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Desempenho alimentar de *Balloniscus sellowii* (Brandt, 1833) (Crustacea: Isopoda: Oniscidea) em folhas de diferentes estágios de decomposição e sua relação com o conteúdo de fenólicos e de flavonoides

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Monografia apresentada à banca examinadora do Instituto de Biociências, Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do grau de Bacharel em Ciências Biológicas.
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SUMÁRIO

APRESENTAÇÃO

Os isópodos terrestres (Isopoda: Oniscidea), comumente conhecidos como “tatuzinhos”, são crustáceos que obtiveram grande sucesso na colonização do ambiente terrestre. Fazem parte da macrofauna de solo e estão fortemente envolvidos nos processos de detritivoria (Hassall et al., 1987; Sousa et al., 1998; Zimmer & Topp, 1999; Kautz & Topp, 2000). Habitam grande variedade de ambientes, sendo um dos grupos de detritívoros mais abundantes em diversas comunidades (Hassall & Dangerfield, 1990; Zimmer, 2002a). São de fácil captura e cultivo em laboratório, característica que os fazem ótimos modelos para um maior entendimento da dinâmica da formação do solo.

Nessa transição para o ambiente terrestre, esse grupo necessitou de adaptações às fontes de alimentação. As espécies de plantas terrestres possuem maior quantidade de lignina e celulose e de compostos secundários, sendo necessárias, diversas enzimas para a quebra de compostos complexos (Zimmer, 2002b). Entre as estratégias utilizadas por esses animais estão a aquisição de bactérias endossimbiontes que contribuem para a digestão de lignina e celulose (Zimmer & Topp, 1998; Zimmer et al. 2001; Zimmer, 2006) e o comportamento coprofágico para um melhor aproveitamento nutricional da fonte de alimento ingerida primariamente (Hassall & Rushton, 1982; Gunnarson & Tunid, 1986; Szlávecz & Pobozny, 1995).

Por serem detritívoros, eles servem de ligação entre produtores primários e níveis tróficos mais altos, contribuindo nos processos de decomposição por consumir grandes quantidades de serapiheira e por promover atividade microbiana (Zimmer, 2002b). Eles retornam grande quantidade de matéria na forma de fezes, o que contribui para a formação do húmus do solo e para o crescimento das florestas

(Knoepp et al., 2000; Förster et al., 2006) e aumentam a qualidade do solo (Kautz & Topp, 2000). Hassall et al. (1987), combinando dados de pesquisadores, sugeriram que cerca de 80,8% da serapilheira seria consumida pela fauna de solo.

Oniscidea inclui aproximadamente 3600 espécies de tamanho pequeno a médio (1,2-30mm) distribuídas por todo o mundo (Schmalfuss, 2003). Para o Brasil, cerca de 120 espécies são registradas (Leistikow & Wägele, 1999), sendo o gênero *Balloniscus* Budde-Lund, 1908 representado por *B. glaber* Araujo & Zardo, 1995 e *B. sellowii* (Brandt, 1833), ambas as espécies nativas.

Estudos sobre nutrição de invertebrados têm sido realizados em sua maioria com insetos (Appel, 1993; Harborne, 1993). Os estudos com isópodos, realizados na maioria na Europa (Gunnarsson, 1987; Szlávecz, 1992, 1993, Szlávecz & Pobozny, 1995, Rushton & Hassall, 1983a,b; Souza et al., 1998; Zimmer, 2002, Ihnen & Zimmer, 2008), incluem espécies de *Porcellio* Latreille, 1804 e *Armadillidium* Brandt 1833, as quais apresentam distribuição mundial. Estudos com espécies neotropicais são mais escassos, não obstante sua importância para o entendimento da dinâmica de solo, precisando estes, portanto, serem encorajados.

OBJETIVOS DO TRABALHO

Sabe-se que organismos detritívoros são de grande importância para a formação do solo. Para isso, esse trabalho foi realizado para contribuição ao conhecimento sobre a nutrição de isópodos terrestres.

Objetivos específicos:

- comparar o desempenho alimentar (taxas de consumo, de egestão e de assimilação) em folhas de diferentes estágios de decomposição;
- verificar a eficiência de assimilação dos animais nas diferentes folhas e o tempo de passagem do alimento pelo trato digestório;
- verificar o conteúdo de fenólicos totais e de flavonoides das folhas consumidas;
- estimar a quantidade de fenólicos totais e de flavonoides ingeridos pelos isópodos;
- relacionar o desempenho alimentar dos animais com o conteúdo de fenólicos totais e de flavonoides.

"FEEDING PERFORMANCE OF *BALLONISCUS SELLOWII* (ISOPODA: ONISCIDEA) IN LEAF LITTER OF DIFFERENT STAGES OF DECOMPOSITION AND ITS RELATION TO THE PHENOLIC AND FLAVONOID CONTENT"

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ABSTRACT

The goal of this study was to use a terrestrial isopod to compare feeding performance in leaves of different stages of decomposition according to their phenolic and flavonoid content. Leaves from the most visually abundant plants were offered to isopods collected in an urban area. The plant species that presented the highest consumption was used to verify feeding performance in different stages of decomposition. Green leaves were collected from branches and placed in litterbags, left to decompose, collected after one, two and three months. Then they were offered to animals as well as additional green leaves. The total phenolic and flavonoid content was determined for green leaves and other stages of decomposition. The consumption rate was significantly higher when animals fed on *Schinus terebinthifolius* and there was no significant difference between *Lithraea brasiliensis* and *Ricinus communis*. *Schinus terebinthifolius* was tested in four stages of decomposition, and the consumption and egestion rates were significantly higher on two months-old leaves. The assimilation rate was significantly highest in green leaves. There was a correlation between the consumption and egestion rates. The mode time of passage through gut was two hours for all treatments and the beginning of ingestion of leaves occurred after two or three days for green leaves, and in the same day for one, two and three months-old leaves. Assimilation efficiency was 43% for green leaves and about 20% for the other stages. The phenolic content was highest in green and lowest in the third month, and the flavonoid content was highest in green leaves and lowest in the second month. The estimated phenolic amount ingested by animals was significantly highest when consumption rate was also highest. The estimated flavonoid amount ingested followed the same trend as the assimilation rate, being significantly higher in green leaves. The mean flavonoid content of each decomposition stage was correlated with the assimilation efficiency. The highest consumption (optimal palatability) occurred on two months-old leaves and not when the phenolic content was lowest. The flavonoids represented a large portion of the total phenolics and the estimated amount of flavonoid consumed by animals was almost the same for leaves with one, two and three months of decomposition. It seems that animals increase consumption to maintain a minimum intake of the flavonoids in leaves with lower flavonoid content, suggesting that they might use these flavonoids as a food parameter.

