1 The role of the freshwater shrimp *Atyaephyra desmarestii* in leaf litter breakdown in
2 streams

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Abstract

In aquatic ecosystems, microorganisms and invertebrates provide critical links between plant detritus and higher trophic levels. *Atyaephyra desmarestii* is an omnivorous decapod that inhabits freshwaters and exhibit high tolerance to temperature oscillations and high ability to colonize new habitats. Although A. *desmarestii* is able to ingest a variety of foods, few studies were conducted to elucidate the feeding preferences of this freshwater shrimp on allochthonous detritus. In this study, *A. desmarestii* was allowed to feed on conditioned or non-conditioned alder and eucalyptus leaves in microcosms, in which the animals had access or not to fecal pellets. At the end of the experiment, total body length of the animals was measured, and the remaining leaves and fecal pellets were used for dry mass quantification and assessment of bacterial and fungal diversity by denaturing gradient gel electrophoresis (DGGE). Cluster analyses of DGGE fingerprints indicated that the major differences in microbial communities on leaves or fecal pellets were between leaf types and conditioned and non-conditioned leaves, respectively. The consumption rate by the shrimp did not differ between leaf types, and was significantly higher on leaves conditioned by microorganisms and in treatments without access to feces. In treatments without access to feces, the production of feces and fine particulate organic matter was also significantly higher for conditioned leaves. Overall, our results support the feeding plasticity of *A. desmarestii* and its potential role in plant litter breakdown in streams. This can have implications to maintain stream ecosystem functioning, particularly when typical sensitive shredders decline.

Keywords: *Atyaephyra desmarestii,* consumption rate, detritus quality, fungi, bacteria

Introduction

In freshwater ecosystems, coarse particulate organic matter (CPOM), mainly constituted by fallen leaves and twigs from riparian vegetation, is the major energy input to low-order forested streams (Allan & Castillo, 2007). The decomposition of CPOM is a complex process and extensively conducted by microorganisms and invertebrates (Suberkropp, 1998; Bärlocher, 2005). Both fungi and bacteria play a central role, not only in decomposing plant material, but also transforming it into a more palatable and nutritious food source for invertebrates. Among microorganisms, fungi, especially aquatic hyphomycetes, are reported to dominate microbial decomposition in streams due to notable adaptations such as the ability to i) produce large amounts of sigmoid and

49 tetraradiate conidia that facilitates adhesion to substrates in turbulent waters (Bärlocher,
50 2009), ii) be active at low temperatures, and iii) produce lignocellulose-degrading
51 enzymes that break the structural compounds of plant material (Suberkropp, 1998;
52 Bärlocher, 2005).
53 Although in streams there are several functional feeding groups of benthic invertebrates
54 (Cummins & Klug, 1979), a major role in litter breakdown is attributed to shredders.
55 Shredders have mouthparts adapted for the maceration of CPOM converting it to fine
56 particulate organic matter (FPOM), which can be consumed by collectors ensuring the
57 transference of carbon and energy in aquatic detritus food webs (Graça, 2001; Alan &
58 Castillo, 2007). The feeding behaviour of many shredder species on plant litter is well
59 established (Graça et al., 2001). However, several typical shredders are sensitive to
60 environmental change, such as eutrophication (Pascoal et al., 2003; 2005), temperature
61 increase (Ferreira et al., 2010) and alteration in riparian vegetation (Graça et al., 2002).
62 Plant litter decomposition may be compromised when shredders decline, unless more
63 tolerant species with broader feeding plasticity will drive the process. In eutrophic
64 temperate streams, the decline of shredders on decomposing leaves can be accompanied
65 by an increased density of oligochaetes which contribute to litter breakdown due to their
66 movements and feeding behaviour (Pascoal et al. 2003; 2005). Also, in tropical streams,
67 where typical shredders are scarce or absent, organic matter breakdown is driven by
68 decapods able to play a range of trophic roles (e.g. Crow et al., 2001; Covich et al.,
69 2003).
70 *Atyaephyra desmarestii* Millet (1831) is a small decapod with a wide geographic
71 distribution in freshwater habitats, spanning from North Africa to the Middle East, a
72 large part of Europe and some Mediterranean islands (Muñoz et al., 2009; Straka &
73 Špaček, 2009). In Portugal, this species occurs in different habitats, such as rivers,
74 temporary streams, reservoirs, rice fields, lakes, and coastal lagoons (Fidalgo &
75 Gerhardt, 2003). A variety of food sources, namely algae, mud, fecal pellets and plant
76 litter, are reported to be used by *A. desmarestii* (Fidalgo, 1985; 1990a; 1990b; Fidalgo
77 & Gerhardt, 2003; Callisto, 2006), and this species constitutes an important food item
78 for fish (García-Berthou & Moreno-Amich, 2000). Because this species is quite tolerant
79 to temperature and salinity variations and is very successful in colonizing new aquatic
80 environments (Gauthier, 1924; Van den Brink & Van der Velde, 1986; Fidalgo, 1989;
81 Fidalgo & Gerhardt, 2003; Janssens de Bisthoven et al., 2006; Straka & Špaček, 2009),
82 we expect that *A. desmarestii* may occupy vacant ecological niches when sensitive

