CHAPTER 50

IDENTIFYING STREAM INVERTEBRATES AS PLANT LITTER CONSUMERS

LUZ BOYERO[1], RICHARD G. PEARSON[2], RICARDO J. ALBARIÑO[3], MARCOS CALLISTO[4], FRANCISCO CORREA-ARANEDA[5], ANDREA C. ENCALADA[6], FRANK MASESE[7], MARCELO MORETTI[8], ALONSO RAMÍREZ[9], APRIL SPARKMAN[10], CHRISTOPHER M. SWAN[10], CATHERINE M. YULE[11] & MANUEL A. S. GRAÇA[12]

[1]University of the Basque Country, Leioa, Spain & Ikerbasque, Bilbao, Spain; [2]James Cook University, Townsville, Australia; [3]National University of Comahue-CONICET, Quintral, Argentina; [4]Federal University of Minas Gerais, Belo Horizonte, Brazil; [5]Universidad Autónoma de Chile, Chile; [6]University of San Francisco de Quito, Quito, Ecuador; [7]University of Eldoret, Eldoret, Kenya; [8]University of Vila Velha, Vila Velha, Brazil; [9]University of Puerto Rico, San Juan, USA; [10]University of Maryland, Baltimore, USA; [11]Monash University, Selangor, Malaysia; [12]MARE – Marine and Environmental Sciences Centre & Department of Life Sciences, University of Coimbra, Coimbra, Portugal.

1. INTRODUCTION

Invertebrates play a key role in the decomposition of plant litter (i.e., leaf litter and wood) in streams (Graça 2001) through their feeding, case-making and burrowing activities. Animals in the shredder functional feeding group (Cummins 1973, Cummins & Klug 1979), which have mouthparts capable of cutting and chewing pieces of litter (Ramírez & Gutiérrez-Fonseca 2014), make the greatest invertebrate contribution to litter decomposition. Other functional feeding groups can also contribute by scraping leaf surfaces (scrapers), or by making tunnels in leaf mesophyll (miners) or wood (borers). Here we focus on all invertebrates that consume plant litter as a substantial proportion of their diets at some time in their life cycle. Hence, rather than adopting a functional feeding group perspective – which is based on feeding mode and relies on mouthpart morphology and feeding behaviour (Ramírez & Gutiérrez-Fonseca 2014) – we focus on the food items consumed, usually determined through gut content analysis (e.g., Cheshire et al. 2005, Chará-Serna et al. 2012). We thus use the term litter-consuming invertebrates to include invertebrates specialized in leaf shredding, scraping or mining, and wood shredding or boring, but also more generalist consumers that feed on a range of other materials such as fine particulate organic matter (FPOM) or periphyton while including a substantial proportion (usually ≥40%) of litter in their diets (Cheshire et al. 2005).

We describe the methods for gut content analysis, as this is the most straightforward way to determine whether and to what extent an invertebrate feeds on litter. However, on some occasions, material in the gut may not be readily identifiable, and mouthpart examination or behavioural observations may be required (e.g., Albariño & Díaz-Villanueva 2006, Mayer et al. 2008). Another useful approach to identify the origin of assimilated food (plant vs. animal, allochthonous vs. autochthonous) is stable isotope analysis (e.g., Mantel et al. 2004), although this method does not differentiate litter from FPOM and it does not identify material that is rapidly metabolised but not assimilated (Schmidt et al. 2017). DNA-based methods have proved successful in determining the diets of other invertebrates, but have not yet been used to identify benthic freshwater invertebrates as litter-consumers (e.g., Blankenship & Yayanos 2005, Hardy et al. 2010, Carreon-Martínz & Heath 2010).

