Freshwater meiofauna in Mediterranean lotic systems: community structure, adaptations and contribution to functional processes

Meiofauna d'aigua dolça en sistemes lòtics mediterranis: estructura de les seves comunitats, adaptacions i contribució en els processos funcionals

Ainhoa Gaudes Saez

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Departament d'Ecologia
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Ainhoa Gaudes Saez
2011

TESI DOCTORAL

Departament d'Ecologia

Universitat de Barcelona

Programa de doctorat: Titulació d'Estudis Avançats en Ecologia

Bienni: 2002-2004

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Memòria presentada per Ainhoa Gaudes Saez per optar al grau de doctor per la Universitat de Barcelona

Ainhoa Gaudes Saez

Barcelona, abril de 2011

Vist-i-plau del director de tesi

Dra. Isabel Muñoz Gracia

Professora titular del Departament d'Ecologia

Universitat de Barcelona

A les dues dones

que m’han ensenyat

el més important de la vida

(26/8/2009-6/8/2010)

No existe ningún buen biólogo , cuyos trabajos fueran coronados con el éxito, que no haya sido llevado hacia su profesión por aquel placer interior que deriva de contemplar las bellezas de las criaturas vivas, y que al mismo tiempo no sienta aumentar su placer en la Naturaleza y en el trabajo a medida que se amplían sus conocimientos profesionales

KONRAD LORENZ

Hablaba con las bestias, los peces y los pájaros
2ª edició. Ed. Labor. 1994
(Traduït per Ramón Margalef)

Hi havia una vegada una noia que es deia Ainhoa que pul·lulava per la facultat de Biologia. Anava de classe en classe i de tant en tant passava pel bar (o era a l'inrevés?). Un cop va fer unes pràctiques d'Eco2 i a la sortida a Torredembarra van trobar una pilota i es va posar a jugar a futbol amb en Jose, el seu professor. Ell li va comentar que hi havia unes noies del seu departament que també volien jugar: la Montserrat Vidal, l'Ester Nin i la Marta Comerma. Amb elles i amb uns quants amics més de la carrera (Laura, Montse, Iria, Marcs, Xavis, Jordis, Nuri i les supporters: Irene, Esther, Annes i Montse F.) es va fundar l'equip Panmixia (l'únic mixte a la competició!). Allà es van enfrontar a temibles guerrers-ecòlegs: en Xavi dP, en Joan Gomà, els Miguel Angel i en Pere Renom. (...)

(...) Van arribar unes pràctiques de limnologia amb uns professors que es deien Sergi, Helena i Narcís. Aquella experiència va marcar l'Ainhoa ja que es va trobar a gust tot mirant animalets pel microscopi, entre jocs, acudits i molts i molts moments divertits. I vet aquí, que en tornar, va agafar al seu amic Julio i un altre noi i se’ls va endur a mostrejar pel Penedès. Així, va ser com l'Ainhoa va entrar a la sala de lupes on aviat va poder conversar amb dues noies molt simpàtiques de cabell ben curtet, la Rosa i la Mi.

Però això no l'omplia prou...i al final entre bromes, en Sergi li va proposar d'ajudar a la Isabel que tot just acabava d'obtenir la plaça de titular i tenia moooooltes ganes de fer coses noves. I així, ajudant també la Bet Vilalta, l'Enrique Navarro, l'Helena Guasch i l'Anna Romaní al camp, als experiments i mirant mostres, l'Ainhoa es va començar a interessar molt pels rius. A partir d'aquí van venir les pràctiques a URS (amb en Miquel, la Isabel, la Montserrat, la Glòria, etc) i l'estada a Bilbao amb l'Arturo i en Jesús (on va conèixer també l' Oihana, l'Aitor, el Xanti, l'Ainhoa, els Javis, en Joseba i en Raúl). En tornar, l'Isabel va proposar a l'Ainhoa encetar el camí que ara s’acaba...

Els millors anys van arribar: el DEA. Nous companys (Ernesto, Blanca, Gerardo, Gonzalo) i vells coneguts (Biel, Neus, Eusebi, Josep i Enric). Un grup tan heterogeni però amb el qui va passar la nit més divertida de la seva vida: milers d'acudits i moooolt alcohol (va por ti, Hugo!). I a partir d'aquí, va compartir molts bons moments amb la gent del departament. Els moments *laboratori amb: la Mary, la Neus i en Biel. Els moments despatx amb: en Jaime, en Luciano, en Rafa, la Susanna, l'Eusebi (ànims!), en Gonzalo, la Marta i la Bet. Els moments del camp: amb la Bet Vilalta, l'Elena Guerra, la Txell Omella, la Rut, l'Isis, l'Esther Mas, en Cristophe, i en Julio (mi fichaje estrella!) Els moments sala de lupes amb: els Sabineros (Tureta, Blanca, Cesc, Mireia, Rosa, Raul i Iraima), la Caro, la Núria Cid, en Pau, la Mia, el Miguel i la Núria Bonada. Els moments dinars i cafès amb: l'Olga, en Pere, en Xavi dP, en Salva, la Izaskun, la Silvia, la Tania, la Isabel Dorado, l'Elena i la Montse de forestals, la Gemma, en Carles, l'Oriol, Lidia, Dani...I amb la gent d'arreu: l'Alba, en Dani i en Guillermo de Blanes; la Sandra, en Joan i l'Elisabet de Girona; en Tom, l' Annick, en Giovanni, la Tania i la Maria de Gent (Dank-U-well and muito obrigada for your always kind attention, for offering me your lab, your home and your friendship!); gent del departament i tots els amics i amigues (colla, Oriol, CAU, bus, grup de mames, etc) de Sant Sadurní ....SSSSHHHHHHH!!*

Ara que ja dorm la criatura, s'acaba el conte i us diré un secret a cau d'orella. Tota aquesta gent m'ha ajudat en algun moment o altre de la tesi, ja fos directament en la feina, amb un cafè o un somriure...Amb alguns d'ells, és cert, he viscut moments molt especials, organitzant trobades dels JI-AILS (Biel i Núria amb vosaltres sempre hi ha hagut xispa i smiles&kissess!!!), amb visites a l'estranger (Neus, Julio i Mary la vostra visita va ser genial i no podia estar millor acompanyada!), etc. Gràcies també pels moments de passadís/font on hem pogut parlar, pels innombrables vídeos comunitaris al despatx, massatges i "gamberrades" varies. I bé, també en els moments més importants de la vida, com són l'arribada i l'adéu d'un esser estimat també m'he sentit molt estimada per tots i cada un de vosaltres.

Vull agrair especialment a la Isabel, per l'ajuda, comprensió i paciència que has tingut amb mi aquests anys, i sobretot per la teva confiança! Als meus pares que em van ensenyar des de petita els valors més importants per aquesta tesi, la curiositat i la constància. Al Guillem per tot l'amor, l'ajuda i la comprensió durant aquests anys (i especialment aquests darrers!). A la seva família, les meves tietes i cosins que m'han ajudat molt i molt...i a tu, petita, per fer-me tirar endavant!

Ainhoa Gaudes Saez
Cal Tut, 2 d'Abril de 2011
(Dia internacional del llibre infantil)

CONTENTS

GENERAL INTRODUCTION AND OBJECTIVES

Meiofauna

Some history about the definition

The prefix meio- (from the Greek word for "smaller") was first coined by Mare (1942) while she was studying the fauna associated with estuarine mud in Plymouth. Thus, the word meiofauna was used to describe those metazoans ranging between 100 and 1000 µm, thus covering the intermediate size between micro and macrofauna (as highlighted in Fig. I.1). The upper and lower limits for meiofauna have not been truly standardised. In the beginning, this category was functionally referred to as "those animals that pass through a 500-µm sieve but are retained on a 42-µm sieve" (Fenchel, 1978; Higgins and Thiel, 1988). Giere (1993) modified the limits to 1000 and 63 µm. The groups and their habitat have also been changed depending on the author. For example, some have included large protists in their meiofaunal definition (e.g. Schmid-Araya, 1994). There has also been a misconception regarding the habitat occupied by meiofaunal communities, relegating them only to hyporheic habitats in freshwater systems (e.g. Giere, 1993). In this thesis we follow Fenchel's definition, which will be applied only for epibenthic metazoans.

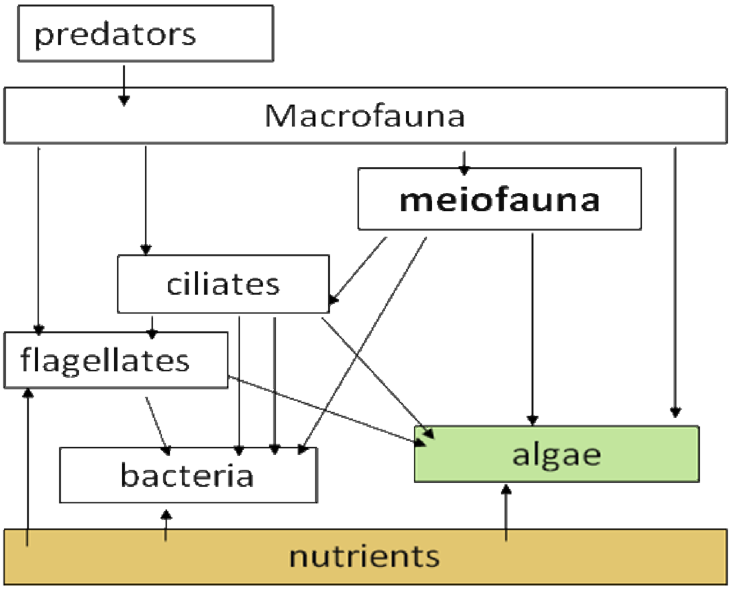

Fig I.1. Modified from Hillebrand et al., 2002.

Temporary and permanent meiofauna

Meiofauna includes metazoans that remain within the meiofaunal size range throughout their life cycle (permanent meiofauna) and the smallest stages of macrofauna, which are of meiofaunal size (temporary meiofauna). In marine environments most invertebrate Phyla include permanent and temporary representatives (Table I.1).

Table I.1. Modified from Robertson et al. 2000b. P=Permanent meiofauna; T= Temporary meiofauna

Phyla	Marine	Freshwater
Cnidaria	P,T	P
Platyhelminthes (Turbellaria)	P	P
Gnathostomulida	P	-
Nemertinea	P	-
Nematoda	P	P
Gastrotricha	P	P
Kinorhyncha	P	-
Priapulida	P,T	-
Loricifera	P	-
Rotifera	P	P
Sipuncula	P	-
Mollusca	T	T
Annelida	P,T	P,T
Tardigrada	P	P
Crustacea	P,T	P,T
Arachnida (Acari)	P	P
Arthopoda (other)	-	T
Echinodermata	P,T	-

Freshwater systems may contain fewer permanent meiofaunal Phyla, but many macroinvertebrate groups contribute to temporary meiofauna (Table I.2).