KEY WORDS: leaf decomposition, Oniscidea, total phenolics, flavonoids.

RESUMO

O objetivo do trabalho foi utilizar um isópodo terrestre para verificar o desempenho alimentar de folhas em diferentes estágios de decomposição e a sua relação com o conteúdo de fenólicos e de flavonoides. Folhas das plantas mais abundantes do local foram oferecidas aos isópodos coletados em uma área urbana. A espécie de planta que obteve o maior consumo foi utilizada para verificar o desempenho alimentar em diferentes estágios de decomposição. Folhas verdes foram coletadas dos galhos e colocadas em *litter bags* que foram recolhidas após um, dois e três meses e oferecidas aos animais, assim como folhas verdes adicionais. O conteúdo de fenólicos totais e de flavonoides foi determinado para folhas verdes e em cada estágio de decomposição. A taxa de consumo foi significativamente maior quando os animais consumiram *Schinus terebinthifolius* e não houve diferença significativa entre *Lithraea brasiliensis* e *Ricinus communis*. *Schinus terebinthifolius* foi testada em quatro estágios de decomposição e as taxas de consumo e de egestão foram significativamente maiores em folhas de dois meses. A taxa de assimilação foi significativamente maior em folhas verdes. Houve correlação entre as taxas de consumo e de egestão. A moda do tempo de passagem pelo trato digestório foi de duas horas para todos os tratamentos e o início da ingestão das folhas ocorreu após dois ou três dias para folhas verdes, e no mesmo dia para folha de um, dois e três meses. A eficiência de assimilação foi de 43% para folhas verdes e em torno de 20% para os demais estágios. O conteúdo de fenólicos foi maior nas folhas verdes e menor no terceiro mês e o conteúdo de flavonoides foi maior nas folhas verdes e menor em folhas do segundo mês. A quantidade estimada de fenólico ingerido pelos animais foi significativamente maior quando o consumo foi maior. A quantidade estimada de flavonoides ingeridos apresentou o mesmo padrão das taxas de assimilação, sendo significativamente maior em folhas verdes. A média do conteúdo de flavonoide de cada mês foi correlacionada com a eficiência de assimilação. O maior consumo (palatabilidade ótima) ocorreu em folhas de dois meses e não quando o conteúdo de fenólicos era mais baixo. Os flavonoides representaram uma grande porção do conteúdo dos fenólicos totais e a quantidade estimada de flavonoides consumidos pelos animais foi quase a mesma com folhas de um, dois e três meses de decomposição. Parece que os animais aumentaram o consumo para ingerir uma quantidade mínima de flavonoides quando o conteúdo de flavonoides era mais baixo, sugerindo que eles possam utilizar os flavonoides como um parâmetro alimentar.

PALAVRAS CHAVE: decomposição de folhas, Oniscidea, fenóis totais, flavonoides.

INTRODUCTION

Litter dynamics is of great importance in the functioning of the ecosystems and it is influenced by many different organisms. Isopods, earthworms, lumbricids, diplopods, dipteran larvae and termites are detritivores of organic soil litter that play a major role in the cycling of nutrients. Detritivores have low assimilation efficiency (Szláveczs and Pobozny, 1995) contributing more indirectly to leaf litter decomposition by returning great amounts of the consumed litter as feces (Quadros and Araujo, 2008) which present increased surfaces that are readily colonized by microbial populations (Hassall et al., 1987, Loureiro et al., 2006).

Detritivores present feeding preference that has been related to leaf senescence (Wieser, 1984; Hassall et al., 1987; Yeates and Barmuta, 1999), nutrient content of food (Graça et al., 2001), microbial colonization (Gunnarsson, 1987; Kautz and Topp, 2000; Zimmer et al., 2003; Ihnen and Zimmer, 2008) and the presence of unpalatable or indigestible compounds (Target et al., 1986; Canhoto and Graça, 1999, Lambdon and Hassall, 2005). The isopods contribution to decomposition depends on leaf litter degradation and may be influenced by food preference (Van Wensem et al. 1993).

Changes in the chemical composition of the litter due to decomposition increase its palatability to detritivores (Cameron and LaPoint, 1978; Neuhauser and Hartenstein, 1978; Rushton and Hassall, 1983; Hassall and Rushton, 1984; Hassall et al., 1987; Wan Wensen et al., 1993). During leaf senescence, there is a reduction in the phenolic content due to action of microorganisms and leaching (Zimmer, 1999b; Zimmer, 2002*a*) that increases palatability for detritivores (Cameron and LaPoint, 1978; Hassall and Rushton, 1984).

Phenolics are thought to play a fundamental role in chemical defense of plants against herbivores and pathogens (Harborne, 1993; Kefeli et al., 2003; Gould and Lister, 2006) although their effects are controversial and not fully understood (Appel, 1993; Johnson and Felton, 2001). Total phenolic content varies with plant growth and abiotic factors such as temperature and radiation (Salgado et al., 2008) and may affect microbial decomposers since a bulk of phenolics remains present during leaf senescence and after death (Bärlocher and Graça, 2005). Although not well understood, the existence of a phenolic cycle in the plant-soil system has been recorded (Kefeli et al., 2003). Differences in the composition and concentration of resin acids and phenolics during leaf and needle litter senescence have been recorded (Kuiters and Sarink, 1986; Kainulainen and Holopainen, 2002). Hassall and Rushton (1984) observed a rapid degradation of some phenolics (alkaloids and terpenes) during leaf senescence and a negative correlation between isopod feeding preference and the phenol content. However, Neuhauser and Hartenstein (1978) found no relation between leaf palatability and total phenolic content, and Kasurinen et al. (2007) found a weak or inconsistent correlation between detritivore feeding performance and chemical parameters of leaf litter.