2. EQUIPMENT AND MATERIALS

- Analytical balance (± 0.1 mg)
- Dissecting microscope (at least 50× and preferably higher magnification)

- Compound microscope
- Scalpel
- Forceps
- Dissection needles (insect mounting pins mounted in a cork or a glass or wooden rod)
- Glass slides and coverslips
- Polyvinyl alcohol-lactophenol or an alternative mountant

3. PROCEDURES

3.1. Invertebrate collection

1. Locate litter packs in the stream bed and capture all litter-consuming invertebrates by scooping the litter with a dip net. If the relative abundance of litter-consuming invertebrates is important for the study, collect with regular methods of sampling benthic invertebrates (*e.g.*, Surber or Hess sampler).
2. Very large and mobile litter consumers such as crayfish or freshwater crabs may be missed with these methods. Therefore, target such litter consumers separately by using electro-shocking procedures (not efficient for burrowing crayfish) or baited traps (in which case the gut contents tend to include the bait!).
3. If particular invertebrate species are to be collected, inspect individual leaves in litter packs retained in different areas of the stream bed and collect the invertebrates of interest with forceps or a small soft brush. For example, cased caddisflies are often located in depositional areas, while large tipulids are more common in riffle areas.
4. Although the sample size and level of replication will be dictated by the particular purpose of each study, ideally observe no less than 20 specimens.

3.2. Invertebrate abundance and biomass

1. To determine invertebrate abundance and biomass, pick all individuals from each litter sample and separate them into species or morphospecies (hereafter called “species”). This can be done with live or frozen samples (see also Chapter 49).
2. Divide invertebrates of each species into size classes (e.g., small, medium, large), at least for initial analysis, to determine whether there is an ontogenetic shift in diet. If all sizes are found to have similar diets, this requirement may be dispensed with, or particular size groups may be targeted.
3. For each species, select a group of at least 5 specimens from each size class in each sample, ideally with full guts. This will result in at least 20 individuals per species per site and time. This could include all size groups if all have the same diet.
4. Remove cased caddisflies from their cases. Surface dry individuals on tissue paper, then weigh them collectively to the nearest 0.1 mg. Note the weight and the number of individuals weighed to give a mean weight per individual for each species, sample unit and, where appropriate, size class.
5. Oven-dry the litter sample (50 °C, 72 h) and weigh to the nearest 0.01 g.
6. Incinerate the litter sample in a muffle furnace (550 °C, 4 h) and reweigh to obtain an estimate of litter ash-free dry mass to express densities or biomass of animals per unit of litter dry mass.

3.3. Gut mounting

1. Examine each individual animal under a dissecting microscope.
2. Where possible, remove the gut, place it on a microscope slide, and squeeze out the contents.

3. For small individuals where guts cannot be removed, detach the head and squash the whole animal to eject the gut contents on the slide. For small animals without a sclerotized or calcareous exoskeleton covering the body, such as chironomids, entire animals may be mounted to assist with identifying the gut contents. Note that retaining the heads can help with identification (e.g., for chironomids).
4. Add a drop of polyvinyl alcohol-lactophenol to a slide, then add the gut contents and finally a coverslip. This mountant will allow slides to be usable for some days or months, but is not permanent.

3.4. Gut content analysis

1. Examine the slide under 100× magnification, using a graticule with a 10×10 grid. Discard slides where guts appear empty.
2. Visually estimate the proportion (%) of grid cells containing vascular plant tissue, which is identified by the presence of cell walls (Fig. 50.1). Use averages for species and size classes.
3. Assign feeding categories as follows: when ≥40% of gut contents consist of vascular plant tissue, classify the specimen as a litter-consuming invertebrate; consider animals with 40-70% of vascular plant tissue in their guts as generalists; and regard those in which vascular plant tissue represents >70% of their gut contents as specialists.

4. FINAL REMARKS

Table 50.1 presents a summary of families recorded as litter-consuming invertebrates in studies from across the globe. The list includes not only families in which most or many species are litter consumers, but also families where this type of diet is occasional or exceptional. Here we focus on the family level of taxonomic resolution because of space limitations, but a table of known genera and species is provided online, together with feeding modes (leaf shredder, leaf scraper, leaf miner, wood shredder/borer, or generalist) and a full reference list (LINK).

