

Leaf litter as a possible food source for chironomids (Diptera) in Brazilian and Portuguese headwater streams

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ABSTRACT. Our objective was to evaluate the potential use of leaf detritus by chironomid larvae. Field and laboratory experiments were performed using leaves and chironomid species collected in Portugal and Brazil. Laboratory experiments under controlled conditions were done using microbial conditioned senescent leaves of *Alnus glutinosa* (L.) Gaertn, *Nerium oleander* L., *Protium heptaphilum* (Aubl.) March, *Protium brasiliense* (Spreng) Engl., *Myrcia guyanensis* (Aubl.) DC and *Miconia chartacea* Triana. Laboratory experiments were performed using specimens collected from leaf litter in local streams. Whenever possible, after the experiments, chironomids were allowed to emerge as adults and identified. In Portugal the following taxa were identified: *Micropsectra apposita* (Walker, 1856), *Polypedilum albicorne* (Meigen, 1838), *Eukiefferiella claripennis* Lundbeck (1898), *Rheocricotopus (Psilocricotopus) atripes* Rempel (1937) and *Ablabesmyia* Johannsen (1905) (Diptera, Chironomidae). Consumption rates ranged from 0.15 ± 0.10 mg (AFDM) of leaf animal⁻¹ day⁻¹ (*Micropsectra apposita* feeding on *Alnus glutinosa*) up to 0.85 ± 0.33 mg (AFDM) of leaf animal⁻¹ day⁻¹ (*Polypedilum albicorne* feeding on *Miconia chartacea*). In Brazil, the following taxa were identified from leaves: *Phaenopsectra* sp., *Chironomus* spp. and *Polypedilum* sp. and maximum consumption rates reached 0.47 ± 0.28 (AFDM) of leaf mg.animal⁻¹.day⁻¹ (*Chironomus* Meigen (1803) feeding on *Protium heptaphilum*). Feeding experiments with laboratory cultured specimens, revealed that some chironomids were unable to feed on decomposing leaves (e.g., *C. xanthus* Rempel (1939) on *P. brasiliense* and *M. guyanensis*). Our results suggest that some stream chironomids (not typical shredders) can use leaf litter of riparian vegetation as a complementary food source.

KEY-WORDS. Leaf feeding; shredders; tropical and temperate experiments.

RESUMO. Detritos foliares como possível fonte de alimento para Chironomidae (Diptera) em riachos de cabeceira brasileiros e portugueses. O objetivo foi avaliar o potencial uso de detritos foliares por larvas de Chironomidae. Foram realizados experimentos em campo e em laboratório utilizando folhas e larvas de Chironomidae coletadas no Brasil e em Portugal. Foram realizados experimentos em laboratório em condições controladas utilizando detritos foliares (*Alnus glutinosa* (L.) Gaertn., *Nerium oleander* L., *Protium heptaphilum* (Aubl.) March, *Protium brasiliense* (Spreng) Engl., *Myrcia guyanensis* (Aubl.) DC e *Miconia chartacea* Triana) após a colonização microbiana. Experimentos laboratoriais de consumo foram realizados com espécies coletadas em detritos orgânicos em riachos locais. Depois dos experimentos, quando possível, os exemplares foram deixados atingir o estágio adulto e identificados. Em Portugal foram identificadas as seguintes espécies: *Micropsectra apposita* (Walker, 1856), *Polypedilum albicorne* (Meigen, 1838), *Eukiefferiella claripennis* Lundbeck (1898), *Rheocricotopus (Psilocricotopus) atripes* Rempel (1937) e *Ablabesmyia* Johannsen (1905). As taxas de consumo variaram entre $0,15 \pm 0,10$ mg de folha animal⁻¹ dia⁻¹ (*Micropsectra apposita* alimentando-se de *Alnus glutinosa*) e $0,85 \pm 0,33$ mg de folha animal⁻¹ dia⁻¹ (*Polypedilum albicorne* alimentando-se de *Miconia chartacea*). No Brasil foram identificados os seguintes taxa: *Phaenopsectra* sp., *Chironomus* spp. e *Polypedilum* sp.; a taxa máxima de consumo foi $0,47 \pm 0,28$ de folha mg.animal⁻¹ dia⁻¹ (*Chironomus* Meigen (1803) alimentando-se de *Protium heptaphilum*). Foram igualmente feitos testes com estirpes laboratoriais de Chironomidae, algumas das quais não foram capazes de alimentarem-se de folhas em decomposição (e.g. *C. xanthus* Rempel (1939) em *P. brasiliense* e *M. guyanensis*). Os resultados sugerem que alguns Chironomidae (não tipicamente fragmentadores) podem utilizar detritos foliares de vegetação ripária como fonte complementar de alimento.