Flavonoids are phenolic compounds commonly found in plants and might interfere with feeding, molting and reproduction of insects (Oberdörster et al., 2001; Simmonds, 2001; Boué and Raina, 2003; Gould and Lister, 2006). Some flavonoids play an important role in the protection of plants from harmful UV-B levels (Gould and Lister, 2006) and several classes of flavonoids show antioxidant activity towards a variety of readily-oxidizable compounds (Gryglewski et al., 1987; Dixon and Steele, 1999; Zhishen et al., 1999).

The ability to digest phenolics such as tannins and lignin is essential in the use of litter (Zimmer, 2002*b*) and studies have demonstrated that isopods are capable of oxidizing (Stevenson, 1961; Zimmer and Topp, 1998; Zimmer, 1999b; Zimmer et al, 2002) or hydrolyzing ingested phenolics (Zimmer, 1999*b*; Zimmer et al, 2002). Cameron and LaPoint (1978) observed decreased mortality and increased leaf consumption after leaching of tannins in *Armadillidium vulgare* (Latreille, 1804) that was related with leaf senescence, and suggested that litter resource cannot be used immediately after leaf fall due to chemical and mechanical defenses of plants.

The goal of this study was to use the terrestrial isopod *Balloniscus sellowii* (Brandt, 1833) as a detritivorous model, to verify feeding performance (consumption, egestion, and assimilation rates and assimilation efficiency) in leaves of different stages of decomposition according to their phenolic and flavonoid content.

MATERIAL AND METHODS

Species and study site

The species *Balloniscus sellowii* (Brandt, 1833) is a common species in Southern Brazil, Uruguay and the region around Buenos Aires in Argentina (Schmalfuss, 2003).

The specimens of *B. sellowii* were collected in a urban area of Porto Alegre, Rio Grande do Sul, southern Brazil. They were kept in laboratory conditions at 20±1ºC, with 12:12 (light: dark) photoperiod. Only animals heavier than 25mg were used in the experiments, excluding ovigerous females.

The source-site consisted of an area where animals were abundant and there were trees characteristic of pioneer vegetation colonization. The three most abundant plant species in the site were *Lithraea brasiliensis* Marchand (Anacardiaceae); *Ricinus communis* Linnaeus (Euphorbiaceae) and *Schinus terebinthifolius* Raddi (Anacardiaceae).

Feeding trials

Leaves from the most abundant plants of the local were offered to isopods to verify feeding preference based on consumption rate. Green leaves from three different plant species were taken from branches and placed into litterbags for decomposition in loco for 14 days. After this period, the leaves were taken to the laboratory and circles of 18mm of diameter were cut and oven dried at 60ºC for 48 hours. The discs were weighed (GIBERTINI E425-B) and remoisten before offered to animals for one week period in individual units consisted of 8cm diameter plastic containers with moist plaster of Paris in the bottom and a net over it to minimize coprophagy (Fig. 1). Animals were kept without food source for two days to empty

gut prior to the experiment. After the experiment, the remaining plant material and feces were oven dried and reweighed, and consumption rates calculated. The control consisted of units containing leaves and no animals. The mean percentage of leaf weight lost due to autogenic changes (weight lost independent from the action of consumers) was subtracted from the amount of plant consumed. The plant species that presented the highest consumption was used to verify feeding performance in different stages of leaf decomposition as well as the phenolic and flavonoid content of leaves.

Feeding performance on leaves in different stages of decomposition

Green leaves were collected from branches in the same site and placed into 20 litterbags (10x15cm) fastened to the soil with small wooden sticks. Litterbags were collected after one, two and three months of decomposition, taken to laboratory and offered to animals, as well as additional green leaves collected from branches when litter bags were placed in the soil. Oven dried leaves from each decomposition stage as well as the remains were stored under refrigeration for phenolic and flavonoid content analysis.

Consumption, egestion and assimilation rates

Experimental units as described above were used in the experiment. Two or three discs of 18mm (or approximate amount for the third month of decomposition, which presented leaves that were too friable to be cut into discs) were oven dried at 60° for 48 hours, weighted, remoisten with distille d water, and offered to animals for ten days. Animals were kept without food two days prior and after the experiment to empty gut. Remaining leaves and feces were collected from units, oven dried and weighted after the experiment to calculate feeding performance. Twenty repetitions and 20 controls were used for each stage of decomposition.

The consumption rates were calculated as mg of ingested leaves (subtracted mean percentage of autogenic losses) in dry weight (DW) per g of body in fresh weight (FW), per day. The egestion rates were calculated as mg of produced feces (DW) per g of body weight (DW) per day. The assimilation rates were calculated as mg of ingested leaves (DW) minus mg of produced feces (DW) per g of body weight (FW) per day (adapted from Souza et al, 1998; Loureiro et al., 2006).

Time of passage through gut and assimilation efficiency

To calculate the assimilation efficiency, the time food was retained in the gut needed to be tested. Animals were kept in individual units containing carrot as a food source for a week. The carrot was used as a marker provided that the fecal pellets derived from its feeding differ in color (Fig. 2). After that, ten animals were exposed to one disc of leaf litter from each decomposition stage, and monitored every two hours for 80 hours. The time that the leaf first appeared fed upon and the time the first feces from feeding on leaves were present in experimental units were recorded.

Animals were fed on carrots for a week and placed in units containing one leaf disc until all of the disc was eaten. Feces were collected, oven dried and weighted to calculate assimilation efficiency. Five units from each decomposition stage were monitored daily and time for the beginning of feeding start was also recorded. The assimilation efficiency was calculated as percentage using mg of ingested leaf minus mg of feces produced per mg of ingested leaf.

Secondary metabolites content

Secondary metabolites were related to content of total phenolics and flavonoids. Total phenolics were determined following Bärlocher and Graça (2005), using tannic acid as standard. Five samples of approximately 100mg of dry leaves were used for each stage of decomposition, totalizing 20 samples.

Determination of the flavonoid content was modified from Zhishen et al. (1999) using quercitin as pattern and five discs of dry leaves or approximate amount (≈150mg) for each stage of decomposition, also totalizing 20 samples.

The mean concentration of phenolics and flavonoids were multiplied by the consumption rates and compared.

Statistical analysis

All data was tested for normality using Kolmogorov-Smirnov test. The consumption, egestion and assimilation rates for each stage of decomposition were compared through one-way Analysis of Variance (ANOVA) followed by Tukey test. Pearson correlations were used to verify association between consumption and egestion rates among treatments. The statistical analyses were performed using InStat 3.01 software.