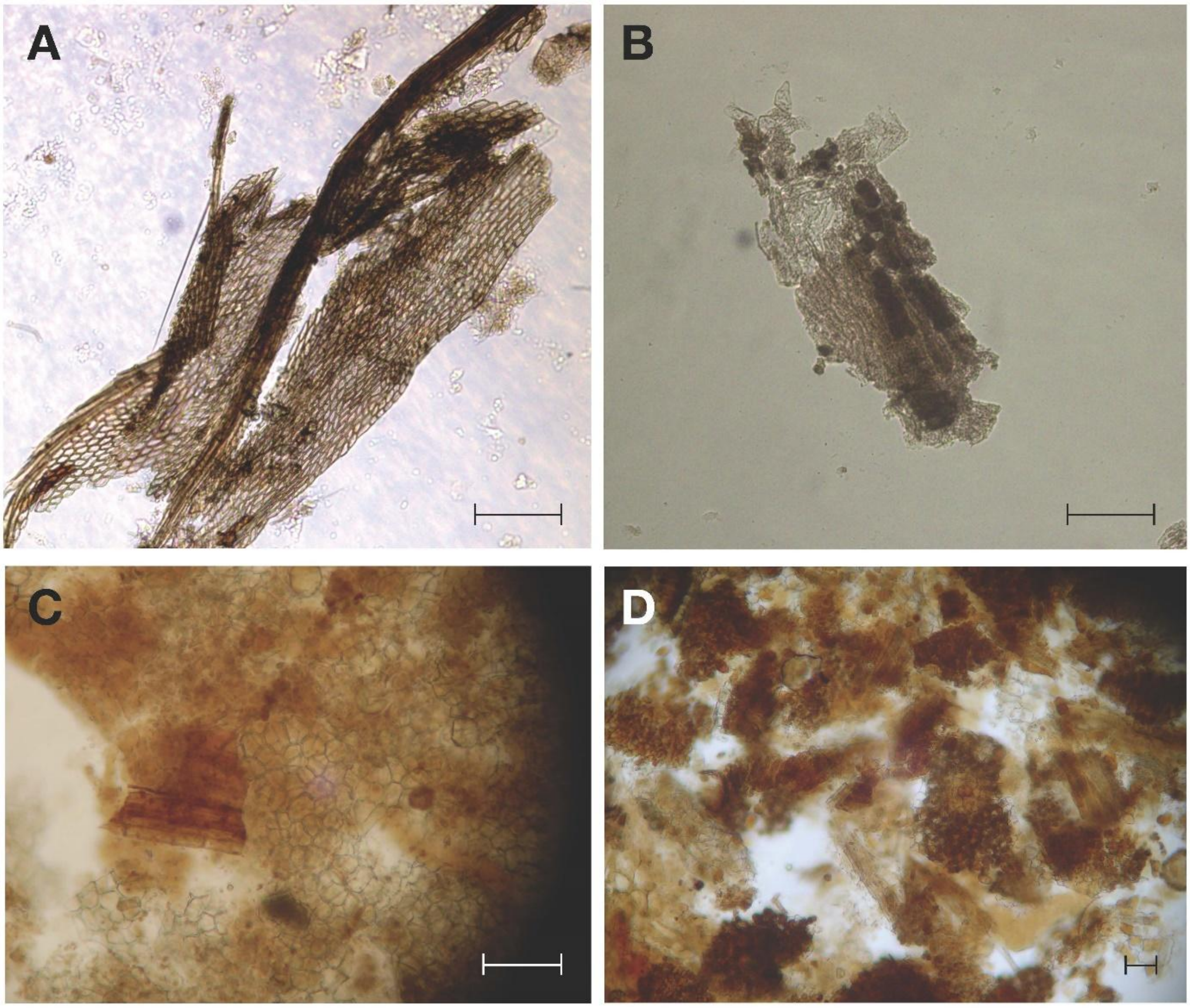

Figure 50.1. Slides photographed under a compound microscope showing gut contents of litter-consuming invertebrates. Plant cell walls are clearly distinguished in some cases (A, C) but not others (B, D). Bar = 100 µm. Photo by Ana Eguiguren (A, B) and Richard Pearson (C, D).