83 invertebrates, such as shredders, decline. This may be particularly relevant under the
84 ongoing global change. However, few studies were conducted to assess the role of this
85 freshwater shrimp in organic matter turnover (but see Calisto 2006). To elucidate the
86 feeding preferences of *A. desmarestii* for particulate organic matter, conditioned or non-
87 conditioned leaves of alder and eucalyptus were exposed to the shrimps in microcosms,
88 in which the animals had access or not to feces. After 16 days, we determined leaf
89 consumption and fecal production by *A. desmarestii*, and fungal and bacterial diversity
90 associated with leaf litter and fecal pellets.
91
92 **Methods**
93 Microcosm setup
94 The shrimps were collected with a hand net (2 mm mesh size) near submerged
95 macrophytes (*Ceratophyllum* sp., *Elodea* sp., *Myriophyllum* sp. and *Potamogeton* sp.) at
96 the left bank of the Minho River (northern Portugal) at about 12 km upstream the mouth
97 of the river. The animals were acclimated to the laboratory for two weeks prior the
98 experiment (water temperature: 15 ± 1 ºC; photoperiod: 12 h light and 12 h dark). The
99 acclimation was done in glass aquaria containing stream water diluted 1:2 with
100 dechlorinated tap water, under constant aeration. The stream water had pH = 7.64 ±
101 0.23, conductivity = 84.00 ± 1.41 µS cm^{-1}, total dissolved solids = 40.50 ± 3.54 mg L^{-1},
102 hardness = 31.00 ± 1.41 mg $CaCO_3$ L^{-1}, nitrate = 0.57 ± 0.06 mg N-NO_3 L^{-1},
103 orthophosphate = 0.12 ± 0.01 mg P-PO_4 L^{-1}, planktonic chlorophyll *a* = 0.40 ± 0.19 mg
104 m^{-3}. During acclimation, animals were fed *ad libitum* on mud, aquatic macrophytes,
105 tetramin and leaves of *Alnus glutinosa* (L.) Gaertn (alder) and *Eucalyptus globulus*
106 Labill (eucalyptus). The animals were starved for two days before the beginning of the
107 experiment.
108 Leaves of alder and eucalyptus, collected in October 2008, were leached for one day
109 and cut into 12 mm diameter disks. Sets of leaf disks were placed in fine-mesh bags (0.5
110 mm; 20 x 20 cm) and immersed in a stream on 25th November 2008 to allow microbial
111 colonization. After 7 days of immersion, the bags were brought to the laboratory and
112 colonized leaf disks were washed to remove debris prior to be placed in microcosms.
113 Microcosms consisted of plastic vessels with 200 mL of filtered (140 µm-pore size
114 membrane) stream water diluted 1:2 as indicated above. Half of the microcosms
115 contained a plastic mesh (1 mm pore size) to separate shrimps and leaf disks from the
116 bottom of the vessel and prevent coprophagy. One animal and ten leaf disks were placed