PALAVRAS-CHAVE. Alimentação de folhas; fragmentadores; experimentos em região tropical e temperada.

Allochthonous organic matter of terrestrial origin is an important component of streams in forested areas and a major energy source consumer (Poza *et al.* 1997, ABELHO 2001). Litter

input to streams can reach up to 1,500 g (AFDM). m⁻². year⁻¹ and standing stock values up to 3,000 g (AFDM). m⁻² have been reported, being leaves the largest litter component (ABELHO 2001).

Leaf litter is generally present all year around in tempered and tropical streams (RAMIREZ *et al.* 1998, GONZALEZ & GRAÇA 2003) and processed in situ by microorganisms and invertebrates (GESSNER & CHAUVET 1994, GESSNER *et al.* 1999, GRAÇA *et al.* 2001). Microbial colonization enhances the palatability of leaves to invertebrate consumers, including trichopterans, plecopterans, amphipods and tipulids (GRAÇA 2001, GRAÇA *et al.* 2001).

Chironomids are an important component of the invertebrate assemblages in virtually all water bodies, and in many cases numerically dominant (COFFMAN & FERRINGTON 1996, RAMIREZ *et al.* 1998, RINCON & CRESSA 2000, GRAÇA *et al.* 2004). In some Portuguese streams GRAÇA *et al.* (2004) referred that chironomids complied around 1,200 individuals m⁻², whereas in pristine Brazilian streams of Serra do Cipó, GALDEAN *et al.* (2000) reported chironomid densities up to 1,600 individuals m⁻². Values up to 2,000 individuals.m⁻² were reported in a Canadian (Ontario) stream (TAVARES-CROMAR & WILLIAMS 1997). Chironomids are among the taxonomic groups with high production in streams (MEYER & POEPPERL 2003). Chironomids are also very diverse and distributed world wide. The number of estimated species ranges from 1,000 to 2,000 in Europe (ILLIES 1978) and c. 10,000 for Brazil (E.J. Fittkau, pers. com.), but less than 10% are already known.

Chironomid larvae are known to ingest a variety of food types, e.g., algae, detritus and associated microorganisms, macrophytes, woody debris and invertebrates (BERG 1995, HIRABAYASHI & WOTTON 1999). Most of the chironomid species are considered gathering-collectors and to a lesser extent predators (Tanytopodinae), miners (e.g., *Stenochironomus* Kieffer, 1919, *Endochironomus* Kieffer, 1921, *Goeldichironomus* Fittkau, 1965, and some Orthoclaadiinae) and shredders (GRUBBS *et al.* 1995, COFFMAN & FERRINGTON 1996). However, several studies have shown that many aquatic invertebrates are opportunistic or generalists (polyphagous), feeding on other sub-optimal food sources (FRIBERG & JACOBSEN 1994, MIHUC & MIHUC 1995, DANGLES 2002) and the same may apply to chironomids in general.