*Table 50.1. List of litter-feeding invertebrate families from different biogeographic regions (Nea: Nearctic; Neo: Neotropical; Pal: Palearctic; Afr: Afrotropical; Ind: Indomalayan; Aus: Australasian). *Families in which most species are litter consumers; §Families in which litter consumers are exceptional.*

ORDER/Family	***Nea***	***Neo***	***Pal***	***Afr***	***Ind***	***Aus***
GASTROPODA						
Ampullaridae		x				
Arctiidae		x				
Hydrobiidae						x
Lymnaeidae			x			
Melanopsidae			x			
Pachychilidae					x	
Planorbidae		x	x			x
Tateinae			x			
Thiaridae		x				
AMPHIPODA						
Corophiidae			x			
Crangonyctidae			x			
Gammaridae*			x		x	
Hyalellidae		x				
Hyalidae						x
Paracalliopiidae						x
Paramelitidae						x
Perthiidae						x
Pontogeneiidae						x
Talitridae			x			
ISOPODA						
Amphisopodidae						x
Asellidae			x			
Cirolanidae					x	
Janiridae		x				x
Oniscidae						x
DECAPODA						
Aeglidae		x				
Astacidae	x		x			
Atyidae §		x				x
Deckeniidae				x		
Gecarcinucidae				x	x	x
Grapsidae			x			
Palaemonidae		x		x		
Paramelitidae						x
Parastacidae		x				x
Parathelphusidae					x	
Potamidae					x	
Potamonautidae				x		
Pseudothelphusidae		x				
Sundathelphusidae					x	

ORDER/Family	***Nea***	***Neo***	***Pal***	***Afr***	***Ind***	***Aus***
Trichodactylidae		x				
Xiphocaridae		x				
EPHEMEROPTERA						
Baetidae		x		x		
Caenidae		x				
Ephemerellidae	x		x		x	
Euthyplociidae		x				
Leptohyphidae		x				
Leptophlebiidae		x			x	x
Melanemerellidae		x				
Oligoneuriidae		x				
Polymitarcyidae		x				
Potamanthidae	x					
Siphlonuridae	x					
Xiphocentronidae		x				
BLATTODEA						
Blaberidae		x			x	
PLECOPTERA						
Austroperlidae		x				x
Brachypterainae	x					
Capniidae*	x		x		x	
Chloroperlidae			x			
Diamphipnoidae		x				
Gripopterygidae		x				x
Leuctridae*	x		x		x	
Nemouridae*	x		x		x	
Notonemouridae		x				x
Peltoperlidae	x				x	
Perlidae§		x				
Perlodidae			x			
Pteronarcyidae	x				x	
Scopuridae					x	
Taeniopterygidae*	x		x		x	
HETEROPTERA						
Corixidae§		x	x			
COLEOPTERA						
Chrysomelidae		x	x			x
Curculionidae	x	x	x	x	x	x
Dryopidae		x	x		x	
Elmidae	x	x	x	x	x	x
Eulichadidae	x				x	
Haliplidae	x	x	x		x	
Helodidae					x	x
Helophoridae		x	x			
Hydraenidae		x	x			x
Hydrochidae		x	x			
Hydrophilidae	x	x	x			x
Lutrochidae		x				
Psephenidae		x				
Ptilodactylidae	x	x			x	x
Scirtidae	x	x	x		x	x
Staphylinidae		x				

DIPTERA						
Axymyiidae	x					
Ceratopogonidae		x				
Cylindrotomidae			x			
Chironomidae§	x	x	x		x	x
Cylindrotominae	x					
Dixidae		x				
Dolichopodidae					x	
Ephydridae	x	x	x		x	
Hydrelliinae	x					
Pelecorhynchidae	x					
Psychodidae			x			
Ptychopteridae	x		x		x	
Scathophagidae	x		x			
Stratiomyidae		x	x			
Tanyderidae						x
Tipulidae*	x	x	x	x	x	x
LEPIDOPTERA						
Coleophoridae	x					
Cosmopterigidae	x					
Crambidae	x	x	x	x	x	
ORDER/Family	***Nea***	***Neo***	***Pal***	***Afr***	***Ind***	***Aus***
Epipyropidae		x				
Musotiminae	x					
Nepticulidae	x					
Noctuidae	x	x			x	
Opostegidae		x				
Pyralidae*		x			x	x
Pyraustinae	x					