117 into each microcosm according to the following treatments (15 replicates): leaf type
118 (alder *versus* eucalyptus), microbial conditioning (colonized *versus* non-colonized
119 leaves) and coprophagy (animal with access or not to feces). Microcosms without
120 shrimps were used to correct leaf mass loss due to factors other than feeding.
121 Microcosms were kept under constant aeration at 15 ± 1 ºC with a photoperiod of 12 h
122 light and 12 h dark for 16 days. Half of the water in each microcosm was replaced every
123 two days. At the end of the experiment, the total animal length was measured; leaf
124 disks, feces and other FPOM were collected and freeze dried for dry mass quantification
125 and microbial diversity assessment.
126
127 Animal biomass
128 Animal dry mass was estimated based on the animal length, measured from the tip of
129 the rostrum to the end of the uropods, as follows: $\ln W = -0.49 + 0.14 L$, where W is the
130 animal dry weight in mg and L is the total body length of the animal in mm (Fidalgo,
131 1983).
132
133 Leaf consumption and fine particulate organic matter production
134 Leaf disks, and fecal pellets and other FPOM were freeze-dried for two days and
135 weighed to the nearest 0.01 mg. Consumption rates were calculated as the difference
136 between dry mass of leaves unexposed and exposed to the animal divided by the
137 exposure time (16 days). Values were expressed as mg leaf dry mass per animal per
138 day. Fecal and FPOM production rates were determined as mg of feces and FPOM,
139 respectively, per animal per day.
140
141 Microbial diversity
142 DNA was extracted from four freeze-dried leaf disks (randomly selected from eight
143 replicates) and from ca. 25 mg of fecal pellets with a soil DNA extraction kit (MoBio
144 Laboratories, Solana Beach, California), according to the manufacturer instructions. The
145 ITS2 region of fungal ribosomal DNA was amplified with the primer pair ITS3GC and
146 ITS4 (Duarte et al., 2010), while bacterial ribosomal DNA was amplified with the
147 primer pair 338GC and 518 (Duarte et al., 2010). For PCR of fungal and bacterial DNA,
148 2x of GoTaq® Green Master Mix (Promega), 0.4 µM of the appropriate primers and
149 1 µL of DNA were used in a final volume of 25 µL.

150 PCRs were carried out in a MyCycler Thermal Cycler (BioRad Laboratories, Hercules,
151 CA, USA) using the following program: initial denaturation at 95 ºC for 2 min; 36
152 cycles of denaturation at 95 ºC for 30 s; primer annealing at 55 ºC for 30 s and
153 extension at 72 ºC for 1 min; and final extension at 72 ºC for 5 min (Duarte et al., 2010).
154 Denaturing gradient gel electrophoresis (DGGE) was performed using a DCode™
155 Universal Mutation Detection System (BioRad Laboratories, Hercules, CA, USA). For
156 fungi, 700 ng of the amplified DNA products with 380-400 bp were loaded on 8% (w/v)
157 polyacrylamide gel in 1x Tris-acetate-EDTA (TAE) with a denaturing gradient from 30
158 to 70% (100% denaturant corresponds to 40% formamide and 7 M urea). For bacteria,
159 700 ng of the amplified DNA products with 200 bp were loaded on 8% (w/v)
160 polyacrylamide gel in 1x TAE with a denaturing gradient from 40 to 75%. The gels
161 were run at 55 V, 56 ºC for 16 h and stained with 1x of GelStar (Lonza, Rockland, Inc.
162 USA) for 10 min. The gel images were captured under UV light in a gel documentation
163 system GenoSmart (VWR International, East Grinstead, UK).
164
165 Statistical analyses
166 A three-way ANOVA was used to test the effects of leaf type (alder or eucalyptus),
167 microbial conditioning (colonized or non-colonized leaves) and coprophagy (animal
168 with access or not to feces) on animals dry mass and leaf consumption rates. A two-way
169 ANOVA was used to test the effects of leaf type and microbial conditioning on fecal
170 and other FPOM production by the shrimps.
171 The DGGE gel was aligned and normalized with GelCompar II (Applied Maths,
172 Belgium), and cluster analyses of banding patterns were done using the unweighted pair
173 group method average (UPGMA) based on the Dice coefficient of similarity.
174
175 **Results**
176 Leaf consumption rate by *A. desmarestii* varied between 0.01 and 0.75 mg leaf dry mass
177 animal^{-1} d^{-1} for non-conditioned alder leaves with access to feces and for conditioned
178 eucalyptus leaves without access to feces, respectively (Fig. 1). Both microbial
179 conditioning and access to feces affected leaf consumption rates by the animals (3-way
180 ANOVA, $P<0.0001$ and $P=0.0002$, respectively; Table 1). The highest consumption
181 rates were found in microcosms with conditioned leaves in which the shrimp had no
182 access to feces, no matter the leaf type.