Table I.2. Groups (Phyla, Orders and Classes) found in the meiofaunal communities described in this thesis.

Temporary meiofauna		Permanent meiofauna	
Chironomidae,	Ephemeroptera	Nematoda,	Ostracoda,
Oligochaeta,	Plecoptera,	Rotifera,	Copepoda,
Trichoptera,	Gasteropoda	Acari,	Cladocera,
Caratopognidae,	Empididae	Hydra,	Gastrotricha,
Culicidae	Other dipterans	Microturbellaria	

Meiofauna vs. macroinvertebrates

Although meiofauna has been neglected in many lotic studies, the interaction between the two communities comprising this group is considerable. Benthic surveys have focused mostly on sampling macroinvertebrates by means of scrapping stones and kick sampling (using >250 µm nets), as examples. In many of these studies, soft sediments on river bottoms or sandy shores have been poorly sampled, thus giving the impression that they are inhabited only by some species of oligochaetes and midges. On the contrary, these habitats host a smaller world that remains to be discovered. During this thesis, we sampled a range of habitats (cyanobacterial mats, sandy pools and leaf packs) in which meiofauna dominated in terms of density (Fig I.2).

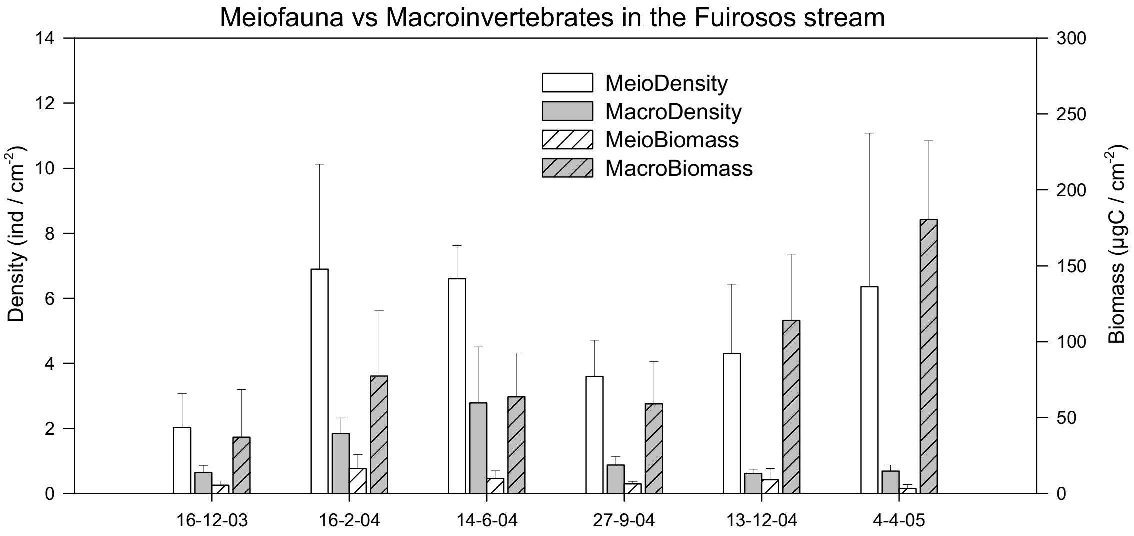

Fig I.2. Meiofauna and Macroinvertebrate density and biomass in sandy sediments of Fuirosos during the sampling period.

In marine environments, some meiobenthic predators (e.g. microturbellarians) feed on macrofaunal juveniles, thereby influencing the benthic recruitment and subsequent population densities of this community (Watzin, 1983). However, several species have developed planktonic larvae that settle when they are larger than permanent meiobenthos. In fresh waters, and especially in lotic systems, this strategy is not feasible, and during their early stages macroinvertebrates interact intensely with meiofauna. Even the most reophil organisms (e.g. gastropods) usually spend their first stages in slow-moving pools, in which meiofauna may dominate and therefore compete or predate on these macroinvertebrates.

Meiofaunal organisms are important trophic links between bacteria and larger fauna such as macroinvertebrates and vertebrates, and are crucial for the regulation of decomposition via microbial grazing (see Schmid-Araya and Schmid, 2000 and references therein). On the basis of these trophic interactions, some authors (Schmid and Schmid-Araya, 2002; Woodward et al., 2005) have stressed that the inclusion of meiofaunal organisms in food web studies results in an increased complexity of these networks (Fig I.3.)

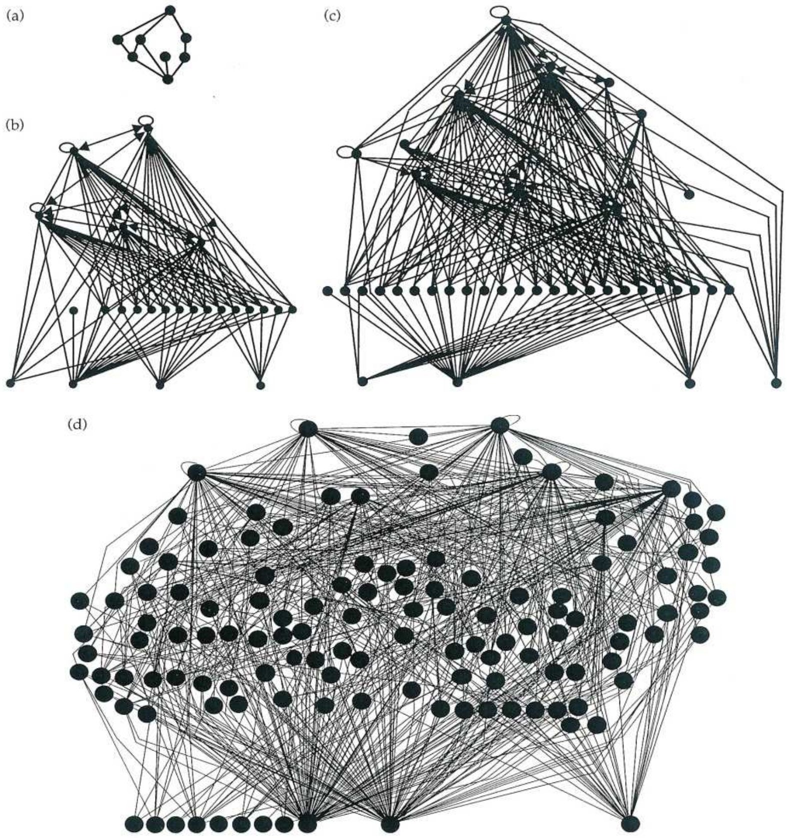

Fig I.3. Increasing complexity of food webs, from (a) to (c), depending on the taxonomic resolution. (d) Food web includes meiofauna and large protists. From Woodward et al. 2005

Research on freshwater meiofauna

Studies in marine and freshwater habitats have arisen independently using different sampling methods and nomenclatures. Research on freshwater meiobenthic communities started in the first half of the 20th century with several studies on rivers and lakes (e.g. those performed by Sassuchin, Wiszniewski, Wilson and Pennak) but these sank into oblivion. Although attention to meiofaunal biology and ecology has intensified over the last 20 years, the basic insights into this field have been gained from research in marine environments. During the obscure years for freshwater meiofauna research, studies in marine habitats resulted in an enhanced understanding of patterns and processes of meiobenthic assemblages (see Coull and Bell, 1979; Higgins and Thiel, 1988; Giere, 1993). Nevertheless, a direct transfer of this knowledge to freshwater habitats is difficult because of fundamental habitat differences (e.g., the unitary nature of the seas and the absence of tidal activity in freshwaters). However, this transfer should be possible at a general level of processes (either in the lab or in the field), and should also be a source of inspiration for further research.

For many years, meiofauna research in fresh waters was relegated to hyporheic and groundwater studies. Therefore, freshwater scientists were more familiar with terms such as psammic, hyporheic and stygobiontic than meiofaunal and meiobenthic. During the 90s, several authors began to move this concept to epigean sediments. Several studies in North America (performed by Palmer, Borchardt, Bott, Morin, Boulton, among others) and Europe (e.g. Schmid-Araya, Robertson, Traunspurger, and Bretschko) and the inclusion of a complete chapter on meiofauna in the book “Methods in stream ecology” (Palmer and Strayer, 1996) impelled meiofaunal research in freshwaters. However, this was merely an impulse for the next step: a special issue on lotic meiofauna in the journal “Freshwater Biology” in 2000; and a text book “Freshwater Meiofauna: Biology and Ecology”, edited by Simon Rundle, Anne Robertson and Jenny Schmid-Araya in 2002. Richard Warwick, a well-known marine meiobenthologist, foreworded this text book as follows :“(…) It represents the ‘coming of age’ of freshwater meiobenthology as a discipline (…)”. But has this actually happened?

The last 10 years of freshwater meiofaunal research

Since the publication of the special issue on lotic meiofauna in 2000, over 120 articles have focused on freshwater meiofauna. These selected papers were conducted in inland waters (excluding interactions with brackish waters or estuaries) and meiofauna itself was the objective (excluding dietary inventories of fish studies). Of these studies, 53 were conducted in lotic habitats, 32 in lentic habitats and one in both systems. The others were as follows: experiments conducted in the lab (15), taxonomic studies (7), theoretical approaches (7), and studies of hyporheic systems or caves (9). Of all these studies, 85% were published in journals with Impact factor while the remaining 15% appeared in non-listed journals.

To measure the impact of the special issue on lotic meiofauna (Table I.3), the number of times cited (last verified 14/3/2011) indicates that microcrustaceans are the most cited group as well as the studies related to meiofaunal trophic relationships. Further down the ranking are studies on the role of meiofauna in ecosystem functional processes and small-scale patterns affecting communities. These two topics are highly related to the development of the present thesis and our current understanding of them in broader contexts is still poor. The last paper of the special issue (Robertson et al., 2000a) summarised current knowledge of lotic meiofauna at that time, stressing the weak points to be addressed by further research. The final dissertation dealt mainly with the under-representation of manipulative experiments and studies on the influence of large-scale processes on community assemblages.

