunha and Moreira (2005) also verified such scenario, suggesting the existence of a consistent and high female reproductive effort.

Most of the females were found carrying eggs on the first developmental stage, and some females contain more than one stage inside its marsupium. Kuris (1991) stated that embryogenesis is synchronous among amphipods. Then, the different stages observed in the same female probably is related to eggs that were not developing but female had not eliminated them yet. But this low frequency of more developed eggs may also have resulted from the migration of females to more secure places in the stream, because the females are more vulnerable to predation when the juveniles are hatching, as suggested by Castiglioni and Bond-Buckup (2009).

The low percentage of females carrying more developed eggs, or even juveniles, indicates their low residence time and low or absence of maternal care. As soon eggs developed, less time each stage lasts. The combination of such factors enables a rapid brood succession (SUBIDA; CUNHA, MOREIRA, 2005). According to Sainte-Marie (1991), gammarideans present one of the highest reproductive potential among amphipods, probably as a result of rapid succession of consecutive broods.

Comparing the number of eggs among stages, significant differences were observed only for *H. longistila*, for which a negative correlation was recorded between these two variables. This was expected since, as eggs develop the available space decreases, what causes the premature loss of some eggs along the incubation period (KOCH, 1990). For *H. carstica* neither differences among the number of eggs in different stages nor correlations were observed. This in part may be explained by some females that oviposit even if copulation has not occurred, and therefore their eggs do not develop. Such unfertilized eggs generally are released from the brood pouch within few days (BOROWSKY, 1991), but if such females had already lost some eggs and after were included accidentally among females with embryos this may have prejudiced results.

Although a positive relation was observed between the fecundity and size of both *H. longistila* and *H. carstica* females, the determination coefficients were not high when compared, for example, with results of Appadoo and Myers (2004). They found more than 50% of explanation of the variance observed among the number of eggs produced per female according to their size. Several other studies have reported the relationship between body size and egg production (MOORE, 1981; SHEADER, 1981; STEELE; STEELE, 1991; CUNHA et al., 2000; MARANHÃO; MARQUES, 2003).

Castiglioni and Bond-Buckup (2009) studied Brazilian species of *Hyalella* and even though *H. castroi* is larger than *H. pleoacuta*, authors observed lower production of eggs by the former species. But it was observed that the lower fecundity was compensated by the production of larger eggs. A trade-off usually is observed: increasing fecundity (in terms of number of eggs) or increasing the juvenile survival (producing less large eggs) (KOLDING; FENCHEL, 1981). Increases on egg size causing the decrease in fecundity was also observed on other aquatic animal besides amphipods (STEELE; STEELE, 1981), like crayfish (ABRAHAMSON, 1971) and fish (WARE, 1975)

This was also observed by Wellborn and Cothran (2004) for other sympatric species of *Hyalella*, but from Michigan, USA. When comparing the number of eggs produced by *H. longistila, H. carstica, H. castroi* and *H. pleoacuta*, the mean fecundity of the sympatric species is three-fold superior, and these eggs were also larger when compared to the species of the present study. Although all of such them are Brazilian subtropical species, their habitats are quite different.

Hyalella castroi and *H. pleoacuta* inhabits artificial ponds build in order to raise trout. Thus, since fishes are fed continuously, there is no limitation of food resource along the year. *Hyalella longistila* lives on a natural stream where apparently there is a continuous availability of food, since the population was found in a large macrophyte stand where there are abundant organic matter and detritus on the bottom. However, *H. carstica* inhabits an intermittent stream where although macrophytes are present during a period of the year, amphipods have to deal with a more unstable environment. Despite such speculations, further researches are necessary to justify with accuracy the differences regarding fecundity based on the availability of food resources.

It is important to mention that *H. castroi* and *H. pleoacuta* are sympatric species that live on ponds that raise trout. It is known that fishes are size-selective predators of amphipods (WELLBORN, 1995; 2011). Sastry (1983) affirmed that large eggs produce larger juveniles which present more competitive ability to feed and survive. High fecundity or the production of large eggs may be related to a great availability of food or may represent an attempt to optimize juveniles survival, given the high risks to which they are submitted (CASTIGLIONI; BOND-BUCKUP, 2007; 2008; 2009). Since the ichthyofauna was not assessed in the present study, it is not possible to state if *H. longistila* is subject to predation by fish. But it was observed a low frequency of juveniles throughout the year (except on October/12), so it is possible that they had been preyed by large invertebrates like nymphs of odonates. The direction and degree of size selectivity depends on odonate size, but Wellborn (1994) have already recorded substantial mortality rates on *Hyalella* populations caused by such invertebrates.