183 Fecal production rates varied between 0.24 and 0.44 mg dry mass animal^{-1} d^{-1} for non-
184 conditioned eucalyptus leaves and conditioned alder leaves, respectively, when shrimps
185 had no access to feces (Fig. 2A). Fecal production rates were significantly affected by
186 microbial conditioning but not by leaf type (2-way ANOVA, P=0.01 and P=0.3,
187 respectively; Table 2). The production of fine particulate organic matter (FPOM),
188 excluding the feces, varied between 0.04 and 0.12 mg animal^{-1} d^{-1} for non-conditioned
189 eucalyptus leaves and conditioned alder leaves in microcosms without access to feces
190 (Fig. 2B). FPOM production rates were significantly affected by both leaf type and
191 microbial conditioning (2-way ANOVA, P=0.0003 and P=0.04, respectively; Table 2).
192 At the end of the experiment, mean shrimp biomass varied between 8.9 mg for non-
193 conditioned alder leaves with access to feces and 11.7 mg for non-conditioned
194 eucalyptus leaves with access to feces (Fig. 3), but these differences were not significant
195 (3-way ANOVA, P>0.05; Table 1). However, the interaction between leaf type and
196 access to feces was significant (P=0.02).
197 The analysis of fungal communities based on PCR-DGGE showed a higher number of
198 operational taxonomic units (OTUs) on leaves (up to 33 OTUs on conditioned alder
199 with access to feces) than in feces (up to 15 OTUs in microcosms with conditioned
200 alder leaves) (Fig. 4A). Cluster analysis of fungal DGGE fingerprints showed the
201 presence of two major groups: fungal communities on leaves and feces in eucalyptus
202 microcosms separated from those in alder microcosms (Fig. 4B). In each group, and
203 especially for alder, communities on leaves and feces in microcosms with conditioned
204 leaves separated from those with non-conditioned leaves (Fig. 4B).
205 A high number of bacterial OTUs was associated with both leaves and feces (up to 38
206 OTUs on conditioned alder leaves with access to feces) (Fig. 5A). Cluster analysis of
207 bacterial DGGE fingerprints indicated the presence of two major groups: communities
208 on leaves and feces from microcosms containing conditioned eucalyptus leaves
209 separated from the remaining treatments. Further, bacterial communities on leaves or
210 feces in microcosms with conditioned alder leaves separated from those with non-
211 conditioned leaves (Fig. 5B).
212

213 **Discussion**

214 In the present study, the shrimp *A. desmarestii* was able to feed on alder or eucalyptus
215 leaves and the leaf type did not affect its consumption rate. Moreover, consumption
216 rates of conditioned leaves were 75 times higher than those of non-conditioned leaves.

217 Microorganisms when growing on leaves degrade the recalcitrant polymers of plant-cell
218 wall and immobilize nutrients (e.g., nitrogen or phosphorus) either from plant material
219 or surrounding water, making plant litter more nutritious to consumers (Suberkropp,
220 1998). Several species of shredders are likely to selectively ingest fungal patches on
221 leaves (Bärlocher, 1985; Arsuffi & Suberkropp, 1988); consequently, leaf conditioning
222 can be even more important than leaf type in determining food preference and
223 consumption rate by invertebrates (Graça et al., 2001).
224 The lack of preference between leaf types was somehow surprising because lower
225 consumption rates of eucalyptus relatively to alder are reported for typical shredder
226 species. Indeed, in the laboratory, the shredders *Sericostoma vitattum* and *Tipula*
227 *lateralis* rejected conditioned eucalyptus leaves over other leaf species (Canhoto &
228 Graça, 1995; 1999). Eucalyptus leaves contain large amounts of oils, polyphenols and a
229 waxy cuticle that can restrain leaf consumption by invertebrate shredders (Canhoto &
230 Graça, 1999). However, Chironomidae larvae caused considerable mass loss of
231 eucalyptus leaves, which was attributed to their small size that allow these animals to
232 selectively consume leaf mesophyll while leaving the oil glands intact (Canhoto &
233 Graça, 1999). The putative ability of *A. desmarestii* to avoid oil glands when feeding on
234 eucalyptus leaves is currently unknown and is worth of further investigation.
235 In our study, consumption rates of conditioned alder leaves (0.67 mg leaf dry mass
236 animal^{-1} d^{-1}) by the shrimp *A. desmarestii* were lower than previously reported (1.24 mg
237 leaf dry mass animal^{-1} d^{-1}; Callisto, 2006). This may be due to differences in i) time of
238 leaf immersion in the stream (1 week in our study *versus* 3 weeks in the cited study)
239 and/or ii) water chemistry used in the experiment, aspects that are known to affect
240 microbial colonization of leaf litter (Pascoal et al., 2005). Indeed, the amount of
241 microbial biomass and the presence of certain microbial taxa on leaves are considered
242 important factors for determining food preferences of invertebrate shredders
243 (Suberkropp, 1998; Graça, 2001; Alan & Castillo, 2007).
244 Here, the production of feces by *A. desmarestii* varied between 0.24 and 0.44 mg dry
245 mass animal^{-1} d^{-1} in non-conditioned eucalyptus and conditioned alder leaves,
246 respectively. These values are similar to those found in a previous study in which *A.*
247 *desmarestii* fed on conditioned alder leaves (0.25 mg dry mass animal^{-1} d^{-1}; Callisto,
248 2006). Many consumers of CPOM produce large amounts of feces that are the dietary
249 mainstay for some animals, while for others, feces work as a food supplement
250 (Frankenberg & Smith, 1967; Wotton, 1980). Coprophagy has been reported in *A.*