Table I.3. Papers of the Special issue on Lotic Meiofauna and number of times cited

SPECIAL ISSUE IN LOTIC MEIOFAUNA (Freswater Biology: 44 (1). 2000)	Times cited
An introduction to a special issue on lotic meiofauna	9
The biology and ecology of lotic microturbellarians	7
The biology and ecology of lotic rotifers and gastrotrichs	26
The biology and ecology of lotic nematodes	26
The biology and ecology of lotic water mites (Hydrachnidia)	23
The biology and ecology of lotic microcrustaceans	58
The biology and ecology of lotic Tardigrada	15
What drives small-scale spatial patterns in lotic meiofauna communities?	33
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Source: ISI Web of Knowledge (last visited: 14/03/2011)

Functional processes in lotic ecosystems

Lotic ecosystems are regulated mainly by geomorphology, hydrology, water temperature and riparian influence (e.g. Margalef, 1960; Minshall et al., 1983; Elwood et al., 1983 among others). Hydrology and the riparian influence are the major organising functions for biotic associations in running waters (Cummins et al., 1984) and also the most affected by human activities. From the late 1950s, a holistic view of stream ecosystem (mainly influenced by Odum and Margalef's seminal papers) impelled integrative studies that sought to generalise the processes that occur in streams. From these beginnings, functional ecology started replacing taxonomic descriptions by functional criteria used to depict processes. For example, among other brilliant scientists, Cummins (1974) focused on the efficient conversion of organic matter to $CO_{2,}$ relegating to a secondary position the contribution of primary producers, which had their golden age in lotic studies performed during the first half of the 20th century. After this first move, lotic scientists agreed that both terrestrial plant debris and aquatic primary production were large sources of simple carbon compounds and that they commonly complemented one another (Minshall et al., 1985). Thus, in 1996, a functional classification based on mechanisms used by invertebrates to acquire food (Functional Feeding Groups, FFG) was developed by Merritt and Cummins. These groups were found to be exportable from one system to another in integrative theories such as the River Continuum Concept (Vannote et al., 1980).

In the literature, processes like decomposition, nutrient dynamics, stream metabolism (relating algal standing crop and heterotrophic respiration) and secondary production have been described in stream ecosystems. All these processes are related and probably part of just one lumping process. The inclusion of meiofauna in these processes has been scant. For example, the meiofaunal community has not been clearly classified into functional groups like that of the FFG, probably because in some cases (mainly in temporary meiofauna) body structures are not well developed and taxonomic resolution to species level is not possible. However, a number of groups, like nematodes, now have their own classification on the basis of buccal morphology (Moens et al., 2006). The taxonomy of other "conflictive" groups like protozoans has also been simplified to include groups on the basis of their cilliar oral bands (e.g. Foissner and Berger, 1996, Franco et al., 1999).

Several recent studies have included meiofauna in secondary production studies, demonstrating that although their biomass may be relatively small, their high turnover rate (among the highest of all metazoans) deserves consideration (e.g. Stead et al., 2005; Lemke and Benke, 2009).

Riparian influences and sources of organic input for the meiofaunal community

After Pomeroy's seminal paper on the marine microbial loop, several lotic scientists, like Cummins (1974), recognised the crucial contribution of bacteria and fungi as decomposers and nutritional resource for insect consumers. However, Cummins acknowledged only protozoans and diatoms as having significant biomass and metabolic activity relative to bacteria and fungi, and discarded the influence of meiofauna. In 1994, Meyer adapted the well-known marine microbial loop to lotic systems (Fig. I.4) and included all sources of organic carbon available to consumers, including microfauna, meiofauna and macroinvertebrates. However, the whole range of interactions in lotic systems has not been fully described. For example, although bioturbation and coprophagy of meiofaunal organisms has received great attention in marine environments, in lotic systems these processes have often been ignored (Hakenkamp and Morin, 2000).

On the whole, the distribution of stream meiofauna is closely linked to patches of organic matter (Swan and Palmer, 2000); consequently, these associations may have considerable ecological effects. Meiofauna can feed directly on organic matter (e.g. some temporary meiofauna with already esclerotised buccal appendages), but they generally feed on associated-biofilm that is decomposing this matter. Meiofauna are mostly bacterial specialists (Meyer, 1994; Borchardt and Bott, 1995), and they can exert significant grazing pressure on bacteria and fungi that colonise organic matter (Perlmutter and Meyer, 1991). However, the organic matter patches in which meiofauna live and feed can also act as shelter during hydrological perturbations (e.g. maintaining moisture during droughts or creating dams during floods) and also as rafts for the recolonisation of downstream reaches (Robertson and Milner, 2001).

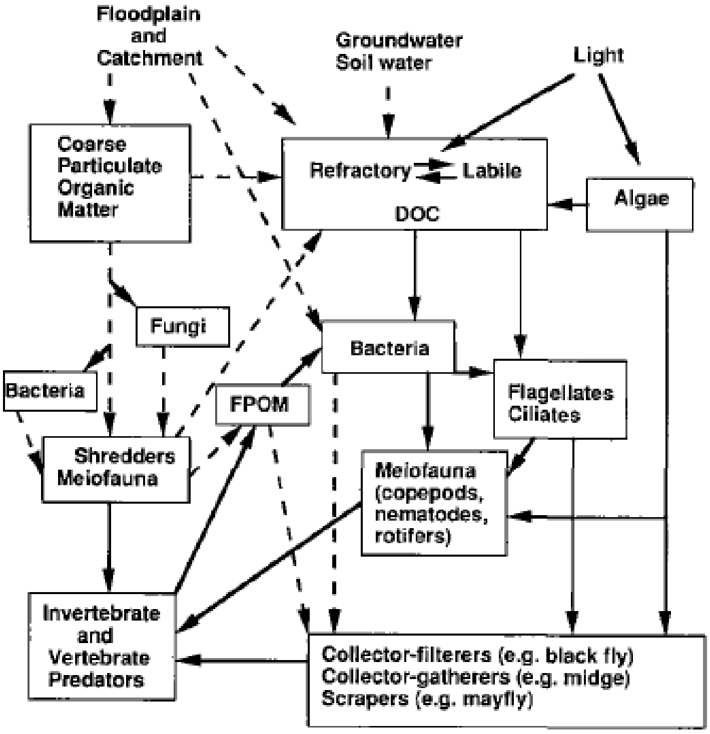

Fig I.4. Scheme of the microbial loop by Meyer, 1994

Mediterranean streams

On all continents, certain coastal regions in the middle latitudes, most extending between 30° and 40° north and south of the equator, are governed by a symmetrical atmospheric circulation that produces a climate characterised by mild, wet winters and hot, dry summers (Aschmann, 1973). The moderating ocean influence keeps winter temperatures mild, with mean monthly minima ranging from about 8° to °C, and frost infrequent except at high altitude or well inland; summer mean monthly maxima usually vary between 18° and 30°C (Dell et al., 1989).

The discharge regime of Mediterranean-type streams generally follows that of the rainfall pattern, and consequently exhibits strong seasonal and annual variability (Davies et al., 1994; Resh et al., 1990; Sabater et al., 1995) (e.g. **Chapter 2**, Fig 2.1). In these streams, high flows abruptly commence in autumn or early winter and floods occur during a few months in late autumn, winter and early spring. Mediterranean systems exhibit high intra- and inter-annual hydrological variability (Acuña et al., 2005), reflecting the intensity and frequency of floods and droughts. Streams in these systems are shaped physically, chemically and biologically by these events (Gasith and Resh, 1999; Lake, 2003). Wet and dry years alternate, and the stream channel may dry up entirely or partially depending on the year (Sabater et al., 2001; Butturini et al., 2003). These streams expand and contract hydrologically (Stanley et al., 1997).

The effects of hydrological disturbances on the macroinvertebrate community have been thoroughly studied (e.g. Gasith and Resh, 1999; Bonada et al., 2007). Concerning droughts, direct effects include loss of water, loss of habitat for aquatic organisms, and loss of stream connectivity. Indirect effects include the deterioration of water quality, alteration of food resources, and changes in the strength and structure of interspecific interactions (Lake, 2003). When streams start to dry up, oxygen and nutrients are rapidly consumed, thereby resulting in strong competition between organisms (Acuña et al., 2005; Beche and Resh, 2007). In contrast, flooding episodes enhance dissolved oxygen availability and lixiviation of watershed soils, which increase nutrient content in stream water (Bernal et al., 2003; Vázquez et al., 2007). Floods also drastically change flow-related parameters (i.e. current velocity, nutrient concentration, conductivity), thus affecting the benthic substrata distribution and retention of dissolved organic matter (DOM) and particulate organic matter (POM) in the stream (Bernal et al., 2002; Acuña et al., 2004). Increases in water current velocity severely affect the structure of benthic microbial communities (Sabater et al., 2006), and indirectly influence the habitat conditions for microbe development (Stevenson, 1996; Artigas et al., 2009). Microbial biofilms are mostly a food resource to meiofaunal organisms. Thus, changes in this microbial compartment have a knock-on effect on the meiofaunal community.

Study sites

Fuirosos

Fuirosos is an intermittent third-order Mediterranean forested stream that drains a 18-Km^2 siliceous basin (90% granite) in north-eastern Spain (Sala and Franch, 1980). Most of the water course of this stream falls in a Natural Park range (Parc del Montnegre-Corredor), in which agricultural impact is very low (only small forest plantations and management of open patches to increase biodiversity Figure I.5) and no industrial activities are present.

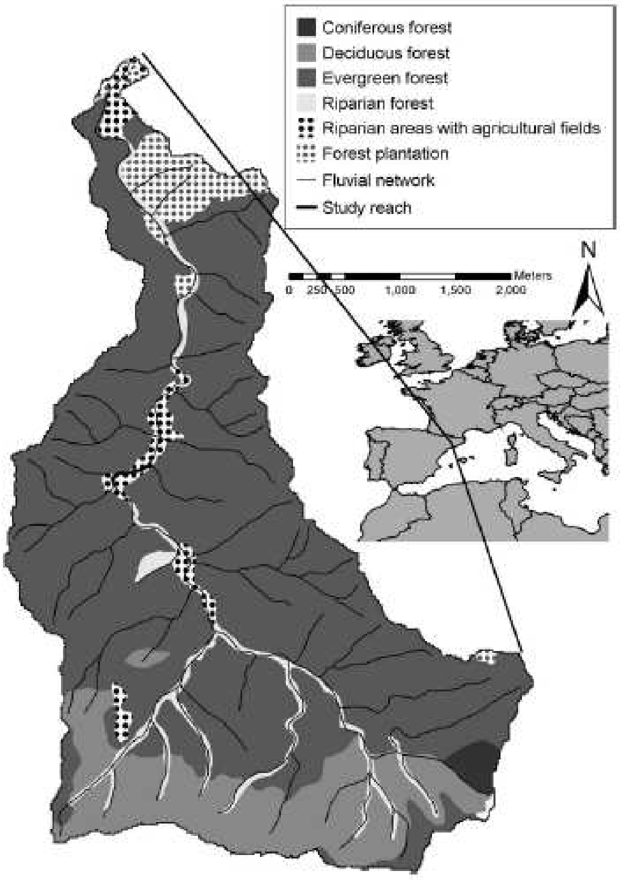

Fig1.5. The Fuirosos catchment (Acuña et al. 2007).