Only *H. longistila* exhibited significant positive assortative mating, what was indicated by the correlation observed between the size of paired males and females. This is common among amphipods, and there are several hypothesis that attempt to explain such correlation (PLAISTOW; BOLLACHE; CÉZILLY, 2003; WELLBORN; BARTHOLF, 2005; CASTIGLIONI; BOND-BUCKUP, 2008b). The much larger the male is, the less likely it is to lose their mates during conflicts with other males that attempt to take over the guarded females, or even to support the female during the whole pre-copulatory period. This consists on the male-male competition hypothesis, which states that larger males are better competitors in this sense (WARD 1983), hypothesis and seems to be a probable cause of the observed correlation between paired males and females of *H. longistila*. Furthermore Wen (1992) had pointed that larger females could enhance the male reproductive success due to their higher fecundity, what have favored the paring males with larger females. Castiglioni and Bond-Buckup (2009) had also observed such size-assortative mating for *H. castroi* and *H. pleoacuta,* while Wellborn (1995) observed it for a population of *H. azteca.*

Size-assortative mating was not observed for *H. carstica,* since the correlation between the size of paired males and females was not significant, what means that both large and small males paired with any female, with no clear pattern between their sizes. *Hyalella carstica* males did not seem to be selective when guarding females, what may have been caused by the hydric regime of the stream where they inhabit. The breeding activity of such species in this place is more intense just after the dry months. As suggested in the second chapter of this work, adults (including ovigerous females) came from upstream populations and are responsible for the recolonization of the local population. During this intense reproductive period, male probably do not choose females, pairing randomly with any female it found, irrespective to their sizes. But since males have to carry the female until the molt, what may last from hours to days, some loading constraints may exist (ADAMS; GREENWOOD, 1983). Othman and Pascoe (2001) also did not observe a size-assortative pattern, but in the population studied by them larger females were always elected, despite the male size, probably to optimize the male reproductive success.

Paired adults were larger, in means, than non-paired, what was also observed for *H. castroi* and *H. pleoacuta* (CASTILIONI; BOD-BUCKUP, 2009), *Gammarus pulex* (WARD, 1983), and *H. azteca* (WELLBORN, 1995;

WELLBORN; BARTHOLF, 2005). Such differences on size may be explained by the same reasons that lead larger males to pair with larger females. Larger males are better competitors both when fighting against another male for a female, and when defending the couple from take-over attempts by other unpaired males (WARD, 1983; ELWOOD; DICK, 1990), while larger females tend to be more fecund (WEN, 1992).

As well as Wellborn (1995) observed for *H. azteca*, the pairing and reproductive success of *H. longistila* and *H. carstica* was greater on intermediate size classes. The males reproductive success is determined both by the frequency of paired males of a given size and the expected fecundity of the female with which it is paired. Thus, probably there is competition for larger and more fecund females, which may occur by interference or exploitation. As large males are, greater is the competition among themselves (FRANCESCHI et al., 2010), especially on *H. longistila* and *H. carstica* in which populations sex ratio was skewed to male as could be noted on the previous chapter.

Pairing success on females does not depend on body size, since females of all sizes can mate, as observed for *H. longstila* in the present study and for *Gammarus pulex* by Ward (1983). Moreover, male availability does not limit the female reproductive success even it is low. Their reproductive success is determined only by the female fecundity, since one male can fecundate several females in the same reproductive season, which once fecundated will incubate their eggs and ensure their reproductive success. The observed increase on reproductive success of *H. longistila* may be related to their increasing fecundity as larger they are, and also because of the males preferences of larger females on this population. However, *H. carstica* presented a different pattern, with greater reproductive success for intermediate size classes. It is necessary to consider that a female success when reproducing is not restrict to her size and/or fecundity, but also is related to how neat to the next molt it is (ELWOOD; DICK, 1990), in which females closer to molt are more likely to be guarded (DUNHAM; ALEXANDER; HURSHMAN, 1989). The frequency with which individuals molt decrease with their development, thus the largest females molt less than intermediate ones and this may increase their probability to be taken by a male. Furthermore, the largest females may pair with fewer males, given the loading constraints, while smaller females may pair with a higher proportion of males.