RESULTS

Feeding preference

The consumption rate was significantly higher when animals fed on *S. terebinthifolius* (52.891±9.000 mg $g^{-1}day^{-1}$) ($F_{2,26}$=9.395; p<0.001) and no significant difference was recorded when animals fed on *L. brasiliensis* (31.749±3.959 mg g^{-1} day^{-1}) and *R. communis* (15.453±2.867 mg $g^{-1}day^{-1}$) (Fig 3). The egestion and assimilation rates for *S. terebinthifolius* were 46.290±9.761 (mg $g^{-1}day^{-1}$) and 11.230±1.948 (mg $g^{-1}day^{-1}$), respectively. For *L. brasiliensis*, the rates were 21.130±2.595 and 14.845±1.623 (mg $g^{-1}day^{-}1$) respectively, and for *R. communis* the amount of fecal pellets were too low to be weighted and therefore egestion rate could not be calculated, and the assimilation rate was 15.453±2.867 (mg $g^{-1}day^{-1}$).

Schinus terebinthifolius in different decomposition stages

The consumption rate was significantly higher on two months-old leaves ($F_{3,58}$=8.59; p<0.001) and there were no significant differences between green, one month-old and three months-old leaves. The egestion rate also presented significant difference, being highest with two months-old leaves ($F_{3,58}$=14.171; p<0.05). There were no significant difference, neither between green and one month-old leaves, nor between one month-old and three months-old leaves. The assimilation rate was significantly highest when the consumption rate was lowest (green leaves) and presented no significant difference between one, two and three months-old leaves ($F_{3,58}$=5.302; p <0.005) (Table 1).

There were significant correlations between consumption and egestion rates for all decomposition stages. The correlation was stronger for three months-old leaves (r^2=0.9258; p<0.0001) followed by one month-old leaves (r^2=0.8959;

$p<0.0001$), two months-old leaves ($r^2=0.8346$; $p<0.0001$) and green leaves being the weakest ($r^2=0.7241$; $p<0.0002$) (Fig. 4).

The time of passage through gut did not present difference among stages of decomposition. In all stages, the mode time for appearance of feces from leaf consumption was two hours. However, the beginning of ingestion of leaves occurred in the same day as the beginning of the experiment for most units from one, two and three months of decomposition, whereas the beginning of ingestion of green leaves occurred after two or three days. For three out of the ten units containing green leaves, the consumption was not noticed after 80h of experiment.

The calculated assimilation efficiency was 43.06±3.84% for green leaves (n=6), 19.74±2.39% for one month-old leaves (n=4), 20.33±1.55 for two months-old leaves (n=5) and 19.51±2.78% for three months-old leaves (n=2).

The phenolic content was highest in green leaves (0.0336±0.0019 mg of tannic acid equivalent per mg of dry leaf) and very low after three months of decomposition (0.0035±0.0002 mg mg^{-1}) ($F_{3,15}=35.259$; $p<0.0001$). There was no significant difference between one and two months-old leaves (0.0226±0.00144 and 0.0202±0.0043 mg mg^{-1} respectively). The flavonoid content was significantly higher in green leaves and lowest in the second month ($F_{3,16}=37.31$; $p<0.001$). There was no significant difference between the content of the first and third month of decomposition (Fig. 5).

The estimated phenolic amount ingested by animals was significantly different ($F_{3,58}=28.934$; $p<0.0001$), being highest when consumption rate was also highest. The estimated flavonoid amount ingested by the animals presented the same trend as the assimilation rate among the stages of decomposition, being significantly

higher in green leaves but not differing between one, two, and three months of decomposition ($F_{3,58}$=10.252; p<0.0001) (Fig. 6).

As the flavonoid content was not tested for every experimental unit, the mean average of the content of each month was tested for correlation with the mean average of the assimilation rate for each stage of decomposition, resulting in a high correlation (r^2=0.9907; p=0.0046, n=4).

DISCUSSION

Numerous studies analyze the effects of secondary metabolites in herbivores, but few studies have been conducted to understand the role of these compounds in detritivore and decomposer organisms, besides the presence of unpalatable or indigestible compounds (Cameron and LaPoint, 1978; Hassall and Rushton, 1984; Target et al., 1986; Canhoto and Graça, 1999) and their feeding preference related to leaf senescence (Cameron and LaPoint, 1978; Yeates and Barmuta, 1999). Our study site presented plant species characteristic of a successional stage that do not present mechanical structures to avoid herbivores other than lignin, suggesting that chemical defenses are key for plant protection. Tropical plants inhabiting resource-poor environments seem to invest heavily in chemical defenses such as various phenolics (Agrawal, 2006). *Ricinus communis* presented the lowest consumption rate and very little amount of fecal pellets egested. This is a plant that has been associated with a large amount of secondary metabolites, being gallic acid, quercetin, and rutin some of the major phenolic compounds responsible for the antioxidant activity of its dry leaves (Singh et al., 2009) and its decomposition after two weeks resulted in viscous leaves.

Lithraea brasiliensis also presents a large amount of secondary metabolites (Correia et al., 2006) and it is an easily found leaf in the leaf litter in Brazil although its consumption by *B. sellowii* was significantly lower than *S. terebinthifolius* and did not differ in consumption when compared to *R. communis*.

Schinus terebinthifolius is known as a source of terpenoids, simple phenolic derivatives and flavonols and the anti-oxidant activity of its aerial parts extract has been described (Velázquez et al., 2003). Leaf extracts contain triterpene acids

(Campelo and Marsaioli, 1974) and the ethanolic extract of the leaves is a source of simple phenolics, several flavonoids (flavones flavanones leucoanthocyanidins), xanthones and free steroids (Lima et al., 2006). After the beginning of decomposition, leaves start to curl and might be used by animals for shelter as well as for feeding.