251 *desmarestii* (Fidalgo, 1990b; Fidalgo & Gerhardt, 2003) and this was supported in our
252 study by an increased consumption of both leaf types when animals had no access to
253 feces. The production of fecal pellets and other FPOM was higher in treatments with
254 conditioned than non-conditioned leaves. Besides the production of FPOM by shrimp
255 feeding activities, microorganisms can also directly contribute to the fragmentation of
256 leaf material and the production of FPOM (Suberkropp, 1998). Additionally, we found
257 higher FPOM production from alder than eucalyptus leaves, which may be explained by
258 the high recalcitrance of eucalyptus leaves (Abelho & Graça, 1996).
259 In our study, the differences in leaf consumption and production of feces and other
260 FPOM by *A. desmarestii* were somehow supported by the structure of microbial
261 communities on leaves, as indicated by cluster analyses of DNA fingerprints of fungi
262 and bacteria. Although substrate is sometimes reported as a minor factor structuring
263 fungal communities (Nikolcheva & Bärlocher, 2005; Das et al., 2007), we found that
264 fungal communities on leaves or in feces in alder microcosms clearly differed from
265 those in eucalyptus microcosms. This separation may mirror differences in leaf quality,
266 since eucalyptus leaves have physical barriers and compounds that retard the
267 colonization and growth of some aquatic hyphomycete species (Canhoto & Graça,
268 1999).
269 We also found that communities of fungi or bacteria on leaves that were previously
270 conditioned in the stream differed from those on non-conditioned leaves. This was
271 expected because recently fallen leaves carry terrestrial fungi (e.g. Nikolcheva et al.,
272 2005) and bacteria that may remain on non-conditioned leaves. But when leaves are
273 immersed in a stream, aquatic species may take advantage leading to shifts in species
274 composition. The number of fungal OTUs was lower in non-conditioned leaves than in
275 colonized leaves, but such differences were not found for bacteria. The high number of
276 bacterial OTUs on non-conditioned leaves can be partially explained by the differences
277 between fungi and bacteria. Bacteria are typically suspended in or attached to a
278 substrate, and its presence on decomposing leaves do not necessarily mean that they are
279 having a role in litter decomposition; substrates can also be used for settling down. On
280 the contrary, saprotrophic fungi are typically filamentous and actively participate in
281 decomposition by the penetration of their hyphae in the substrates (Suberkropp, 1998).
282 In our study, microbial diversity on feces was high (as number of DGGE OTUs), and
283 the structure of fungal and bacterial communities was similar in fecal pellets and leaves.
284 In a recent study, the extraction, amplification and sequencing of DNA from feces of

285 two detritus feeding amphipods also revealed the presence of numerous fungal
286 phylotypes (Sridhar et al., 2010). The authors were able to recover up to 5 aquatic
287 hyphomycete species from invertebrate feces out of 10 species that were colonizing the
288 leaves (Sridhar et al., 2010). Also in our study, the number of fungal DGGE OTUs was
289 lower on feces than on leaves. However, we did not determine which DNA sequences
290 were assigned to aquatic microbes, therefore we cannot discard the hypothesis that some
291 fungal or bacterial OTUs on feces might belong to gut symbionts (Sridhar et al., 2010).
292 Overall results show that the freshwater shrimp *A. desmarestii* was able to consume
293 both alder and eucalyptus leaves at similar rates. Conditioned leaves were preferred
294 over non-conditioned leaves, and feces appeared to be a nutritious food source for this
295 freshwater shrimp. Hence, our results support the feeding plasticity of *A. desmarestii*.
296 Although this shrimp is generally associated with high quality waters, it has been found
297 at sites with compromised water quality (Oscoz & Durán, 2005). Moreover, this shrimp
298 is reported to tolerate notable temperature oscillations (Van den Brink & Van der Velde,
299 1986) and to have a high ability to colonize new freshwater habitats (Van den Brink &
300 Van der Velde, 1986; Oscoz & Durán, 2005; Straka & Špaček, 2009). Because typical
301 shredders are generally sensitive to water quality and warming temperature, their
302 populations may decline under the ongoing global change with impacts on plant litter
303 decomposition in streams (Graça et al., 2002; Pascoal et al., 2003; 2005; Ferreira et al.,
304 2010). For these reasons, it is conceivable that *A. desmarestii* may play a greater role in
305 plant litter breakdown in streams in the near future. Indeed, in some insular streams,
306 freshwater shrimps are the main drivers of litter breakdown following the riparian
307 inputs, acting as shredders, and are well adapted to disturbances rapidly re-colonizing
308 headwater streams following droughts and floods (Crow et al., 2001; Covich et al.,
309 2003).
310