Chironomids are sometimes present in large numbers in decomposing leaves (REICE 1980) and in litterbags or leaf packs of decomposing experiments (GRUBBS *et al.* 1995, CASAS *et al.* 2000, MENENDEZ *et al.* 2003). In a Brazilian shallow lake (Jurubatiba lagoon, Rio de Janeiro) 21 genera of Chironomidae were observed in decomposing leaves of *Typha domingensis* Pers. (GONÇALVES *et al.* 2000) and 9 genera in *Nymphaea ampla* (Salisb.) DC (GONÇALVES *et al.* 2003). In a decomposition experiment GRUBBS *et al.* (1995) reported that typically > 75% of invertebrates colonizing leaves were chironomids, but only 1 to 23% were shredders; a total of 37 chironomid genera were identified in decomposing leaves. POPE *et al.* (1999) reported that 24 to 94% of the invertebrates associated to decomposing leaves in a Canadian Lake were chironomids; however, it was assumed that chironomids were using leaves as habitat. There are also been references for consumption of entire leaves by chironomids (BERG 1995, CANHOTO & GRAÇA 1999).

Since leaves in many small streams are conspicuous and chironomids are numerous, it is plausible that many chironomids may use leaf litter as potential alternative energy source. As stated before in Gonçalves *et al.* (2006b), the role of aquatic invertebrate consumers in leaf-litter processing is unclear outside temperate systems. In this paper we evaluated the capability of a range of chironomids to feed on conditioned leaves. Specimens were collected in some streams from two climates: temperate (Portugal) and tropical (Brazil) areas in order to cover two different climatic zones.

MATERIAL AND METHODS

Field experiment

In a first experiment we assessed the numerical importance of chironomids in decomposing litter in streams. Senescent leaves of *Nerium oleander* were collected during autumn, air dried and assembled in portions of 4.0 g (\pm 0.2 g) and allocated into bags with mesh sizes of 10mm (coarse mesh bags), 0.5 mm and 0.25 mm (fine mesh bags). Twenty four bags were prepared and placed in a side channel of the S. João stream, Lousã (Central Portugal). The channel was 10 cm deep, 0.5 m wide and has natural substratum of pebbles and gravel. The bags were recovered after 45 days (February-March 2003), transported to the lab and the invertebrates retrieved in white trays. Invertebrates were counted and classified into two categories: Chironomids and other invertebrates. The remaining leaf material was dried at 50 °C for at least 48 hours and the percentage of remaining mass calculated after correction for air to oven dry mass.

Laboratory experiment

To evaluate the capability of Chironomidae larvae to use conditioned leaves as food we collected specimens from natural leaf packs in local streams. The streams were (1) Portugal (rio Dueça, running from a forested area into the village of Miranda do Corvo, 40°10'N, 8°23'W) and (2) Minas Gerais, Brazil (Indaiá stream in the Serra do Cipó National Park 19°16'N, 43°31'W, Pampulha reservoir in Belo Horizonte 19°50'N, 43°60'W, and Ibitité stream a tributary of the Paraopeba 20°00'N, 44°06'W). Only the Indaiá can be considered pristine; the others are affected by organic pollution. We also used (3) laboratory cultures of *Chironomus riparius* (IAV stock, Universidade de Coimbra), *Chironomus* sp. and *C. xanthus* (Universidade Federal de Minas Gerais).

The field specimens used in feeding trials were collected in March-April 2003 in Portugal, and in May-July 2003 in Brazil and acclimated to laboratory conditions for 1-3 days prior to the experiments. Specimens of chironomids were individually reared in Petri dishes 1.3 cm high containing 20 ml of filtered stream water (GF/C, Whatman), under constant temperature similar to the local streams (i.e., 15°C in Portugal and 22°C in Brazil) and photoperiods of 12:12 h and 14:10h light-dark in Portugal and Brazil, respectively. No aeration was required during the feeding trials; spot checks showed that dis-

solved oxygen was always near saturation throughout the experiments. The number of replicates was initially 20 per species, but replicates where pupation occurred were eliminated. Portuguese chironomids were deposited at Colección del Departamento de Biología Animal, Facultad de Biología, Universidad de Santiago de Compostela (Spain), and Brazilian chironomids were deposited at Reference Collection of Benthic Macroinvertebrates, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (Belo Horizonte, Brazil).