The hydrological features of the Fuirosos stream are largely determined by the Mediterranean climate. Baseline water flow ranges from 5 to 20 L s^{-1} but can increase 100-fold during autumn and spring spates, while in summer it can be reduced to zero (Figure I.6). The main terrestrial input of organic material from the riparian vegetation [*Platanus acerifolia* (Aiton-Willd.), *Populus nigra* (L.) and *Alnus glutinosa* (Gaertn.)] dominated] occurs between summer (because of water stress in the riparian forest) and autumn. Light incidence is generally low, except in spring and winter when the forestry canopy is open (Acuña et al., 2004).

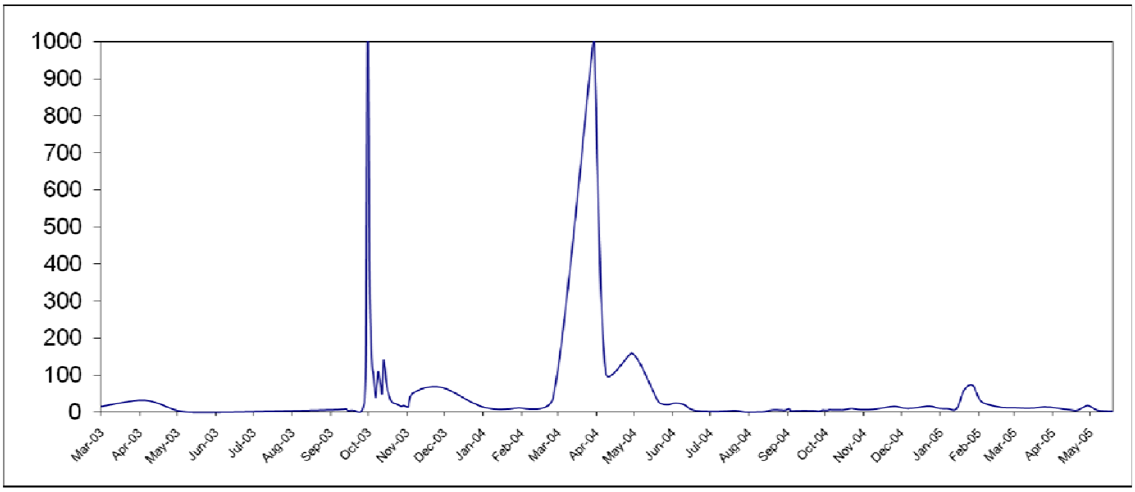

Fig I.6. Fuirosos discharge (L/s) during the study period.

The first studies in the Fuirosos stream date from the early 1980s and consisted of descriptions of geomorphology and lixiviation processes of the watershed soils (Sala and Franch, 1980; Sala, 1983). The phreatic dynamics and its relationship to the riparian vegetation was one of the first issues studied in this stream in the late 90s, as part of the European project entitled “Nitrogen Control by Landscape Structures” (NICOLAS) (Sabater et al., 2003). The relevant role of vegetation on nitrate depletion through the phreatic level, the low denitrification occurring in the dry topsoil, and the intrusion of the stream water into the riparian compartment during recovery from the summer drought were some of the most relevant findings of that project (i. e. Sabater et al., 2001; Butturini et al., 2002; Bernal et al., 2002). Later, the links between hydrology and biogeochemistry were widely explored. The calibration of the INCA (Integrated Nitrogen in CAtchments) model in Mediterranean systems produced a huge data set (Bernal et al., 2004). Recently, research efforts have focused on the effect of seasonality and interannual variability on Dissolved Organic Carbon and Nitrogen (DOC and DON) and nutrient uptake (Vázquez et al., 2007; von Schiller et al., 2008).

The functional ecology of communities has been addressed from many perspectives. The contribution of biofilm and its interaction with the faunal compartment, including protozoans (Domènech et al., 2006; this thesis), meiofauna (this thesis), macroinvertebrates (Acuña et al., 2005), has been explored through descriptive and manipulative approaches. The relation between environmental variations and stream metabolism, organic matter dynamics, algal, meiofaunal, macroinvertebrate and fish dynamics, stoichiometry, trophic relationships, stable isotope signatures, among others, have received considerable attention (e.g. Acuña et al., 2005; Artigas et al., 2009; Mas-Martí et al., 2010). Several *in situ* experiments have been performed in the Fuirosos stream to test the relevance of several of these factors. Among these, nutrient additions (Romaní et al., 2004, Sabater et al., 2011), and sand and leaf litter colonisation (Artigas, 2008; this thesis) are shedding light on how the Fuirosos stream works.

Most of the experiments included in this PhD thesis were carried out in S3 reach*, a 50- m reach (latitude 41° 42'N, longitude 2° 34'), 3-4 m wide and *c*. 10-50 cm in water depth (Fig. I.7). Two other reaches were sampled (**Chapter 1**): S2 was 4000 m upstream from S3, and S1 was in a lateral second-order tributary upstream of the other two reaches. The basin areas drained for each sampling point were 1, 4.2 and 12.3 km^2 (S1, S2 and S3, respectively; *Institut Cartogràfic de Catalunya*, http://www.icc.es, verified 14/3/2011).

All three reaches have similar constrained morphologies as well as slightly different streambed characteristics. Streambed morphology alternates between riffles and pools. Coarse substrata (rocks, boulders and cobbles) are found in the high-current areas of the channel while fine substrata (fine-to-medium gravel and sand) tend to accumulate in pools. Leaves and branches tend to accumulate in the streambed, particularly near the banks and in slow-moving areas.

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* S1, S2 and S3 correspond to reaches sampled in **Chapter 1**. The experiments described in **Chapter 2** were performed in S3. In **Chapter 3**, Impacted and S3 (in the text referred solely as Control) reaches were sampled. The nomenclature used corresponds to an initial BACI design (Before-After-Control-Impacted), which aimed to minimise spatial heterogeneity using 3 control sites to test the impact of nutrient release in water (Impacted reach). However, although sites were similar, the meiofaunal community was too diverse (as demonstrated in **Chapter 1**) to be used as a control.

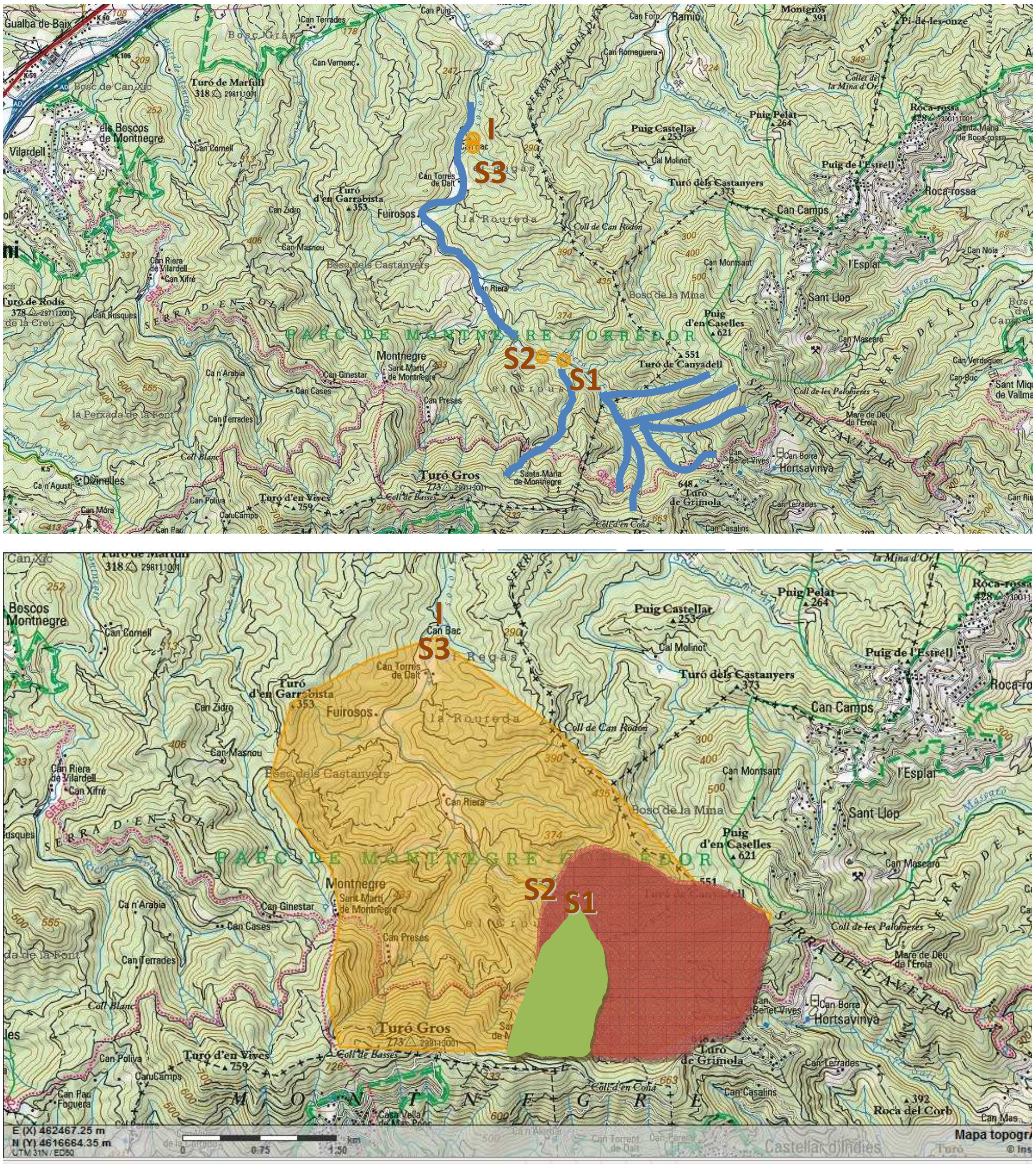

Fig. I.7. (A) Topographic map (1:50.000) in which sampling points (I, S1, S2 and S3) are shown. (B) Different catchment areas. Green:S1; Red: S2; Orange: S3 and I

The Llobregat river

The Llobregat watercourse is characterised by calcareous geology and a typical Mediterranean regime, which causes frequent floods in spring and autumn and minimum flow in summer. The main channel of the river (Fig. I.8) is continuously interrupted by a series of small dams and derivation channels, thereby leading to further instability of the water regime, especially during periods of low flow. Waste waters of industrial and urban origin enter the river in its mid and lower stretches, thus leading to a severe deterioration of water quality (see López-Doval et al., 2010 and references therein).