As already mentioned by Sastry (1983), it is still necessary to understand better how populations interact with the environment, how individuals integrate their functions and behaviors to their habitat, and what the adaptive value of such responses to the reproductive success of the population is. The knowledge regarding such point may allow the better comprehension of the evolution of life histories and reproductive strategies in different environments. And this is fundamental, especially for *Hyalella* species, since most of the studies regarding their ecology only were developed on temperate regions.

Castiglioni and Bond-Buckup (2009) pointed that each species is able to develop a specific reproductive strategy depending on the conditions to which is submitted (physical or biological conditions). In the present study the differences observed on the reproductive strategies of these two *Hyalella* species probably may be attributed to the distinct hydric regimes to which they are submitted. The knowledge regarding such issues subsidize future studies, which are promising since amphipods present great potential to be used as model organisms for applied studies, either as bioindicators, as experimental organisms for ecotoxicological tests, or even on other studies that elucidate some unknown ecological functionality, besides the link they represent between lower and higher levels on trophic webs.

REFERENCES

ABRAHAMSSON, S. A. A. Density, growth and reproduction in populations of *Astacus astacus* and *Pacifastacus leniusculus* in an isolated pond. **Oikos,** Buenos Aires, v. 22, p. 373–380, 1971.

ADAMS, J.; GREENWOOD, P. J. Why are males bigger than females in pre-copula pairs of *Gammarus pulex*? **Behavioral Ecology and Sociobiology**, New York, v. 13, p.239-241, 1983.

APPADOO, C.; MYERS, A. A. Reproductive bionomics and life history traits of three gammaridean amphipods, *Cymadusa azteca* Savigny, *Ampithoe laxipodus* Appadoo and Myers and *Mallacoota schellenbergi* Ledoyer from tropical Indian Ocean (Mauritius). **Acta Oecologica,** Paris, v. 26, p. 227-238, 2004.

BACELA, K.; KONOPACKA, A.; GRABOWSKI, M. Reproductive biology of *Dikerogammarus haemobaphes*: an invasive gammarid (Crustacea: Amphipoda) colonizing running waters in Central Europe. **Biological Invasions,** v. 11, p. 2055–2066, 2009.

BORGMANN, U.; RALPH, K. M.; NORWOOD, W. P. Toxicity test procedures for *Hyalella 106zteca* and chronic toxicity of cadmium and pentachlorophenol to *Hyalella 106zteca*, *Gammarus fasciatus* and *Daphnia magna*. **Bulletin of Environmental Contamination and Toxicology**, New York, v. 18, p. 756–764, 1989.

BOROWSKY, B. Patterns of reproduction of some amphipod crustaceans and insights into the nature of their stimuli,. In: BAUER, R.T. & W. MARTIN (Eds). **Journal of Crustacean Sexual Biology**. New York, 1991. P. 33-66.

CALOW, P. The cost of reproduction – a physiological approach. **Biological Review**, Cambridge, v. 54, p. 23-40, 1979.

CASTIGLIONI, D. S.; BOND-BUCKUP, G. Reproductive strategies of two sympatric species of *Hyalella* Smith, 1874 (Amphipoda, Dogielinotidae) in laboratory conditions. **Journal of Natural History**, London, v. 41, n. 25/28, p. 1571-1584, 2007.

CASTIGLIONI, D. S.; BOND-BUCKUP, G. Pairing and reproductive success in two sympatric species of *Hyalella* (Crustacea, Amphipoda, Dogielinotidae) from southern Brazil. **Acta Oecologica**, Paris, v. 33, n. 1, p. 49-55, 2008.

CASTIGLIONI, D. S.; BOND-BUCKUP, G. Egg production of two sympatric species of *Hyalella* Smith , 1874 (Crustacea, Amphipoda, Dogielinotidae) in aquaculture ponds in southern Brazil. **Journal of Natural History**, London, v. 43, p. 1273–1289, 2009.