There has been controversy on results of assimilation efficiency on high and low quality food. Authors have hypothesized that some foods might present high assimilation efficiency due to a slower passage through the gut, which decreases fecal production (Souza et al., 1998; Loureiro et al., 2006). In this experiment, the assimilation efficiency in the experimental units were also high for green leaves, which would be considered a low quality food, and there was no difference in the time for beginning of appearance of feces from leaves in this treatments. However, calculating the assimilation efficiency by total consumption and egestion of a leaf disc with a known mass, the results were more plausible. The latter food source makes the animal empty gut from the former ingested food source. The assimilation efficiency was still higher when animals fed on green leaves, but the value was under 80%. Leaves with one, two and three months of decomposition presented similar assimilations efficiency, as well as assimilation rates. The assimilation rates calculated from total consumption of a disc of leaf with a known mass did not differ as much from the rates calculated in the units of the first experiment. The assimilation efficiency should reduce over time (Rushton and Hassall, 1983, Hassall and Rushton, 1984) due to lower content of metabolites in leaves making them more palatable (Johnson and Feldon, 2001)

Green leaves presented high phenolic and flavonoid concentration taking a long time for the animals to begin feeding, suggesting that they might present

substances that are feeding deterrents and therefore reduce palatability. These phenolic substances are probably lost in the beginning of the decomposition process due to leaching, once the same time for the beginning of feeding was not observed in the other decomposition stages. If substances that cause feeding deterrence and inhibit feeding are lost right after the start of leaf senescence, the faster they will be consumed by detritivores returning to the soil as nutrient and therefore being also an adaptive advantage to the plants. Cameron and LaPoint (1978) observed a rapid leach of tannins that was associated with food inhibition from litter bags in the first week of decomposition.

The green leaves used in this study were oven dried before offered to animals, possibly changing properties and making them more palatable to animals. When given a choice, animals avoid green leaves that present high amounts of secondary compounds (Cameron and LaPoint, 1978). Ingesting decayed leaves is a behavioral strategy used to cope with chemically defended food. This way, the concentration of defensive chemicals is reduced and animals may use this strategy to increase their tolerance to chemically defended food (Glendinning, 2007) as recorded by Roy and Bergeron (1990) observing a small rodent cutting leaves from branches and waiting for some decomposition before ingesting it.

Switching frequently between different types of food and regulating intake of specific defensive chemicals are also behavioral mechanisms to handle chemically defended food (Glendinning 2007). Isopods ingest decayed plant material, and ingest different types of plants in their diet when given a chance (Wieser, 1984). However, experiments of feeding preference between more than two food sources are difficult to analyze (Wieser, 1984; Peterson and Renaud, 1989), and therefore preference was not tested here.

During leaf senescence, the content of secondary metabolites tends to diminish due to leaching (Kuiters and Sarink, 1986). Phenolics and flavonoids in plants has mostly been related to defense against pathogens and herbivores (Dixon and Steele, 1999; Boué and Raina, 2003) and leaves presenting a lower content of total phenolics would be less toxic and more used by isopods once ingested food does not change much in the digestory tract. Hassall and Rushton (1984) predicted that less heavily defended species would reach optimal palatability earlier than climax species that usually present more secondary metabolites for defense. However, what determines detritivore consumption is thought to be phenolic signature rather than total phenolic content (Zimmer et al., 2005), and the optimal palatability occurred on two months-old leaves and not when the phenolic content was lowest.

The flavonoid represented a large portion of the total phenolics in the green, one month-old and two months-old leaves. However, the amount of flavonoids in the third month exceeded the amount of total phenolics. That might have happened due to decomposition of the substance during its extraction or due to sampling. Phenolics can be converted into lignin that is broken down by microorganisms; these fragments contribute to the mineralization of soil and humus formation (Kefeli et al., 2003). Lignin phenolics cannot be isolated from lignocellulose without partial denaturation (Breznak and Brune, 1994, Zimmer, 1999*a*) while flavonoids are non-lignin phenolics (Brisson et al.,1986) and might not have suffered denaturation during analyses, and therefore presented a higher content than the total phenolic content in the third month.

Leaching of substances also occurs in other materials in the litter, and might increase the content of a specific constituent originated from the litter itself (from

absorption) or from the action of microorganisms. In this experiment, the phenolic content decreased throughout the months of the experiment, but the flavonoid content increased in the last month instead of decreasing in relation to the leaves of the previous month.

Although the flavonoid content differed between treatments, the estimated amount of flavonoid consumed by animals was almost the same for leaves with one, two and three months of decomposition. There was a strong correlation between the flavonoid content and the assimilation rate but no significant correlation with the total phenolic content. Neuhauser and Hartenstein (1978) did not find any correlation between phenolic content of leaves and feeding preference. However, it seems that animals increase consumption in order to ingest a minimum amount of flavonoid, suggesting that they might use these flavonoids as a food parameter. The content of phenolics and flavonoids in plants has been mostly related to defense against pathogens and herbivores and only a few times possible benefits for organisms ingesting those substances have been mentioned. Johnson and Felton (2001) using herbivores, also observed a lower consumption and digestibility on plants that were overexpressing phenolics, but no significant reduction on growth and no indications of oxidative stress as a causal factor, suggesting that a beneficial antioxidant property for herbivores. These researchers obtained the same results as this study and suggested that the animals might be using the flavonoids as an antioxidant agent.

Even though the use of flavonoids by herbivore invertebrates is not well documented, this paper suggests that the isopods may also use and discriminate concentrations of flavonoids at some degree since they seem to have increased consumption to maintain a minimum intake of flavonoids in leaves with lower

flavonoid content. To verify the plausibility of this hypothesis, experiment must be conducted in order to understand whether or not animals can discriminate different concentrations of flavonoids and if the true ingestion of this substance is equivalent to the estimate presented in this study.

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Table 1. Feeding performance of *Balloniscus sellowii* on *Schinus terebinthifolius* in different stages of decomposition. Data are expressed as mean value and standard error. The superscript letters indicate significant difference among treatments ($p<0.05$).