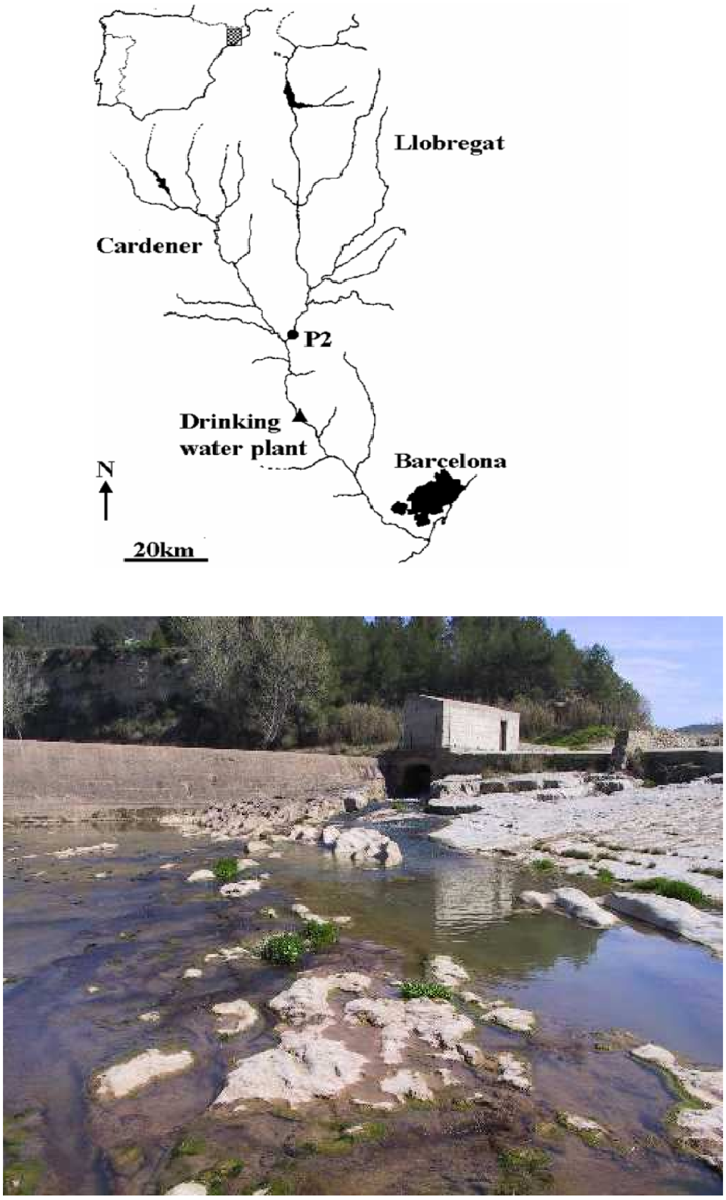

Fig. I.8. The Llobregat catchment and a photo of the sampling point.

The sampling in **Chapter 4** was conducted in Pont de Vilomara (U.T.M. 406502, 4604544), a site in the middle stretch of the river, from January to May 2002. The physical and chemical properties of the water during the study period were fully described in Sabater et al. (2003). Table I.4. summarises some general physico-chemical features of this river.

Table I.4. Comparison between physico-chemical characteristics of both sampling sites, the Fuirosos stream and the Llobregat river.

Variables	Units	Fuirosos	Llobregat
pH		7.18	8.3
Temperature	**°C**	11.52	10.8
Oxygen	**mg/L**	9.75	10.6
Oxygen	**%**	88.65	97.1
DOC	**mg/L**	2.45	3.6
Cl^-	**mg/L**	35	244.1
NO_3^-	**mg/L**	674.74	3900
NH_4^+	**mg/L**	39.22	100
PO_4^{3-}	**mg/L**	24.44	400
N:P		104	18
Conductivity	**µS/cm**	189.71	1496
Discharge	**L/s**	30.83	7500

Objectives and development of this thesis

The main objective of this thesis is to characterise the meiofaunal community in Mediterranean systems and to integrate them as the missing link in in-stream functional processes. Although current knowledge of freshwater meiofauna is still scarce, this thesis does not intend to describe the community assemblages from a taxonomic point of view, but to use functional classifications that could allow their integration in broader contexts.

The thesis is divided into 5 chapters, in which we sampled various substrates in order to study the small-scale processes affecting them and the interactions with other organisms. In these experiments, a number of questions were addressed depending on habitat or site characteristics. We focused essentially on the main lotic ecosystem determinants: hydrology and nutrient inputs (organic and inorganic).

- In **Chapter 1**, we studied the meiofaunal community over 2 years. Its structure reflected high intra- and inter-annual variability, which coincided with the alternating periods of hydrological stability and disturbances. Since flow is a major abiotic determinant of invertebrate distribution patterns in streams and its impact on meiofauna is relatively well known (see Swan and Palmer, 2000 and references therein), the study addressed mainly the adaptive responses of organisms to spates and droughts in a Mediterranean context. These adaptations were measured in terms of advantageous traits (either structural or life strategies) that could confer resistance or resilience to organisms.
- Organic matter input and its availability to consumers is one of the major processes in headwater streams. In **Chapter 2**, we analysed the colonisation pattern of experimentally disposed riparian leaves, including all organisms involved to trace the fate of carbon during the process.
- Eutrophication of lotic systems is one of the negative results of human activity. Therefore we paid special attention to the effects of nutrient availability to meiofauna because their small size and high turnover rates can provide information of interest about their rapid response to these kinds of

disturbances. In Mediterranean systems scarcity can potentiate the negative effects of eutrophication. Therefore, we also explored the interaction of hydrology and nutrient inputs. In **Chapter 3** we analysed the meiofaunal response to an experimental addition of nutrients in an almost pristine headwater stream. For this purpose, we focused on differences in community assemblages and secondary production of microcrustaceans. In that system, hydrological disturbances were the "background noise". In contrast, in **Chapter 4** we explored a nematode community in a eutrophic system and we focused on the stability of substrate (attached vs. free-floating cyanobacterial mats). In **Chapter 5**, using a laboratory experiment, we analysed the fast response of nematode organisms to eutrophication and examined life strategies adopted to adapt to increased nutrient availability.

This thesis is structured as follows:

1. Two organisational levels:
    Community level: meiofauna (**Chapter 1, 2, 3**) and nematode (**Chapter 4**).
    Population level: cyclopoids and ostracods (**Chapter 3**, secondary production), and nematodes (**Chapter 5**).
2. Several functional approaches:
    Resilience (**Chapter 1**)
    Species traits (**Chapters 1** and **5**)
    Secondary production (**Chapters 3** and **5**)
    Colonisation (**Chapters 2, 4** and **5**)
    Carbon allocation (**Chapter 2**)
    Consumption-excretion rates (**Chapter 5**)

CHAPTER 1

Species traits and resilience of meiofauna to floods and drought in a Mediterranean stream

Ainhoa Gaudes, Joan Artigas, and Isabel Muñoz

Marine and Freshwater Research, 2010, 61: 1336–1347

SUMMARY

In Mediterranean streams, droughts and floods are mainly seasonal and predictable, occurring twice or three times a year. Under these conditions, multivoltinism and short life-cycles would be favoured, particularly for organisms with low migratory capacity. The meiofaunal community is therefore hypothesised to have species traits adapted to these hydrological perturbations. However, meiofauna have been neglected in many lotic studies. The present study examined the temporal variability of meiofaunal density and biomass over a 2-year period in three reaches of a low-order Mediterranean stream. Relationships between biological traits and hydrological and environmental characteristics were investigated. Resilience of meiofauna to floods was quantified using regression. Small differences in basin drainage resulted in different responses. The abundance and resilience of the meiofauna were higher in the upstream reach, than those in the downstream communities. A small, worm-shaped body and active locomotor structures conferred higher resilience in the face of natural hydrological disturbances. Low-order reaches are refugia for functionally important meiofauna that can eventually repopulate downstream reach.

1.1. Introduction

Mediterranean systems exhibit high intra- and inter-annual hydrological variability (Acuña et al. 2005). This variability reflects the intensity and frequency of floods and droughts. Mediterranean streams are shaped physically, chemically and biologically by these events (Gasith and Resh, 1999; Lake, 2003). Wet and dry years alternate, and the stream channel may dry up entirely or partially in different years (Sabater et al., 2001; Butturini et al., 2003). These streams expand and contract hydrologically (Stanley et al., 1997), whereby droughts reduce connectivity and floods restore it. Seasonal variability in the Mediterranean climatic areas is considered a selective force that constrains communities and shapes their biological traits (Stamou, 1998; Bonada et al., 2007). The biota of these dynamic hydrological systems has morphological, physiological and/or behavioural adaptations that confer resistance and/or resilience on communities (Humphries and Baldwin, 2003).

Natural disturbances (e.g. floods and droughts) and habitat heterogeneity are the main determinants of community structure in stream ecosystems (Resh et al., 1988; Lake, 2000; Lepori and Hjerdt, 2006). During the past two decades, several theoretical frameworks have been proposed to explain relationships between habitat heterogeneity and disturbances of organisms. For example, the habitat templet theory establishes a tight relationship between habitat characteristics and the biological traits or adaptations of the species present (Townsend and Hildrew, 1994). Species traits help predict changes in community composition as a response to environmental gradients (e.g. Statzner et al., 2005; Bonada et al., 2006).

Many of these studies have focussed on the macroinvertebrate community. However, less attention has been paid to meiofauna (metazoans that can pass through a 500-µm sieve but will be retained by a 32-µm sieve; Fenchel, 1978; Higgins and Thiel, 1988). The contribution of meiofauna to total stream biomass may be relatively small. However, this contribution varies strongly with season and species composition (Hakenkamp and Morin, 2000). Meiofauna can reach higher abundances than macroinvertebrates (Reiss and Schmid-Araya, 2008). Meiofaunal organisms contribute significantly to community dynamics and are intermediate species for macroinvertebrate consumers in the food web. The small size and generally short generation times of meiofaunal organisms promote fast population growth (Stead et al., 2005), and are essential traits for rapid recovery following disturbance (Townsend and Hildrew, 1994; Griswold et al., 2008).