COOPER, W. E. Dynamics and production of a natural population of a freshwater amphipod, *Hyalella azteca*. **Ecological Monographs**, Lawrence, v. 35, p. 377–394, 1965.

CUNHA, M. R.; SORBE, J. C.; MOREIRA, M. H. The amphipod *Corophium multisetosum* (Corophiidae) in Ria de Aveiro (NW Portugal). I. Life history and aspects of reproductive biology. **Marine Biology,** Berlin, v. 137, p. 637–650, 2000.

DUNHAM, P. J.; ALEXANDER, T.; HURSHMAN, A. Precopulatory mate guardingin the amphipod, *Gammarus lawrencianus* Bousfield. **Animal Behaviour**, London, v.34, p. 1680–1686, 1989.

ELWOOD, R.W.; DICK, J.T.A. The amorous *Gammarus*: the relationship between precopula duration and size-assortative mating in *G. pulex*. **Animal Behaviour**, London, v. 39, p. 828-833, 1990.

FRANCESCHI, N.; LEMAÎTRE, J.F.; CÉZILLY, F.; BOLLACHE, L. Size-assortative pairing in *Gammarus pulex* (Crustacea: Amphipoda): a test of the prudent choice hypothesis. **Animal Behaviour,** London, v. 79, p. 911–916, 2010.

GONZÁLEZ-GURRIARÁN, E. Reproductión de la nécora *Macropipus puber* (L) (Decapoda, Brachyura), y ciclo reproductivo em la Ría de Arousa (Galicia, NW España). **Boletín del Instituto Español de Oceanografia**, Madrid, v. 2, n. 1, p. 10-32, 1985.

HARTNOLL, R.G. Growth. In: BLISS, D.E. (Ed.). **The biology of Crustacea:, morphology and genetics.** London Academic Press, London, 1982, p. 111-196.

HYNES, H. B. N. The reproductive cycle of some British freshwater Gammaridae. **Journal of Animal Ecology,** Oxford, v. 24, n. 2, p. 352 – 387, 1955.

KOCH, H. Aspects of the population biology of *Traskorchestia traskiana* (Stimpson, 1857) (Amphipoda, Talitridae) in the Pacific Northwest, USA. **Crustaceana**, Leiden, v. 59, p. 35–52, 1990.

KOLDING, S.; FENCHEL, T. M. Patterns of reproduction in different populations of five species of the amphipod genus *Gammarus*. **Oikos**, Buenos Aires, v. 37, p. 167–172, 1981.

KRUSCHWITZ, L. G. Environmental factors controlling reproduction of the amphipod *Hyalella azteca*. **Proceedings of the Oklahoma Academy of Science**, Oklahoma, v. 21, p. 16–21, 1978.

KURIS AM. A review of patterns and causes of crustacean brood mortality. In: Wenner A, Kuris A., editors. **Crustacean Issues** 7 – Crustacean Egg Production. Rotterdam: A. A. Balkema; p. 117–141, 1991.

MANLY, B.F.J. A model for certain types of selection experiments. **Biometrics,** Washington, v. 30, p. 281- 294, 1974.

MARANHÃO, P., BENGALA, N., PARDAL, M. & MARQUES, J. C. The influence of environmental factors on the population dynamics, reproductive biology and productivity of *Echinogammarus marinus* Leach (Amphipoda, Gammaridae) in the Mondego estuary (Portugal). **Acta Oecologica,** Paris, v. 22, p. 139–152, 2001.

MARANHÃO, P.; MARQUES, J. C. The influence of temperature and salinity on the duration of embryonic development, fecundity and growth of the amphipod *Echinogammarus marinus* Leach (Gammaridae). **Acta Oecologica**, Paris, v. 24, p. 5-13, 2003.

MOORE, P.G. The life histories of the amphipods *Lembos websteri* Bate and *Corophium bonnellii* Milne Edwards in kelp holdfasts. **Journal of Experimental Marine Biology and Ecology**, v. 49, p. 1–50, 1981

NELSON, M. K.; BRUNSON, E. L. Postembryonic growth and development of *Hyalella 108zteca* in laboratory cultures and contaminated sediments. **Chemosphere, v.** 31, n. 4, p. 3129–3140, 1995.