Stage of decomposition	Consumption rate (mg $g^{-1}day^{-1}$)	Egestion rate (mg $g^{-1}day^{-1}$)	Assimilation rate (mg $g^{-1}day^{-1}$)
Green leaves	41.465±5.129 ^{a}	10.742 ± 3.196 ^{a}	30.722±2.936 ^{a}
1 month old leaves	50.185±5.396 ^{a}	29.136 ± 5.669 ^{a,b}	21.049±1.829 ^{b}
2 months old leaves	80.147±6.220 ^{b}	61.297 ± 6.414 ^{c}	18.851±2.633 ^{b}
3 months old leaves	53.391±5.516 ^{a}	33.440 ± 5.068 ^{b}	19.951±1.522 ^{b}

LIST OF FIGURES

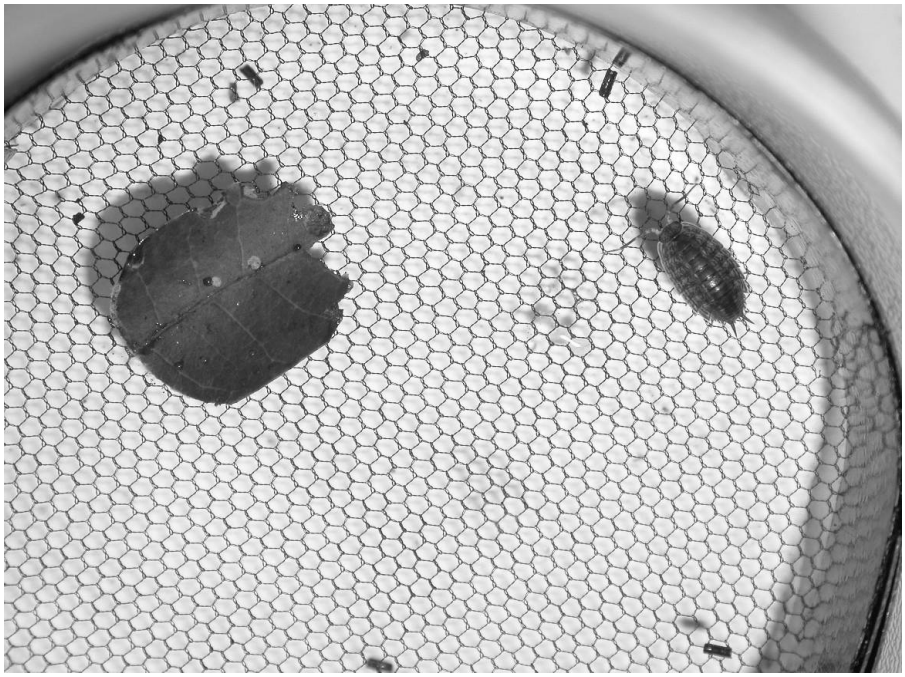

Figure 1. Experimental units with moist plaster of Paris in the bottom and a net over it to minimize coprophagy containing *Balloniscus sellowii* and a disc of leave from *Schinus terebinthifolius*.

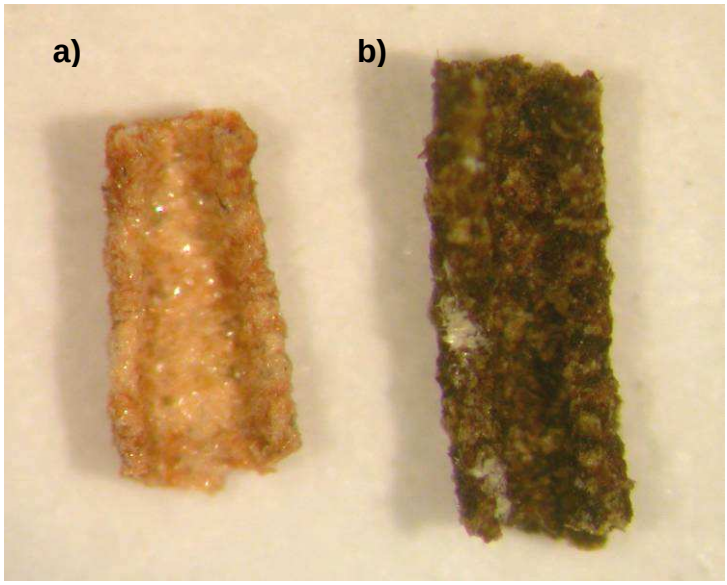

Figure 2. Fecal pellets from the terrestrial isopod *Balloniscus sellowii*. a) fecal pellet from the consumption of carrots and b) fecal pellet from the consumption of leaves.

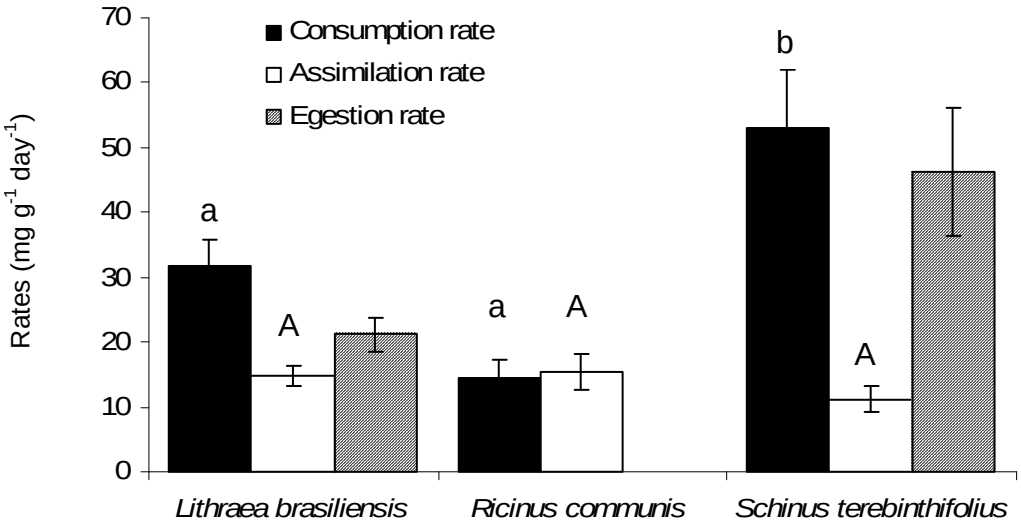

Figure 3. Isopod feeding performance on leaves of different plant species with 14 days of decomposition in litter bags left to decompose in loco. The egestion rate for *Ricinus communis* could not be calculated due to low amount of fecal pellets. The values are mean and SE. Superscript letters indicate significant difference among treatments ($p<0.05$).