Some studies have suggested that patterns of meiofaunal abundance are mainly driven by flow regime and substrate type (Schmid-Araya, 1994; Palmer and Strayer, 1996). Weaker current velocities and finer substrates enable burrowing animals to penetrate the sediments for protection or for feeding (Malmqvist, 2002). Consequently, pools may offer good shelter during summer droughts for those invertebrates that are in their earlier larval stages (temporary meiofauna) and for aquatic invertebrates lacking aerial adult stages (Acuña et al., 2005). During stable flows, these pools can also serve as depositional reservoirs for detritus (Gonzalez and Graça, 2003). The detritus may be densely colonised by bacteria and fungi, which increase its nutritional value (Gaudes et al., 2009) and provide rich food sources for pool detritivores.

Generally, sandy patches are highly susceptible to scour from floods because of the relative instability of the fine substratum (Townsend, 1989). In a Mediterranean climate, seasonality strongly influences stability and structure of these patches. Patch predictability may influence (1) the composition of meiofaunal assemblages, (2) their response to desiccation and (3) their recolonisation capacity after floods (Davies et al. 2005; Williams, 1987, 2006). Species survival after flooding may also result from traits that confer resistance to flow fluctuations. Such traits include streamlined body forms or adhesive mechanisms, as well as traits that confer resilience to disturbance, such as rapid population growth or the use of habitat refugia (Townsend and Hildrew, 1994).

We studied temporal patterns in the meiofaunal community in response to major hydrologic disturbances (floods and droughts) in a Mediterranean stream. We hypothesised that (1) community structure reflects high intra- and inter-annual variability, thus coinciding with the alternating periods of hydrological stability and disturbances that are characteristic of Mediterranean systems, (2) patterns of community structure may be related to species traits (e.g. size, life cycle, body shape, locomotion) and (3) meiofaunal organisms offer rapid recovery capacity (resilience) in response to discharge disturbances, associated with the possession of these traits.

1.2. Study site

The study was performed in Fuirosos, a third-order stream that drains a 15.6-km2 forested granitic basin. Almost all of the catchment is included in the Montnegre–Corredor Natural Park, north-eastern Spain (41°42’N, 2°34’E; 150–400 m asl). Holm oak, coniferous and deciduous forest covers 90% of the total catchment.

Rainfall in this area is highly variable (Fig. 1.1), a typical feature of Mediterranean systems. Precipitation occurs mainly in autumn and spring, with occasional storms in summer. The stream flow at Fuirosos is intermittent. Baseflow ranges from 5 to 20 L s^{-1} but can increase 100-fold during autumn and spring floods. Water flow in summer can drop to zero (Artigas et al., 2009). Summer cessation of flow lasts for several weeks or even months. The stream dried up during 8 of 11 summers from 1997 to 2007 (S. Sabater, unpubl. data).

Sandy pools from three reaches (S1, S2 and S3) of the Fuirosos catchment were sampled monthly for 2 years (May 2003 – June 2005). Two (S3 and S2) of the three reaches were in the main Fuirosos watercourse. S2 was 4000 m upstream from S3. The third reach (S1) was in a lateral second-order tributary upstream from the other two reaches. The basin areas drained for each sampling point were 1, 4.2 and 12.3 km^2 (S1, S2 and S3, respectively; Institut Cartogràfic de Catalunya, http://www.icc.es, verified 19 July 2010).

All three reaches had similar constrained morphologies as well as slightly different streambed characteristics. Previous studies demonstrated that sandy pools had a significantly higher meiofaunal abundance than rocks and cobbles (A. Gaudes, unpubl. data) and were present during the whole year (in contrast to leaves and other organic substrates). Sediment disruption and movement in the Fuirosos occur at flows

>1000 L s^{-1} (Sabater et al., 2008). Therefore, we focussed our surveys on sandy pools with similar water velocity and granulometry. The methods and results for the grain-size analyses from these sampling points are described in Domènech et al. (2006).

1.3. Material and methods

Physical and chemical measurements

Conductivity, temperature, dissolved oxygen and pH were determined in the field with handheld meters (MultiLine F/SET-3, WTW, Weilheim, Germany) on each of the 24 sampling dates. Current velocity was measured with an anemometer (MiniAir2, Schiltknecht, Zurich, Switzerland) in the sandy pools before sample collection. Stream discharge was calculated using the slug injection method with sodium chloride as a tracer (Gordon et al., 1992). Discrete discharge data were also used to complement long-term daily data on rainfall provided by a nearby meteorological station (Diputació de Barcelona) to calculate the amount and duration of precipitation. A 2–3-day event with an accumulated precipitation of 15 mm was enough to produce a bed-moving spate during the study period (A. Butturini, pers. comm.).

Water samples for nutrient-content analysis were collected in triplicate and filtered through pre-combusted glass-fibre filters (Whatman GF/F) before analysis. Ammonium, nitrate and dissolved phosphorus were analysed following standard methods (APHA, 1992).

Streambed description and organic matter content

To estimate the different substrate compositions of the three reaches, the percentage cover of coarse substrata (cobbles, boulders and rocks), fine substrata (gravel and sand), leaves, branches and fine detritus was visually assessed at 20-cm intervals along five transverse transects spaced every 8 m along the reach. At each sampling time, percentages of each substratum along all transects were averaged and related to the total wetted surface area of the reach.

The standing stock of coarse benthic organic matter was estimated from triplicate cores (15-cm diameter) taken at random in the pools. Samples were filtered through a 1-mm-mesh nylon net and dried to a constant weight at 70°C. A subsample of each replicate was kept for further carbon- and nitrogen-content analysis (EA 1108 CHNS-O, Carlo Erba Instruments, Milano, Italy).

Algal and fungal biomass

On each sampling date, six sand samples were collected with a Perspex corer (4.3 cm in diameter, uppermost 2–4 cm) for estimations of fungal and algal biomass (three replicates each). Samples were kept in a cool box for transport to the laboratory and frozen until analysis. Chlorophyll-*a* was used as an estimate of algal biomass (periphyton). Algal samples were sonicated (2+2 min, Selecta, Abrera, Spain, 40 W power, 40 KHz frequency). Chlorophyll was further extracted with acetone (90% final concentration) and its concentration was determined spectrophotometrically (U-2000 Spectrophotometer, Hitachi, Tokyo, Japan) after filtration (GF/F, Whatman) of the extract following the method described by Jeffrey and Humphrey (1975). Algal biomass was calculated on the basis of the ratio carbon : chlorophyll = 60 (Geider et al., 1996). Fungal biomass was estimated from the ergosterol content (Artigas et al., 2009).

Meiofauna densities and biomass

Five cores were taken randomly from the sandy substrate (perspex cylinders 3.15 cm in diameter, uppermost 7–8 cm) of the three reaches. The sandy cores were immediately sieved through 500- and 32-µm nested sieves. The fraction retained by the 32-µm sieve was chilled immediately and stored for counting and identification of the live meiofauna to order level under a dissecting microscope (MZ9.5, Leica, Wetzlar, Germany) within 2 or 3 days of sampling. Pilot experiments showed that community composition and abundance of meiofauna in live samples were similar regardless of whether they were processed immediately or within 3 days after collection.

Meiofaunal soft-bodied organisms were identified and measured alive, whereas the hard-bodied organisms were simply counted, identified and then fixed in formalin for further length and width measurements. Biomass estimations were obtained through power equations applied to body length when available in the literature, or else from biovolumes (Gaudes et al., 2009).

Species traits

Meiofaunal individuals were assigned to functional groups (Claret et al., 1999) on the basis of simple species traits that might indicate adaptation to hydrological disturbances (e.g. floods and droughts), namely locomotor strategy and adult-phase strategy (emergence or non-emergence from the aquatic environment). The mobility categories were slightly modified from Claret et al. (1999), and were as follows: organisms with appendages (actively mobile organisms and considered to be good swimmers, e.g. cyclopoids), organisms with adhesive structures (with passive response to floods, e.g. gastropods), organisms with cilia (slow-moving, e.g. rotifers) and organisms with a worm-shaped body (capable of burrowing within the sediment e.g. nematodes). Additionally, the samples were classified according to size, as follows: permanent meiofauna (invertebrates that even as adults are small enough to pass through a 500-µm sieve) and temporary meiofauna (early macroinvertebrate instars).

Data analyses

Differences between the physical and chemical characteristics of the reaches were analysed with a non-parametric Kruskal–Wallis test. The Mann–Whitney U test was applied to precipitation data to analyse differences between hydrologic years.

Meiofaunal abundance was log (x + 1)-transformed to downweight the influence of the highly abundant orders. One-way ANOSIM and similarity percentage (SIMPER) analyses identified the main taxa that accounted for similarity and dissimilarity among the reaches. Significance levels were assessed using a permutation procedure (Clarke and Gorley, 2006). Inter- and intra-annual variability (with season and years as factors) within the reaches was assessed with 2-way crossed ANOSIM and SIMPER tests. Spearman’s rank correlation coefficient was used to measure the association of environmental and biological variables at each sampling point. Meiofaunal abundance and biomass were grouped into functional categories defined by body or life-history traits to assess adaptation to perturbations. To associate environmental variables with meiofaunal community composition, a non-metric multidimensional scaling (NMDS) ordination plot was calculated. Environmental variables were used to interpret the ordination of samples by a vector-fitting procedure (Oksanen et al., 2008). The direction and length of the resulting plotted vectors often indicate the direction of the most rapid change in the environmental variable and its correlation to the ordination.

The resilience response was calculated using slopes (hereafter referred to as 'm') of linear regressions (Grimm and Fisher, 1989; Uehlinger, 2000) of days required to achieve pre-disturbance meiofaunal density (Schmid-Araya, 1994). Lower values of 'm' mean a weaker resilient response and longer times until densities return to their previous values.

ANOSIM and SIMPER analyses were performed with the software package PRIMER (version 6.1.6; PRIMER-E, Plymouth, UK). Spearman's rank correlation and Kruskal–Wallis tests were applied using the STATISTICA package (version 8.0; StatSoft, Tulsa, Oklahoma, USA). NMDS and vector-fitting were applied using R (R Development Core Team, 2008; Oksanen et al., 2008).

1.4. Results

Hydrological periods and reach characteristics

From May 2003 to May 2005, we detected two periods differentiated by distinct hydrological conditions (Mann–Whitney U test, $P = 0.024$). The first period started just before the summer drying of 2003 (when the streambed was completely dry), followed by a rainy autumn and spring (Fig. 1.1), which led to a wet summer in 2004 (streambed with pools partially disconnected). In the second period, autumn 2004 and spring 2005 had consistently less rainfall and were followed by a second summer drought in 2005. In the first hydrologic year (wet year), ~1000 mm of precipitation was recorded, whereas in the second (dry year), only 500 mm fell. The three reaches significantly differed in conductivity ($H_2 = 37.88$, $P < 0.001$), discharge (H_2

= 13.56, P = 0.001) and phosphate concentration in water (H_2 = 6.39, P = 0.04) (Table 1.1).