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314

315 **References**
316 Abelho, M. & M.A.S. Graça, 1996. Effects of eucalyptus afforestation on leaf litter
317 dynamics and macroinvertebrate community structure of streams in central
318 Portugal. Hydrobiologia 324: 195-204.

319 Alan, J.D. & M.M. Castillo, 2007. Stream Ecology. Springer, Dordrecht, The
320 Netherlands.
321 Arsuffi, T.L. & K. Suberkropp, 1988. Effects of fungal mycelia and enzymatically
322 degraded leaves on feeding and performance of caddisfly (Trichoptera) larvae.
323 Journal of the North American Benthological Society 7: 205-211.
324 Bärlocher, F., 1985. The role of fungi in the nutrition of stream invertebrates. Botanical
325 Journal of the Linnean Society 91: 83-94.
326 Bärlocher, F., 2005. Freshwater fungal communities. In Deighton, J., P. Oudemans & J.
327 White (eds), The fungal community: its organization and role in the ecosystem.
328 Taylor and Francis, CRC Press, Boca Raton, Florida: 39-59.
329 Bärlocher, F., 2009. Reproduction and dispersal in aquatic hyphomycetes. Mycoscience
330 50: 3-8.
331 Callisto, M., 2006. Some laboratory evidences about the Mediterranean shrimp
332 *Atyaephyra desmarestii* feeding on *Alnus glutinosa* (L.) Gaertn leaf detritus. Acta
333 Limnologica Brasiliensia 18: 225-228.
334 Canhoto, C. & M.A.S. Graça, 1995. Food value of introduced eucalypt leaves for a
335 Mediterranean stream detritivore: *Tipula lateralis*. Freshwater Biology 34: 209-
336 214.
337 Canhoto, C. & M.A.S. Graça, 1999. Leaf barriers to fungal colonization and shredders
338 (*Tipula lateralis*) consumption of decomposing *Eucalyptus globulus*. Microbial
339 Ecology 37: 163-172.
340 Covich, A.P., T.A. Crowl & F.N. Scatena, 2003. Effects of extreme low flows on
341 freshwater shrimps in a perennial tropical stream. Freshwater Biology 48: 1199-
342 1206.
343 Crowl, T.A., W.H. McDowell, A.P. Covich & S.L. Johnson, 2001. Freshwater shrimp
344 effects on detrital processing and nutrients in a tropical headwater
345 stream. Ecology, 82: 775-783.
346 Cummins, K.W. & M.J. Klug, 1979. Feeding ecology of stream invertebrates. Annual
347 Review of Ecology and Systematics 10: 147-172.
348 Das, M., T.V. Royer & L.G. Leff, 2007. Diversity of fungi, bacteria, and actinomycetes
349 on leaves decomposing in a stream. Applied and Environmental Microbiology 73:
350 756-767.