Food

Food was provided in the form of conditioned leaves. The European leaf species were *Alnus glutinosa* (L.) Gaertn., and *Nerium oleander* L., this one occurring in the dry Mediterranean areas of South Iberian Peninsula and North Africa. The Brazilian species were *Miconia chartacea* Triana (Melastomataceae), *Myrcia guyanensis* (Aubl.) DC. (Myrtaceae), *Protium brasiliense* (Spreng.) Engl., *P. heptaphyllum* (Aubl.) March (Bursereaceae), *Solanum leucodendron* Sendt. (Solanaceae), *Ocotea* sp. (Lauraceae), all occurring in the riparian zones of highland streams in Minas Gerais State, southeastern Brazil.

Senescent leaves were collected with nets (Serra do Cipó, Brazil) or from the ground just after abscission (Portugal), air-dried and stored dry until needed. Before use in the experiments, leaves were microbial conditioned in local streams for 2 weeks (approximately 5 g of leaves in 0.5 mm mesh bags) or in a laboratory aquarium using a mixture of stream collected leaves as an inoculum and under strong aeration.

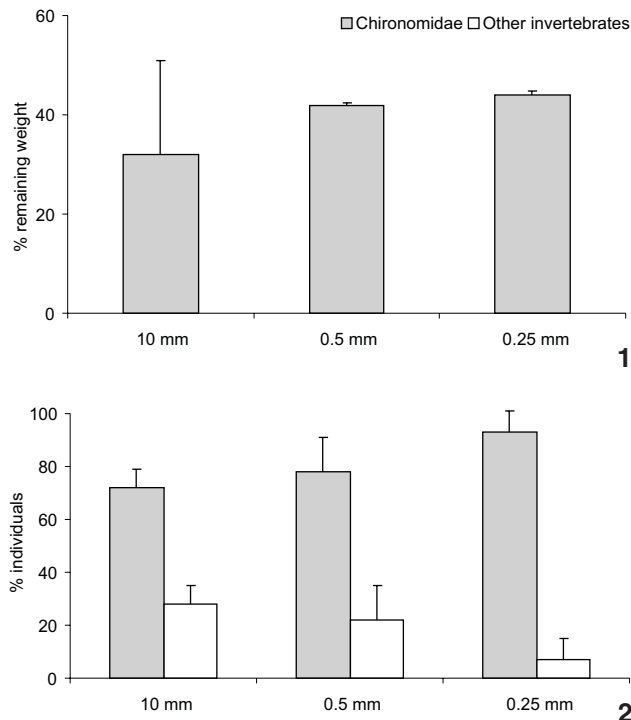
Consumption

Food was provided in the form of leaf discs. Discs were cut in pairs from contiguous areas of a same leaf. One of the discs was exposed to the chironomid, whereas the other was placed in a Petri dish with no invertebrates. Larvae were allowed to feed for a minimum of 3 days and whenever possible, adults were collected for identification. Consumption was calculated as the difference in mass between unexposed and exposed discs divided by the elapsed time in days. Values were expressed as ash free dry mass: discs were dried at 50°C for 2 days, weighed, ashed at 500°C for 5 hours and weighed again.

Feeding was considered to have occurred when the mass of exposed discs was significantly lower (pair t-test, $p < 0.05$) than the corresponding control disc (FRIBERG & JACOBSEN 1994, GRAÇA *et al.* 2001).

RESULTS

After 45 days in the channel, leaves of *Nerium oleander* lost 68 % of their initial mass in coarse mesh bags, with similar results in the 0.5 and 0.25 fine mesh bags (58 % and 56 % respectively). Coarse mesh bags had higher number of invertebrates than fine mesh bags: 1,028 invertebrates g^{-1} in 10 mm, 495 invertebrates g^{-1} in 0.5 mm, and 211 invertebrates g^{-1} in 0.25 mm. Chironomids were more abundant than other invertebrates in all bags (Figs 1 and 2).



Figures 1-2. Remaining leaf weight of *Nerium oleander* leaves after 45 days in a stream (1) and percentage of chironomids and other invertebrates in litter bags differing in mesh size (2). Mean \pm S.E.