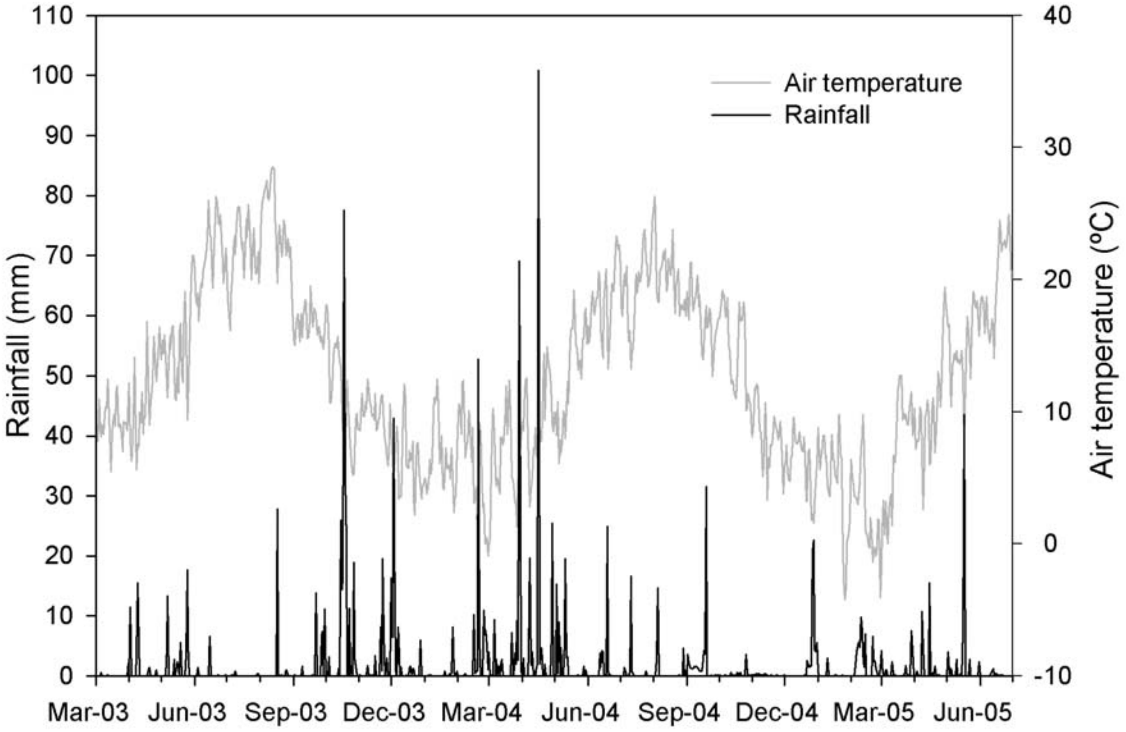

Fig. 1.1. Daily precipitation (black line) and air temperature (grey line) during the study period (March 2003–May 2005).

All three reaches had similar streambed compositions during the study period, namely alternating sands with cobbles and boulders (Table 1.1). In the S1 reach, the percentage of mobile substrates such as sands, leaves and detritus was higher. Variation coefficients in S1 were lower than in the two downstream reaches (except for the coarse substrate).

Table 1.1. Physical and chemical characteristics and substrate composition in the three reaches (S1, S2 and S3) of a Mediterranean stream Means, standard deviations (s.d.), percentages (%) and coefficients of variation (C.V.; s.d./mean) are shown

	S1 (n=22)*		S2 (n=24)		S3 (n=24)	
	Mean ± SD	V.C.	Mean ± SD	V.C.	Mean ± SD	V.C.
PHYSICO-CHEMICAL						
pH	7.33± 0.38	0.05	7.44±0.37	0.05	7.25±0.55	0.08
T (°C)	12.02 ± 3.78	0.31	12.16±4.42	0.36	11.71±5.00	0.43
Oxygen (mg L^{-1})	9.84±2.83	0.29	10.39±2.85	0.27	9.92±2.55	0.26
Oxygen Sat. (%)	87.54±16.70	0.19	93.1±12.90	0.14	90.32±15.93	0.18
Conductivity(µS cm^{-1})	126.48±16.08	0.13	176.19±22.51	0.13	206.74±34.31	0.17
Discharge (L s^{-1})	4.04±5.52	1.37	15.36±20.44	1.33	30.83±43.97	1.43
Ammonia (µg $N\text{-}NH_4^+$ L^{-1})	20.62±18.29	0.89	67.58±137.68	2.04	30.33±32.31	1.07
Nitrate (µg $N\text{-}NO_3^-$ L^{-1})	256.17±222.61	0.87	386.18±548.87	1.42	464.38±525.63	1.13
Phosphate (µg $P\text{-}PO_4^{3-}$ L^{-1})	12.04±16.50	1.37	8.99±12.33	1.37	14.30±18.51	1.29
SUBSTRATA (coverage %)						
Coarse	5.4±2.0 (33.1%)	0.4	17.4±5.0 (49.5%)	0.3	39.8±11.0 (44.5%)	0.3
Fine	4.5±0.9 (27.7%)	0.2	6.8±2.4 (19.3%)	0.4	19.8±8.1 (22.2%)	0.4
Leaves	3.2±2.7 (19.8%)	0.8	5.2±4.5 (14.7%)	0.9	15.7±12.8 (17.6%)	0.8
Wood	0.6±0.4 (3.8%)	0.7	1.3±1.3 (3.7%)	1.1	4.6±6.5 (5.2%)	1.4
Detritus	2.5±1.4 (15.5%)	0.6	4.5±3.9 (12.8%)	0.9	9.5±7.3 (10.6%)	0.8

Effect of spatio-temporal variability on meiofaunal communities

One-way ANOSIM revealed significant differences among the meiofauna communities of the various reaches (Global $R_{density}$ = 0.195, P = 0.001; $R_{biomass}$ = 0.15, P = 0.001) during the study period (Table 1.2). The results from the pairwise test indicated that meiofaunal density and biomass in S1 (mean values, 11.8 individuals cm^{-2} and 17.3 µg C cm^{-2}, respectively) were higher than in the S2 (5.8 individuals cm^{-2} and 11.2 µg C cm^{-2}, respectively) and S3 (4.7 individuals cm^{-2} and 6.7 µg C cm^{-2}, respectively) reaches. Oligochaetes, chironomids and nematodes contributed to 50%

of the similarity in all three reaches (SIMPER analysis), whereas copepods (cyclopoids and harpacticoids), ostracods and microturbellarians contributed to 75% of the similarity.

Table 1.2. Mean abundance (individuals m^{-2}) of the different meiofaunal groups in each sampling point (S1, S2 and S3) Accumulated precipitation of the two hydrological periods is given

UNITS (Ind m^{-2})	May 2003-June 2004 (1069.40 mm acum. precipitation)			July 2004-May 2005 (364.40 mm acum. precipitation)		
Organisms with cilia	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
Rotifera	158.22	177.33	215.86	249.95	313.15	188.64
Gastrotricha	39.55	53.19	69.06	44.97	35.87	47.28
Microturbellaria	315.36	162.95	375.10	644.99	295.27	523.95
Worm-shaped body						
Nematoda	3988.43	1243.41	534.45	4273.77	2598.45	1698.02
Ceratopogonidae	136.89	16.46	3.97	422.43	249.34	73.86
Chironomidae	10643.50	3915.57	1550.06	5241.64	2912.52	1146.47
Oligochaeta	5608.21	1813.51	2654.26	10316.74	5327.83	4576.89
Organisms with appendages						
Tardigrada	42.58	43.15	679.68	12.92	10.24	114.08
Acari	124.44	89.25	28.34	282.41	95.56	43.49
Ostracoda	1567.69	319.86	436.94	3195.31	428.80	657.56
Cyclopoida	524.05	650.52	612.28	621.71	506.45	557.93
Harpacticoida	2332.56	607.81	92.43	7365.40	1253.31	387.85
Nauplii	185.07	125.58	190.05	2229.85	505.00	153.46
Cladocera	206.93	15.81	40.85	842.72	313.27	71.69
Ephemeroptera	40.14	101.53	24.33	296.94	173.78	215.26
Plecoptera	184.29	85.69	113.08	220.58	50.29	18.28
Coleoptera	0.00	3.05	50.11	16.39	41.50	10.05
Trichoptera	3.71	0.00	0.00	5.18	3.73	5.00
Organisms with adhesive structures						
Simuliidae	3.51	3.95	7.54	12.18	19.05	8.56
Gastropoda	0.00	0.00	0.00	3.00	3.73	679.38
Hydridae	0.00	0.00	0.00	7.37	14.11	3.19

The meiofaunal community differed between the ‘hydrological periods’ (higher densities during the dry year and lower during the wet year, Table 1.2) in all reaches (2-way ANOSIM, season and year as factors; R_{S1} = 0.37, P = 0.033; R_{S2} = 0.36, P = 0.034; R_{S3} = 0.35, P = 0.02) but did not show clear seasonality in the S2 and S3 reaches. In contrast, meiofaunal communities were different among the seasons in the S1 reach (R_{S3} = 0.23, P = 0.044). The winter community had higher densities (mainly nematodes, chironomids and microcrustaceans) than the autumn community.

Temporary meiofauna showed a consistently higher density (~60%) and biomass (~80%) than did permanent meiofauna in all three reaches throughout the study period (Figs 1.2, 1.3). Oligochaetes and chironomids were the main contributors to the temporary meiofauna group. Density of the permanent meiofauna (dominated by Copepoda and Ostracoda) varied according to the hydrological stability. This density increased between December and April (Fig. 1.2), attaining biomass similar to that of the temporary meiofauna in the S1 reach only (Fig. 1.3A). In the wet summer of 2004 (June–August), which caused partially disconnected pools, temporary meiofauna had a higher density and biomass in all reaches. In a similar streambed situation, in spring 2005 (just before complete streambed desiccation), these increases were from two to four times higher (Figs 1.2, 1.3). After all flood events, oligochaetes, chironomids and nematodes were the most abundant groups.