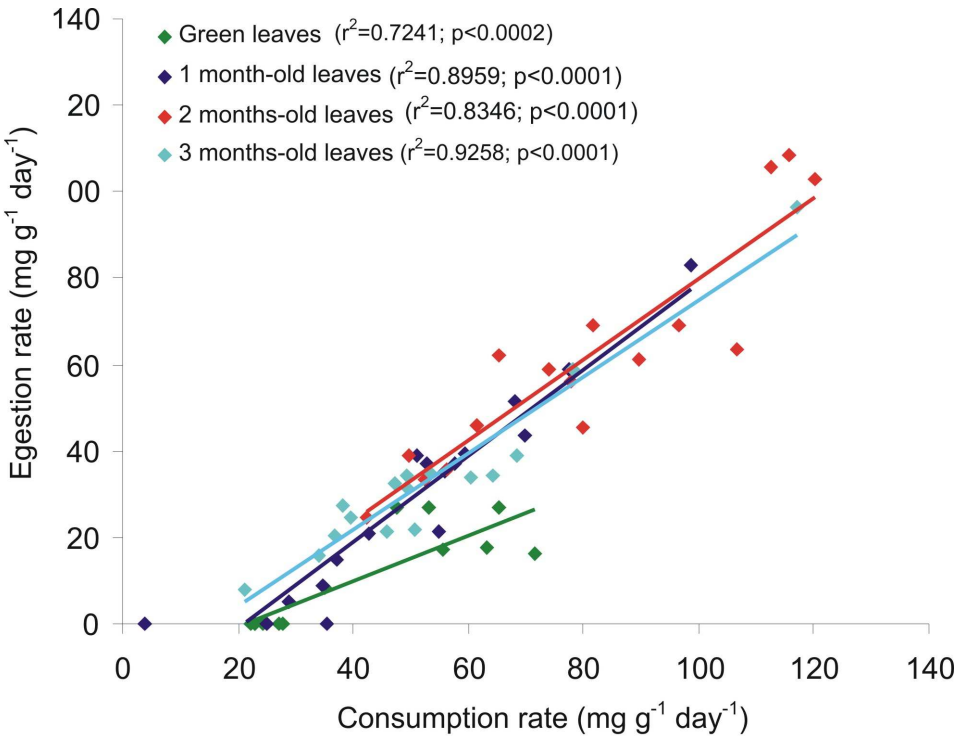

Figure 4. Pearson correlations between consumption and egestion rates of *Balloniscus sellowii* on *Schinus terebinthifolius* leaves in different decomposition stages.

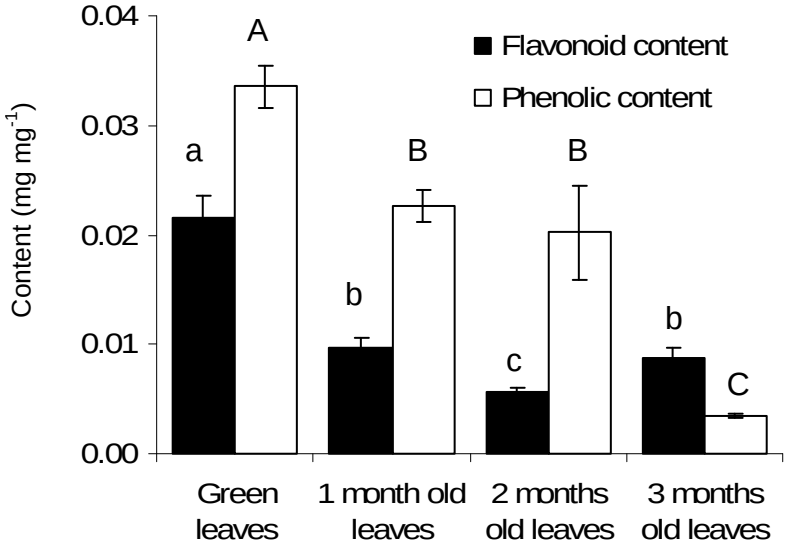

Figure 5. Total phenolic and flavonoid content in leaves of *Schinus terebinthifolius* in different stages of decomposition. The values are mg of equivalent of quercitin (flavonoid) or tannic acid (phenolic) per mg of dry leaf SE. Superscript letters indicate significant differences among treatments ($p<0.05$).

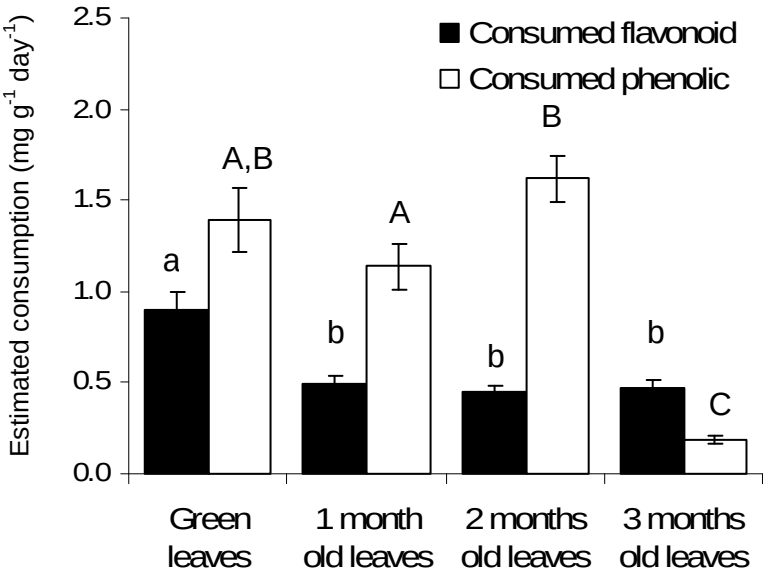

Figure 6. Estimated amount of total phenolics and flavonoids ingested by *Balloniscus sellowii* in leaves of *Schinus terebinthifolius* in different stages of decomposition. The values are mean and SE. Superscript letters indicate significant differences among treatments ($p<0.05$).

CONSIDERAÇÕES FINAIS

Diversos trabalhos têm sido realizados utilizando isópodos terrestres como modelo para entender a nutrição de detritívoros embora não muitos discutam a relação dos metabólitos secundários.