In Portugal the adult chironomids emerging from the feeding experiments turned to be *Micropsectra apposita*, *Polypedilum albicorne*, *Eukiefferiella claripennis*, *Rheocricotopus* (*Psilocricotopus*) *atripes*, and *Ablabesmyia* Johannsen, 1905. In Brazil they included *Phaenopsectra* Kieffer, 1921, *Polypedilum* Kieffer, 1912, and *Chironomus* Meigen, 1803. Mortality ranged from 0 to 40%. Among the survivors, the percentage of larvae causing a reduction in mass of exposed leaves varied between 10% and 100% (Tab. I). Some survivors did not feed for a period of three days (up to 43%).

Under laboratory conditions, most of the specimens consumed leaf discs (Fig. 3). Consumption rate ranged from 0.15 ± 0.10 $mg \cdot animal^{-1} \cdot day^{-1}$ of *Micropsectra apposita* in *Alnus glutinosa* up to 0.85 ± 0.33 $mg \cdot animal^{-1} \cdot day^{-1}$ of *Polypedilum albicorne* in *Miconia chartacea*. For the Brazilian species *Phaenopsectra*, *Chironomus* and *Polypedilum* no consumption was observed (Tab. I). Laboratory specimens had lower consumption rates than wild specimens (Tab. II). Maximum consumption rates reached 0.47 ± 0.28 of leaf $mg \cdot animal^{-1} \cdot day^{-1}$ (*Chironomus* on *Protium heptaphyllum*).

DISCUSSION

In the experiments with wild specimens, survivorship under laboratory conditions ranged from 60 to 100%. Those values are in the range of the reported for other invertebrates

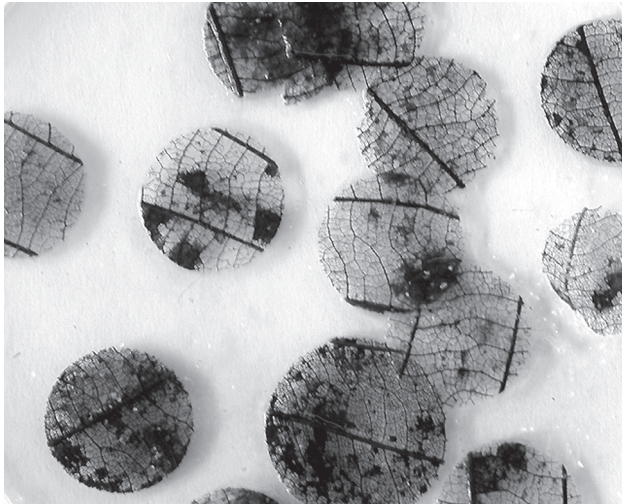


Figure 3. Leaf discs of alder (*Alnus glutinosa*) after being exposed to *Chironomus riparius* for three days under laboratory conditions.

reared under laboratory conditions (FEIO & GRAÇA 2000, GRAÇA *et al.* 2001, GONZÁLEZ & GRAÇA 2003). Feeding rates here reported ($0.06\text{--}0.85\text{ mg animal}^{-1}\text{ day}^{-1}$) are in the upper range of values reported for other food sources: $0.04\text{--}0.27\text{ mg animal}^{-1}\text{ day}^{-1}$ for *Pseudochironomus richardsoni* Malloch, 1915 feeding on algae

(GRESENS 2001), and in the lower range of the reported for true shredders (e.g., $0.47\text{--}1.88\text{ mg animal}^{-1}\text{ day}^{-1}$ for Sericostomatid caddisflies; FEIO & GRAÇA 2000, WAGNER 1990). Results of consumption are probably underestimation of the real capability of larvae to feed on leaves since dead specimens and specimens which pupated had evidences of leaf tissue removal and reduction of mass in discs (not taken into account for our calculations).