OTHMAN, M. S.; PASCOE, D. Growth, development and reproduction of *Hyalella azteca* (Saussure, 1858) in laboratory culture. **Crustaceana,** Leiden, v. 74, n. 2, p. 171-181, 2001.

PILGRIM, W.; BURT, M. D. B. Effect of acute pH depression on the survival of the freshwater amphipod *Hyalella azteca* at variable temperatures: field and laboratory studies. **Hydrobiologia**, The Hague, v. 254, p. 91-98, 1993.

PLAISTOW, S. J.; BOLLACHE, L.; CÉZILLY, F. Energetically costly precopulatory mate guarding in the amphipod *Gammarus pulex*: causes and consequences. **Animal Behaviour ,** v.65, p. 683–691, 2003.

R CORE TEAM R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/. 2013.

RINDERHAGEN, M.; RITTERHOFF, J.; ZAUKE, G. P. Crustaceans as bioindicators. In: Geerhardt A, editor. Biomonitoring of polluted water—reviews on actual topics. Zurich: Trans Tech Publications/Scitech Publications. P 161–194, 2000.

RUPPERT, E. E.; BARNES, R. D. **Zoologia dos invertebrados**. 6ª edição. Editora Rocca Ltda., São Paulo, 1996. 1097p.

SAINTE-MARIE, B. A review of the reproductive bionomics of aquatic gammaridean amphipods: variation of life history traits with latitude, depth, salinity and superfamily. **Hydrobiologia,** The Hague, v. 223, p. 189–227, 1991.

SASTRY, A. N. Ecological aspects of reproduction. In Vernberg, F. J.and Vernberg, W. B. (Ed.). **The Biology of Crustacea**: Environmental adaptations, Academic Press, New York, v. 8, pp. 179-269. 1983.

SHEADER, M. The reproductive biology and ecology of *Gammarus duebeni* (Crustacea: Amphipoda) in southern England. **Journal of Marine Biology Association**, v. 63, p. 517–540, 1983.

STEELE, D.H.; STEELE, V. J. Morphological and environmental restraints on egg production in amphipods. In: WENNER, A.; KURIS, A. (Eds.). **Crustacean Egg production – Crustacean Issues,** v. 7. A. A. Balkema, Rotterdam, 1991. P. 157-170.

STRONG, D. R. Life history variation among populations of an amphipod (*Hyalella azteca*). **Ecology**, v. 53, p. 1103–1111, 1972.

SUBIDA, M. D.; CUNHA, M, R.; MOREIRA, M. H. Life history, reproduction and production of *Gammarus chevreuxi* (Amphipoda: Gammaridae) in the Ria de Aveiro, northwestern Portugal. **Journal of the North American Benthological Society,** v. 24, p. 82–100, 2005.

VAN DOLAH, R.F.; BIRD, E. A comparison of reproductive patterns in epifaunal and infaunal gammaridean amphipods. **Estuarine and Coastal Marine Science**, London, v. 2, p. 593-604, 1980.

WARE, D. M. Relation between egg size, growth and natural mortality of larval fish. **Journal of Fisheries Research Board of Canada,** v. 32, p. 2503–2512, 1975.

WELLBORN, G. A. Size-Biased Predation and Prey Life Histories□: A Comparative Study of Freshwater Amphipod Populations. **Ecology ,** v.75, p. 2104–2117, 1994.

WELLBORN, G. A. Determinants of reproductive success in freshwater amphipod species that experience different mortality regimes. **Animal Behavior,** v. 50, p. 353−363, 1995.

WELLBORN, G. A. The Mechanistic Basis of Body Size Differences Between Two *Hyalella* (Amphipoda) Species. **Journal of Freshwater Ecology ,** v.9, p. 159–168, 2011.

WELLBORN, G.A.; BARTHOLF, S.E.; Ecological context and the importance of body and gnathopod size for pairing success in two amphipods. **Oecologia**, v. 143, p. 308-316, 2005.

WELLBORN, G. A.; COTHRAN, R. D. Phenotypic similarity and differentiation among sympatric cryptic species in a freshwater amphipod species complex. **Freshwater Biology**, Oxford, v. 49, p. 1–13, 2004.