351 Duarte, S., C. Pascoal, A. Alves, A. Correia & F. Cássio, 2010. Assessing the dynamic
352 of microbial communities during leaf decomposition in a low-order stream by
353 microscopic and molecular techniques. Microbiological Research 165:351-362.
354 Ferreira, V., A.L. Gonçalves, D.L. Godbold & C. Canhoto, 2010. Effect of increased
355 atmospheric CO_2 on the performance of an aquatic detritivore through changes in
356 water temperature and litter quality. Global Change Biology 16: 3284-3296.
357 Fidalgo, M.L. & A. Gerhardt, 2003. Distribution of the freshwater shrimp, *Atyaephyra*
358 *desmarestii* (Millet, 1831) in Portugal (Decapoda, Natantia). Crustaceana 75:
359 1375-1385.
360 Fidalgo, M.L., 1983. Oxygen consumption in *Atyaephyra desmaresti* Millet (Crustacea,
361 Decapoda, Natantia). Publicações do Instituto de Zoologia "Dr. Augusto Nobre"
362 176: 1-8.
363 Fidalgo, M.L., 1985. About the assimilation efficiency of the freshwater shrimp,
364 *Atyaephyra desmarestii* Millet (Crustacea, Decapoda). Verh International Verein
365 Limnology 22: 3227-3229.
366 Fidalgo, M.L., 1989. Biology of the freshwater shrimp *Atyaephyra desmarestii* Millet
367 (Decapoda: Natantia) in the river Douro, Portugal. I. Life cycle and individual
368 growth. Archiv für Hydrobiologie 116: 97-106.
369 Fidalgo, M.L., 1990a. Assimilation efficiency of the freshwater shrimp *Atyaephyra*
370 *desmaresti* Millet fed *Microcystis aeruginosa* Kutz. Publicações do Instituto de
371 Zoologia "Dr. Augusto Nobre" 221: 1-6.
372 Fidalgo, M.L., 1990b. Biology of the freshwater shrimp *Atyaephyra desmarestii* Millet
373 (Decapoda: Natantia) in the river Douro, Portugal. II: Feeding rate and
374 assimilation efficiency. Publ. Inst. Zool. "Dr. Augusto Nobre" 223: 1-19.
375 Frankenberg, D. & K.L. Smith, Jr., 1967. Coprophagy in marine animals. Limnology
376 and Oceanography 12: 443-449.
377 García-Berthou, E. & R. Moreno-Amich, 2000. Food of introduced pumpkinseed
378 sunfish: ontogenic diet shift and seasonal variation. Journal of Fish Biology 57:
379 29-40.
380 Gauthier, H., 1924. Recherches sur le développement larvaire *d'Atyaephyra desmaresti*
381 (Millet, 1831) (Décapodes, Natantia, Caridea, Atyidés). Bulletin de la Société
382 d'Histoire Naturelle de l'Afrique du Nord 15: 337-376.

383 Graça, M.A.S, C. Cressa, M.O. Gessner, M.J. Feio, K.A. Callies & C. Barrios, 2001.
384 Food quality, feeding preferences, survival and growth of shredders from
385 temperate and tropical streams. Freshwater Biology 46: 947-957.
386 Graça, M.A.S., 2001. The role of invertebrates on leaf litter decomposition in streams –
387 a review. International Review of Hydrobiology 86: 383-393.
388 Graça, M.A.S., J. Pozo, C. Canhoto & A. Elosegi, 2002. Effects of *Eucalyptus*
389 plantations on detritus decomposers, and detritivores in streams.
390 TheScientificWorldJournal 2: 1173-1185.
391 Janssens de Bisthoven, L., A. Gerhardt, K. Guhr & A.M.V.M. Soares, 2006. Behavioral
392 changes and acute toxiciy to the freshwater shrimp *Atyaephyra desmarestii* Millet
393 (Decapoda: Natantia) from exposure to acid mine drainage. Ecotoxicology 15:
394 215-227.
395 Muñoz, J.E.G., A. Rodríguez, J.E.G. Raso & J.A. Cuesta, 2009. Genetic evidence for
396 cryptic speciation in the freshwater shrimp genus *Atyaephyra* de Brito Capello
397 (Crustacea, Decapoda, Atyidae). Zootaxa 2025: 32-42.
398 Nikolcheva, L.G. & F. Bärlocher, 2005. Seasonal and substrate preferences of fungi
399 colonizing leaves in streams: traditional versus molecular evidence.
400 Environmental Microbiology 7: 270-280.
401 Nikolcheva, L.G., T. Bourque & F. Bärlocher, 2005. Fungal diversity during initial
402 stages of leaf decomposition in a stream. Mycological Research 109: 246-253.
403 Oscoz, J. & C. Durán, 2005. Contribution to the knowledge of the freshwater shrimp,
404 *Atyaephyra desmaresti* (Millet, 1831) (Decapoda, Natantia), in the Ebro basin
405 (Spain). Zoologica Baetica 16: 155-159.
406 Pascoal C., F. Cássio, A. Marcotegui, B. Sanz & P. Gomes, 2005. Role of fungi,
407 bacteria, and invertebrates in leaf litter breakdown in a polluted river. Journal of
408 the North American Benthological Society 24: 784-797.
409 Pascoal, C., M. Pinho, F. Cássio & P. Gomes, 2003. Assessing structural and functional
410 ecosystem condition using leaf breakdown: studies on a polluted river. Freshwater
411 Biology 48: 2033-2044.
412 Sridhar, K.R., M. Beaton & F. Bärlocher, 2010. Fungal propagules and DNA in feces of
413 two detritus feeding amphipods. Microbial Ecology, DOI 10.1007/s00248-010-
414 9732-4