Not all populations consumed leaves at the same extent. In general, laboratory populations were not able to feed on leaves. The specimens sampled in the Indaiá stream performed badly on decomposing leaves. This could be an indication that leaf litter is not an energy source for chironomids in that system or for the assayed species. Consistently, invertebrate shredders are almost absent from this stream (CALLISTO *et al.* 2001, GONÇALVES *et al.* 2006b), probably due the low quality (chemical composition) of local riparian leaves in terms of hardness (MORETTI *et al.* 2007) and due to flush flow regime of the stream (GONÇALVES *et al.* 2006a).

The field taxa *Micropsectra apposita*, *Polypedilum albicorne*, *Eukiefferiella claripennis*, *Ablabesmyia* sp. and *Rheocricotopus (Psilocricotopus) atripes* were able to feed on leaves. According to the literature, those species are considered gathering collectors and, to a lesser extent engulferers, piercers (just *Ablabesmyia*) and shredders (GRUBBS & CUMMINS 1994, TAVARES-CROMAR & WILLIAMS 1997). In a related study GRUBBS *et al.* (1995) also sampled *Eukiefferiella claripennis* (3–12% of total chironomids), *Micropsectra*

Table 1. Survivorship, percentage of chironomids feeding on leaves and leaf consumption (\pm s.d.; $n = 20$) of wild specimens on conditioned leaves. (Pt) Portuguese populations, (Bz) Brazilian populations, (Ind) Indaiá stream, (Ibi) Ibitiré stream.

Chironomid species	Plant species	% survivorship	% chironomid feeding	Consumption (mg.animal ⁻¹ .day ⁻¹)	Origin
<i>Micropsectra apposita</i> (Walker, 1856)	<i>Alnus glutinosa</i> (L.) Gaertn	75	100	0.15 ± 0.10	Pt
<i>Polypedilum albicorne</i> (Meigen, 1838)	<i>Alnus glutinosa</i> (L.) Gaertn	75	100	0.17 ± 0.04	Pt
<i>Polypedilum albicorne</i> (Meigen, 1838)	<i>Miconia chartacea</i> Triana	69	65	0.85 ± 0.33	Pt
<i>Eukiefferiella claripennis</i> Lundbeck, 1898	<i>Alnus glutinosa</i> (L.) Gaertn	77	65	0.16 ± 0.05	Pt
<i>Eukiefferiella claripennis</i> Lundbeck, 1898	<i>Nerium oleander</i> L.	80	90	0.23 ± 0.06	Pt
<i>Ablabesmyia</i> sp. and <i>Eukiefferiella claripennis</i> Lundbeck, 1898*	<i>Alnus glutinosa</i> (L.) Gaertn	100	57	0.14 ± 0.03	Pt
<i>Rheocricotopus (Psilocricotopus) atripes</i> Kieffer, 1913	<i>Alnus glutinosa</i> (L.) Gaertn	73	70	0.17 ± 0.06	Pt
<i>Phaenopsectra</i> sp. Kieffer, 1921	<i>Miconia chartacea</i> Triana	100	0	no consumption	Bz-Ind
Chironominae genera varia	<i>Miconia chartacea</i> Triana	100	35	0.38 ± 0.27	Bz-Ind
<i>Chironomus</i> spp. Meigen, 1803	<i>Miconia chartacea</i> Triana	60	0	no consumption	Bz-Ind
<i>Polypedilum</i> spp. Kieffer, 1912	<i>Miconia chartacea</i> Triana	70	0	no consumption	Bz-Ind
<i>Chironomus</i> Meigen, 1803	<i>Miconia chartacea</i> Triana	100	80	0.29 ± 0.16	Bz-Ibi
	<i>Protium heptaphilum</i> (Aubl.) March	45	45	0.47 ± 0.28	Bz-Ibi
	<i>Ocotea</i> sp. (Schott) Mez	100	100	0.35 ± 0.25	Bz-Ibi
	<i>Solanum leucodendrum</i> (TRO)	100	90	0.33 ± 0.28	Bz-Ibi
	<i>Myrcia guyanensis</i> Aubl.	40	40	0.32 ± 0.20	Bz-Ibi

* *Ablabesmyia* sp. and *Eukiefferiella claripennis* were mixed in the obtained material.