Schoenobiinae	x					
Spilomelinae	x					
Tortricidae	x	x				
TRICHOPTERA						
Anomalopsychidae		x				
Apataniidae	x		x			
Beraeidae			x			
Brachycentridae	x		x			
Calamoceratidae*	x	x	x	x	x	x
Calocidae						x
Conoesucidae						x
Ecnomidae		x		x		x
Glossosomatidae		x				
Helicopsychidae		x				
Hydropsychidae		x				
Hydroptilidae	x	x			x	
Lepidostomatidae*	x	x	x	x	x	
Leptoceridae*	x	x	x	x	x	x
Limnephilidae*	x	x	x		x	x
Limnocentropodidae					x	
Odontoceridae	x	x	x		x	
ORDER/Family	***Nea***	***Neo***	***Pal***	***Afr***	***Ind***	***Aus***
Oeconesidae						x
Parasericostomatidae*		x				
Phryganeidae	x		x		x	
Pisuliidae				x		
Polycentropodidae	x	x			x	
Philopotamidae		x				
Psychomyiidae			x			

Ptilocolepidae	x		x			
Rhyacophilidae	x		x			
ORDER/Family	***Nea***	***Neo***	***Pal***	***Afr***	***Ind***	***Aus***
Rossianidae	x					
Sericostomatidae*	x	x	x	x	x	
Tasiimidae		x				x

Theliopsychinae	x					
Xiphocentronidae		x				
Nº of known families	51	70	50	15	43	38

[1]Includes the subfamily Limoniinae.

5. REFERENCES

Albariño R.J. & Díaz-Villanueva V. (2006). Feeding ecology of two plecopterans in low order Andean-Patagonian streams. *International Review of Hydrobiology,* 91**,** 122-135.

Blankenship L.E. & Yayanos A.A. (2005). Universal primers and PCR of gut contents to study marine invertebrate diets. *Molecular Ecology,* 14**,** 891-899.

Carreon-Martínz L. & Heath D.D. (2010). Revolution in food web analysis and trophic ecology: diet analysis by DNA and stable isotope analysis. *Molecular Ecology,* 19**,** 25-27.

Chará-Serna A.M., Chará J.D., Zúñiga M.D.C., Pearson R.G. & Boyero L. (2012). Diets of leaf litter-associated invertebrates in three tropical streams. *International Journal of Limnology,* 48**,** 139-144.

Cheshire K., Boyero L. & Pearson R.G. (2005). Food webs in tropical Australian streams: shredders are not scarce. *Freshwater Biology,* 50**,** 748-769.

Cummins K.W. (1973). The utilization of leaf litter by stream detritivores. *Ecology,* 54**,** 336-345.

Cummins K.W. & Klug M.J. (1979). Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics,* 10**,** 147-172.

Graça M.A.S. (2001). The role of invertebrates on leaf litter decomposition in streams – a review. *International Review of Hydrobiology,* 86**,** 383-393.

Hardy C.M., Kurull E.S., Hartley D.M. & Oliver R.L. (2010). Carbon source accounting for fish using combined DNA and stable isotope analyses in a regulated lowland river weir pool. *Molecular Ecology,* 19**,** 197-212.

Mantel S.M.K., Salas M. & Dudgeon D. (2004). Foodweb structure in a tropical Asian forest stream. *Journal of the North American Benthological Society,* 23**,** 728-755.

Mayer G., Maier G., Maas A. & Waloszek D. (2008). Mouthparts of the Ponto-Caspian invader *Dikerogammarus villosus* (Amphipoda: Pontogammaridae). *Journal of Crustacean Biology,* 28**,** 1-15.

Ramírez A. & Gutiérrez-Fonseca P.E. (2014). Functional feeding groups of aquatic insect families in Latin America: a critical analysis and review of existing literature. *Revista de Biología Tropical,* 62**,** 155-167.

Schmidt K., Blanchette M., Pearson R.G., Alford R.A. & Davis A.M. (2017). Trophic roles of tadpoles in tropical Australian streams. *Freshwater Biology,* 62**,** 1929-1941.