415 Straka, M. & J. Špaček, 2009. First record of alien crustaceans *Atyaephyra desmarestii*
416 (Millet, 1831) and *Jaera istri* Veuille, 1979 from the Czech Republic. Aquatic
417 Invasions 4: 397-399.
418 Suberkropp, K., 1998. Microorganisms and organic matter decomposition. In Naiman
419 R.J. & R.E. Bilby (eds), River ecology and management: lessons from the Pacific
420 Coastal Ecoregion, Springer, New York: 120-143.
421 Van den Brink, F.W.B. & G. Van der Velde, 1986. Observations on the seasonal and
422 yearly occurrence and the distribution of *Atyaephyra desmaresti* (Millet, 1831)
423 (Crustacea, Decapoda, Natantia) in the Netherlands. Hydrobiological Bulletin 19:
424 193-198.
425 Wotton, R.S., 1980. Coprophagy as an economic feeding tactic in black fly larvae.
426 Oikos 34: 282-286.
427

428 **Tables**

429 **Table 1.** Effects of leaf type, microbial conditioning and access to feces on the biomass
430 of *A. desmarestii* and leaf consumption rates. Values were compared by a 3-way
431 ANOVA.

Treatment	Animal biomass			Leaf consumption rates		
	df	F	P	df	F	P
Leaf type	1	0.2	0.6	1	2.4	0.1
Microbial conditioning	1	1.5	0.2	1	30.9	<0.0001
Access to feces	1	1.1	0.3	1	15.3	0.0002
Leaf type* microbial conditioning	1	0.9	0.3	1	0.03	0.8
Leaf type*access to feces	1	5.7	0.02	1	0.1	0.7
Microbial conditioning*access to feces	1	0.04	0.8	1	0.5	0.5
Leaf type*microbial conditioning*access to feces	1	0.2	0.7	1	0.05	0.8

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434

435 **Table 2.** Effects of leaf type and microbial conditioning on fecal and other fine
436 particulate organic matter (FPOM) production by *A. desmarestii*. Values were compared
437 by a 2-way ANOVA.

Treatment	Fecal production			FPOM production		
	df	F	P	df	F	P
Leaf type	1	1.0	0.3	1	14.6	0.0003
Microbial conditioning	1	6.4	0.01	1	4.4	0.04
Leaf type*microbial conditioning	1	0.09	0.8	1	0.2	0.6

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439

440 **Figure legends**

441 Figure 1. Leaf consumption rates by *A. desmarestii* in microcosms with conditioned and
442 non-conditioned leaves of alder (white bars) and eucalyptus (black bars), with access or
443 not to feces. Mean ± SEM, n =15.

444

445 Figure 2. Production rates of feces (A) and other fine particulate organic matter (FPOM)
446 excluding feces (B) in microcosms with *A. desmarestii* feeding on conditioned or non-
447 conditioned leaves of alder (white bars) and eucalyptus (black bars). Mean ± SEM, n
448 =15.

449

450 Figure 3. Biomass of *A. desmarestii* after 16 days in microcosms with conditioned and
451 non-conditioned leaves of alder (white bars) and eucalyptus (black bars), with access or
452 not to feces. Mean ± SEM, n =15.

453

454 Figure 4. DGGE fingerprints (A) and cluster dendograms (B) of fungal communities on
455 leaves (L) and *A. desmarestii* feces (F). Dendograms were constructed from UPGMA
456 analysis based on the Dice coefficient of similarity. M, mixture of DNA of 5 aquatic
457 hyphomycete pure cultures.

458

459 Figure 5. DGGE fingerprints (A) and cluster dendograms (B) of bacterial communities
460 on leaves (L) and *A. desmarestii* feces (F). Dendograms were constructed from
461 UPGMA analysis based on the Dice coefficient of similarity. M, mixture of DNA of 5
462 bacterial pure cultures.

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