Table II. Survivorship, feeding and leaf consumption ($X \pm s.d.$; $n = 20$) of laboratory chironomid species on conditioned leaves. (UC) University of Coimbra stock, (UFMG) Federal University of Minas Gerais stock.

Chironomid species	Plant species	% Survivorship	% chironomid feeding	Consumption (mg.animal ⁻¹ .day ⁻¹)	Origin
<i>Chironomus riparius</i> Meigen, 1804	<i>Alnus glutinosa</i> (L.) Gaertn	80	60	0.06 ± 0.07	UC
<i>Chironomus xanthus</i> Rempel, 1939	<i>Miconia chartacea</i> Triana	100	30	0.14 ± 0.05	UFMG
	<i>Protium heptaphilum</i> (Aubl.) March	100	20	0.10 ± 0.12	UFMG
	<i>Protium brasiliensis</i> (Spreng.) Engl.	100	0	no consumption	UFMG
	<i>Myrcia guyanensis</i> Aubl.	100	0	no consumption	UFMG
<i>Chironomus</i> Meigen, 1803	<i>Ocotea</i> sp. (Schott) Mez	50	10	no consumption	UFMG
	<i>Protium brasiliensis</i> (Spreng.) Engl.	30	30	0.31 ± 0.22	UFMG
	<i>Protium heptaphilum</i> (Aubl.) March	30	30	0.25 ± 0.21	UFMG

sp. (2-19%) and *Polypedilum* spp. (in lower densities) in decomposing leaves and suggested that shredding chironomids can contribute to the decomposition of fast decay rate leaves, but not to slow decay rate plant species. *Eukiefferiella* sp. was also reported in high densities in severely decayed wood in a stream by COLLIER & HALLIDAY (2000). GONÇALVES *et al.* (2000, 2003, 2004) reported that Chironomids reached over 50% of total invertebrate abundance among 29 taxa colonizing detritus of aquatic macrophytes. *Chironomus*, *Goeldichironomus*, *Parachironomus* Lenz, 1921, *Polypedilum* and *Rheotanytarsus* Thinemann & Bause *in* Bause, 1913 were the most abundant genera, reaching over 50% (40-70%) of detritus associated macrofauna.

We used late (3rd-4th) instar larvae in our experiments, but in an earlier experiment with 2nd instar of *Chironomus riparius* none of the individuals were able to survive up to pupation (data not shown). According to PINDER & REISS (1983) 1st instar larvae of several chironomids fed mainly by diatoms, but probably because of particle size rather than diatoms per se; later instars of *Eukiefferiella ilkleyensis* Edwards, 1929 seem to switch to macrophytes (*Ranunculus calcareous* Butcher) in the 3rd and 4th instars. Shifts in food items in chironomids during development have also been reported by other authors (TAVARES-CROMAR & WILLIAMS 1997). Therefore it is plausible that many chironomids in streams may be able to feed on decomposing leaves in instars, especially fast decomposing and well conditioned leaves. They therefore may have a contribution to decomposition of coarse particulate organic matter in streams, particularly in the summer (GRUBBS & CUMMINS 1994, MENENDEZ *et al.* 2003) and when their densities are high.

It was frequently observed that many chironomid larvae eat the leaf tissue from the inside, between the abaxial and adaxial epidermis (CALLISTO *et al.* 1996). Besides surface feeding, we also observed a "mining" behavior in some leaves, the construction of cases with faecal pellets, and pupation within some leaves. A set of laboratory experiments with *Psectrocladius limbatellus* Holmgren, 1869 processing organic matter estimated that 59% was incorporated into tubes, 39% was present in faecal pellets (HIRABAYASHI & WOTTON 1999).

In summary, our results show that some chironomids are able to use well conditioned leaves as food source; their role on leaf decomposition depends on their density, quality of leaves and the presence of other consumers with high efficiency using coarse particulate organic matter as food source.

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