WEN, Y. H. Life history and production of *Hyalella azteca* (Crustacea, Amphipoda) in a hypereutrophic prairie pond in southern Alberta. **Canadian Journal of Zoology**, Ottawa, v. 70, p. 1417-1424, 1992.

CONSIDERAÇÕES FINAIS

Sabe-se da relativa facilidade com que anfípodos são coletados e cultivados, sua sensibilidade a contaminantes e seu curto ciclo de vida, atributos que favorecem seu uso como bioindicadores de qualidade de água e organismos teste em experimentos de toxicologia. Até o presente conhecia-se para *Hyalella longistila* e *H. carstica* apenas a descrição taxonômica e registros de ocorrência. O conhecimento da ecologia e biologia das espécies possibilita seu uso em estudos de cunho aplicado, assim os resultados do presente trabalho constituem informação de base para pesquisas envolvendo tais anfípodos como organismos experimentais.

O cultivo de *H. carstica* foi bem sucedido em laboratório, e os detalhes deste estudo estão em fase de elaboração para publicação. Todavia, tentativas de cultivo de *H. longistila* também foram feitas e os procedimentos adotados foram os mesmos que os utilizados para *H. carstica*. No entanto,, embora alguns organismos tenham sobrevivido e até reproduzido, isto ocorreu com baixa frequência e os juvenis nascidos no laboratório não sobreviveram por longo prazo. Diversas estratégias foram adotadas a fim de descobrir as melhores condições que permitissem a manutenção desta espécie em laboratório, mas não obtivemos sucesso com tais tentativas. Possivelmente, *H. longistila* apresenta maior sensibilidade a variações no ambiente quando comparada à *H. carstica* que naturalmente está submetida a condições ambientais mais instáveis. Caso esta maior sensibilidade seja comprovada, *H. longisila* pode representar um bioindicador de boa qualidade do ambiente. No entanto, mais estudos ainda são necessários para elucidar a tolerância desta espécie a distúrbios no ambiente e assim de fato corroborar tal possibilidade.

Observou-se que o regime hídrico local parece ter influência significativa sobre a dinâmica populacional de *H. carstica.* Como mencionado no presente trabalho, acreditamos que ocorra um processo de recolonização neste córrego com indivíduos provenientes de centros de abundância populacional à montante. Esta possibilidade ainda deve ser mais bem investigada acompanhando a dinâmica da população em longo prazo no campo. Além disso, em laboratório estes crustáceos podem ser cultivados para testar sua capacidade de estabelecimento de uma nova população ou restabelecimento desta, tamanhos populacionais mínimos viáveis para a permanência da população, efeito da presença de predadores, do aumento da densidade populacional e consequente aumento da competição por recursos, alterações na razão sexual e as consequências para o sucesso reprodutivo dos indivíduos, entre inúmeros outros estudos que ainda podem ser desenvolvidos.

Hyalella longistila aparentemente está sujeita a condições mais constantes no ambiente no que diz respeito ao volume de água disponível ao longo do ano. Possivelmente as variações observadas na dinâmica populacional e nas estratégias reprodutivas adotadas estão relacionadas às interações bióticas as quais não foram mensuradas neste trabalho para que tal afirmação seja feita com maior acurácia. Em campo a presença de alguns peixes e invertebrados maiores foi observada, os quais são retratados na literatura como potenciais predadores de *Hyalella*. Estes predadores podem influenciar desde o tamanho corporal até as estratégias adotadas pelos anfípodos para otimizar a reprodução e sobrevivência, portanto, estudos para elucidar tais questões ainda podem e precisam ser desenvolvidos.

Embora o conhecimento sobre as espécies brasileiras de *Hyalella* ainda seja incipiente, pesquisadores da área tem se dedicado para que o grupo seja mais bem compreendido quanto à sua origem, identificação das espécies, biologia molecular e distribuição do gênero no país. Os estudos para o presente trabalho vem agregar conhecimento à ecologia e biologia reprodutiva de algumas espécies deste gênero e fornece subsídio para que estudos posteriores sejam desenvolvidos, permitindo então, compreender melhor os mecanismos que atuam sobre a diversidade, distribuição e abundância do gênero, assim também como sobre as relações entre os organismos e as condições do ambiente em que vivem.