Nesse trabalho, observou-se a relação do conteúdo de fenólicos presentes em uma planta durante sua decomposição e a sua relação com o desempenho alimentar do isópodo terrestre *Balloniscus sellowii*. Dentro dos fenólicos, o conteúdo de flavonoides teve uma relação ainda mais forte, sendo a quantidade de flavonoides ingeridos em folhas de um, dois e três meses de decomposição muito similares e sem diferença significativa. Para isso, a taxa de consumo foi mais alta quando o conteúdo de flavonoides das folhas era mais baixo. Com esse resultado, surgiu uma dúvida e uma proposta de um novo experimento a fim de testar se os animais possuem a capacidade de discriminar diferentes concentrações de flavonoides. O experimento consistirá de um disco de gelatina com diferentes concentrações de flavonoides oferecido aos animais. Desta forma, poderemos calcular mais precisamente a quantidade de flavonoides ingeridos em um período de tempo e qual a concentração ótima e inibitória de flavonoides para os animais.

Sabe-se que além do conteúdo de metabólitos secundários, diversas características das folhas são de grande importância na escolha e preferência dos animais sendo, a dureza, também importante na escolha e aproveitamento do recurso. Esta característica leva-nos a outro questionamento, relacionado ao desgaste das mandíbulas. Assim, outro experimento será conduzido, utilizando-se discos foliares dos diferentes estágios de decomposição utilizados no experimento (armazenados em refrigeração), os quais serão re-umedecidos com água destilada

e oferecidos a animais que acabaram de realizar a ecdise. Esses animais receberão discos daquele estágio de decomposição até começarem a armazenar cálcio na forma de placas de cálcio, o que indica que estão se preparando para realizar nova ecdise, e serão acondicionados em álcool 70%. Os animais serão dissecados para a retirada das mandíbulas e preparados para microscopia eletrônica de varredura (MEV). A cada ecdise, os animais trocam as peças bucais e com isso saberemos que aquele desgaste foi devido à alimentação oferecida no tratamento. As imagens das mandíbulas serão analisadas e o desgaste dos diferentes tratamentos comparados.

A análise dos dados do trabalho permitiram a proposição de experimentos que possam confirmar as hipóteses levantadas na discussão. Desta forma, a sua continuação torna-se importante para uma complementação deste trabalho.

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ANEXO 1

Normas do periódico *Journal of Crustacean Biology*.

Disponível em < http://web.vims.edu/tcs/?svr=www>. Acesso em: 23 maio de 2009.

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Information for Contributors

Content

The *Journal of Crustacean Biology* contains papers of broad interest dealing with any aspect of crustacean biology, biographies of notable carcinologists, notices of business transacted at meetings of The Crustacean Society, book reviews of works on Crustacea, obituary and memorial pieces, and pertinent announcements. We also can entertain submissions from time to time on other groups of marine arthropods such as pycnogonids and merostomes. Papers will be published in English only, but abstracts or summaries in French, German, Portuguese, or Spanish may be added when appropriate. Descriptions of single new species may be accepted if accompanied by significant information on zoogeography, ecology, phylogenetic relationships, or other biological concerns.

You do not have to be a member of the Society to publish in the Journal of Crustacean Biology, but there are benefits of membership that are specified below.

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The cost of printing color figures is in addition to all other charges. Authors must pay the full cost of printing color figures after the manuscript has been accepted but before it is published. For recent issues of the journal, the costs of printing color figures has ranged from US$692.50 to US$832.50 for **each signature in the final complied journal**. The cost varies depending on the size and composition of the figures and whether or not they are submitted as hard copy or as electronic image files.

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Authors will be billed for all charges in paragraphs 1-3 on a single invoice, which will be sent immediately after publication of the paper (payments for color plates, see below).

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Authors who fail to pay a promised page charge (full or partial) or the mandatory charges (figures, tables, alterations in proof) will not have other papers published in JCB until the deficit is paid. **JCB will not publish papers from authors who will not pay the nominal page charges but offer to pay for color plates or excess illustrations or tables.** Authors who pay no page charges or pay only partial page charges must be aware that their manuscript may not be published as quickly as those from authors paying in full.

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While embedded figures can suffice for the review process, by the final revision process, separate text and TIF-figure files will be needed saved at the appropriate resolution.

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The title page should give the running head; the title of the paper, typed in all capital letters; and the author name(s), followed by affiliation(s) including e-mail address(es). In the case of multiple authorship, list all authors' names first, then each author's initials should be enclosed in parentheses before the appropriate mailing address; the e-mail address should be within parentheses immediately following the mailing address. The running head should contain the first author's surname, a colon, and an abbreviated title in the case of a single author. When there are two authors, include both authors' surnames before the colon. In the case of three or more authors, use only the first author's surname followed by - et al. The running head should not exceed about 52 characters.

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Tables and figures should be self-explanatory, not requiring reference to the text. Each table should start on a separate page and must be double-spaced throughout, even if it extends onto multiple pages. Headings and format must be consistent with the style used in *Journal of Crustacean Biology;* see previous issues for format. Vertical rules and excessive horizontal rules should not be used. For treatment of large tables of gene sequences, see below.

Figures must be proportioned to fit nicely within the journal's margins when reduced. Figures will be reduced to either 176 mm width (full page) or 84 mm width (column width) and a maximum height of 237 mm (including the space for caption beneath). For figures with multiple parts (A, B, C, etc.), all of the parts must be together on one figure, not spread over multiple separate photographs. For electronic submission of figures, see below.

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Smith and Brown, 1998, followed by Smith and Jones, 1996, followed by Smith and Jones, 1997. Use only the authors' surnames and initials in the References; place a space between an author's initials. Names of periodicals should be written out in full and should not be italicized. Do not use issue numbers of continuously paginated volumes. Use a hanging indent for multiple lines within one citation (see below for more about hanging indents). All citations in the article must be in the References, INCLUDING the authors of taxonomic names.

A sample citation of an article by a single author in a serial journal follows:

Smith, J. Q. 1981. The distribution of swimming crabs. Journal of Crustacean Biology 1: 105-119.

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Martin, G., and P. Juchault. 1999. Androgenic hormone specificity in ten species of lobsters. Journal of Crustacean Biology 19: 684-689.

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Garth, J. S. 1991. Taxonomy, distribution, and ecology of Galapagos Brachyura, pp. 123-125. In, M. J. James (ed.), Galapagos Marine Invertebrates. Plenum Publishing Company, New York.

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Do not justify the right margin.

Use italics only for the scientific names of genera and lower categories; do not use underlining.

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Last updated: 16 April 2009.