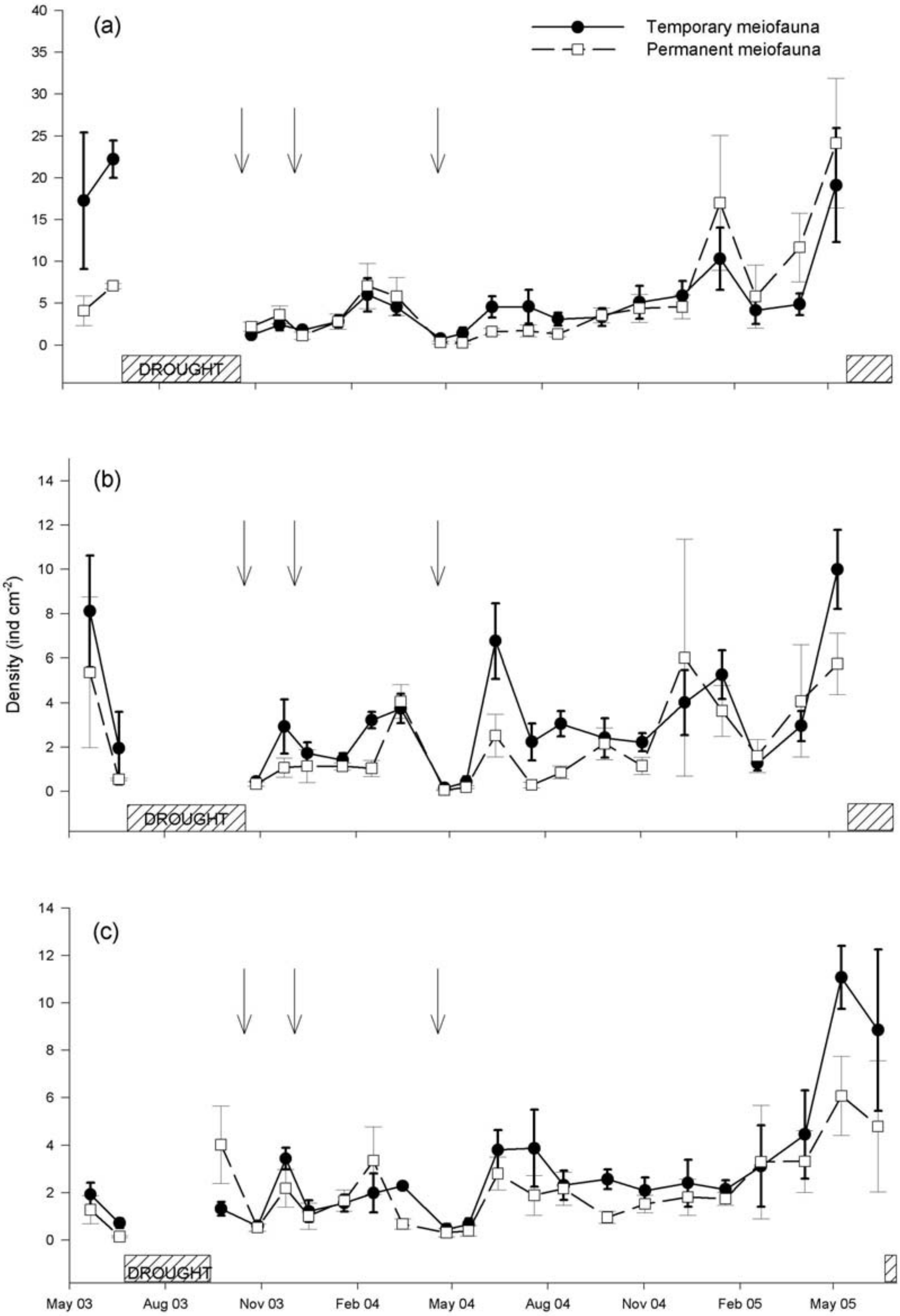

Fig. 1.2. Mean (±s.e.) density of temporary and permanent meiofauna in the following three reaches of a Mediterranean stream: (a) S1, (b) S2 and (c) S3. Boxes with an oblique pattern correspond to summer droughts and arrows to flooding events (>3m^3 s^{-1})

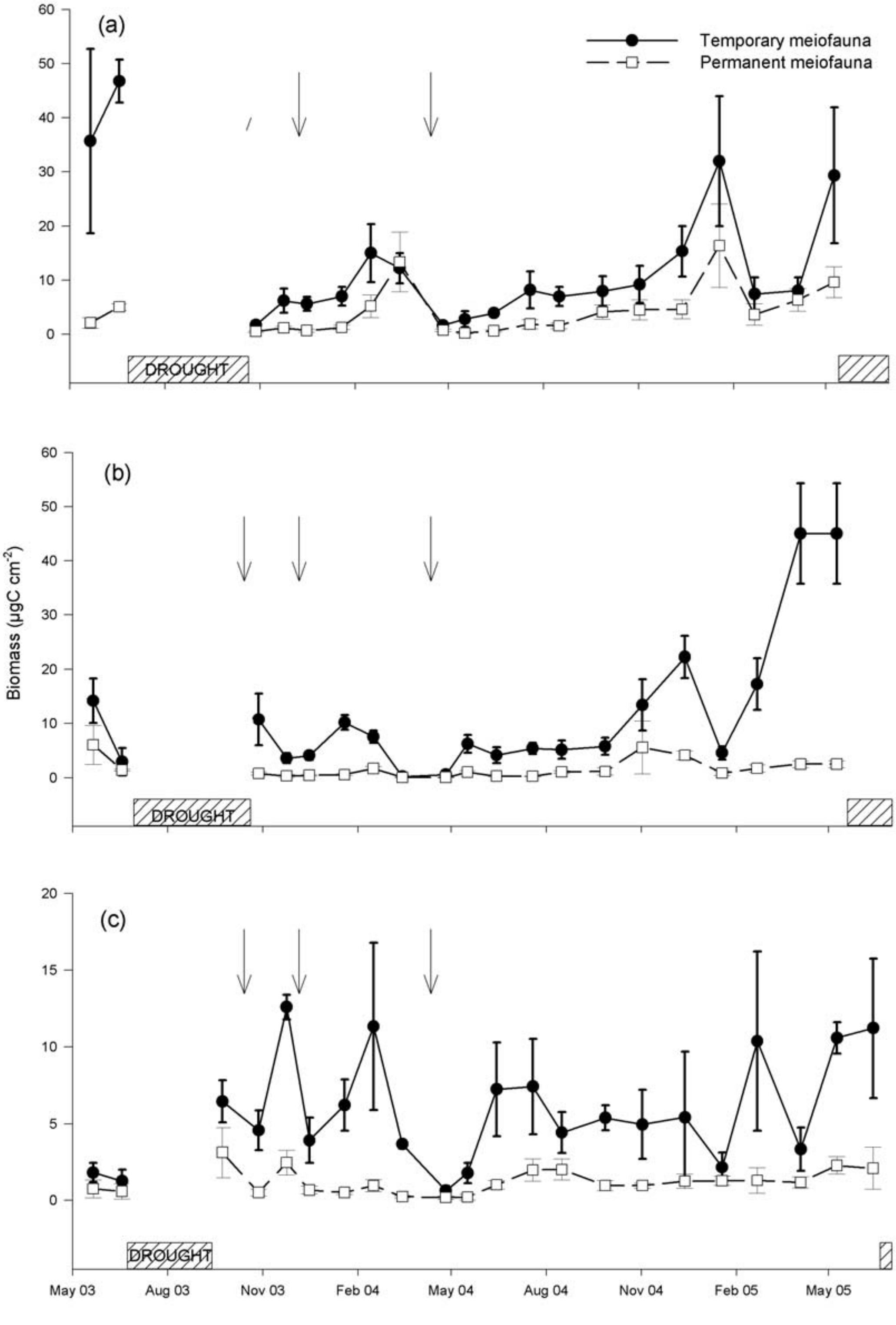

Fig. 1.3. Mean (± s.e.) biomass of temporary and permanent meiofauna in the following three reaches of a Mediterranean stream: (a) S1, (b) S2 and (c) S3. Boxes and arrows as in Fig. 1.2.

Biological traits and environmental variables

A strong dependence of meiofaunal communities on stream hydrology was observed (Table 1.3) in all reaches, particularly in S1 and S2. All locomotion and life-history strategies appeared to be affected by changes in discharge. Low precipitation (measured as accumulated precipitation during the previous 15 days) and periphyton chlorophyll favoured density and biomass of almost all groups, except for organisms with cilia. The small size of rotifers, gastrotrichs and microturbellaria could be an advantage to these organisms if they must find a refuge in sand substrate to avoid hydrological changes. Biomass of individuals with aerial adults in the S3 reach showed a positive correlation with discharge, perhaps indicating the use of an emergency strategy to avoid flooding events. In this reach only, the organisms with adhesive structures (gastropods and black flies) maintained positive correlations of densities with hydrological stability parameters. This result contrasts with the presence of larger individuals (macroinvertebrates) of these taxa in riffles. This trait evidently represents an adaptation for resisting intense water flow. The early life-history stages would require the protection furnished by pools and would respond negatively to discharge. Higher C and N content of the organic matter and fungal biomass showed no broad relationships with meiofauna.

Table 1.3. Spearman's rank correlation coefficient (r) between density (d) and biomass (b) of traits and environmental variables for the three sampling points.

	TRAITS	gC cm^{-2}	gN cm^{-2}	Chl*a*	Disch	ppacum15
S1 reach	worm-shaped			d+b+	d-b-	d+b+
	appendix			d+b+	d-b-	d+b+
	cilia				b-	
	adhesive	b-	b-	b+		
	aerial ad.			d+b+	d-b-	d+
	aquatic ad.			d+b+	d-b-	d+b+
	temporary			d+b+	d-b-	d+b+
	permanent			d+b+	d-b-	d+b+
S2 reach	worm-shaped			d+b+	d-b-	d+b+
	appendix			d+b+		b+
	cilia					
	adhesive				b-	
	aerial ad.			d+b+		b+
	aquatic ad.			d+b+	d-b-	b+
	temporary			d+b+	d-b-	b+
	permanent			d+b+		d+b+
S3 reach	worm-shaped			d+		
	appendix					
	cilia					
	adhesive			d+	d-	d+
	aerial ad.			b-	b+	
	aquatic ad.					
	temporary					
	permanent			b+	b-	

Non-metric multidimensional scaling (NMDS) did not show a clear aggregation among density samples (Fig. 1.4). In the NMDS plot, there were three vectors related to discharge (discharge, magnitude of the precipitation event and conductivity; P < 0.001). Samples from the S1 reach tended to aggregate in a direction opposite to discharge and magnitude. These data produce a dense cluster of sampling dates. In contrast, the sampling dates for the S2 and S3 reaches showed a slight dispersion, depending on the hydrological year in which the samples were taken.

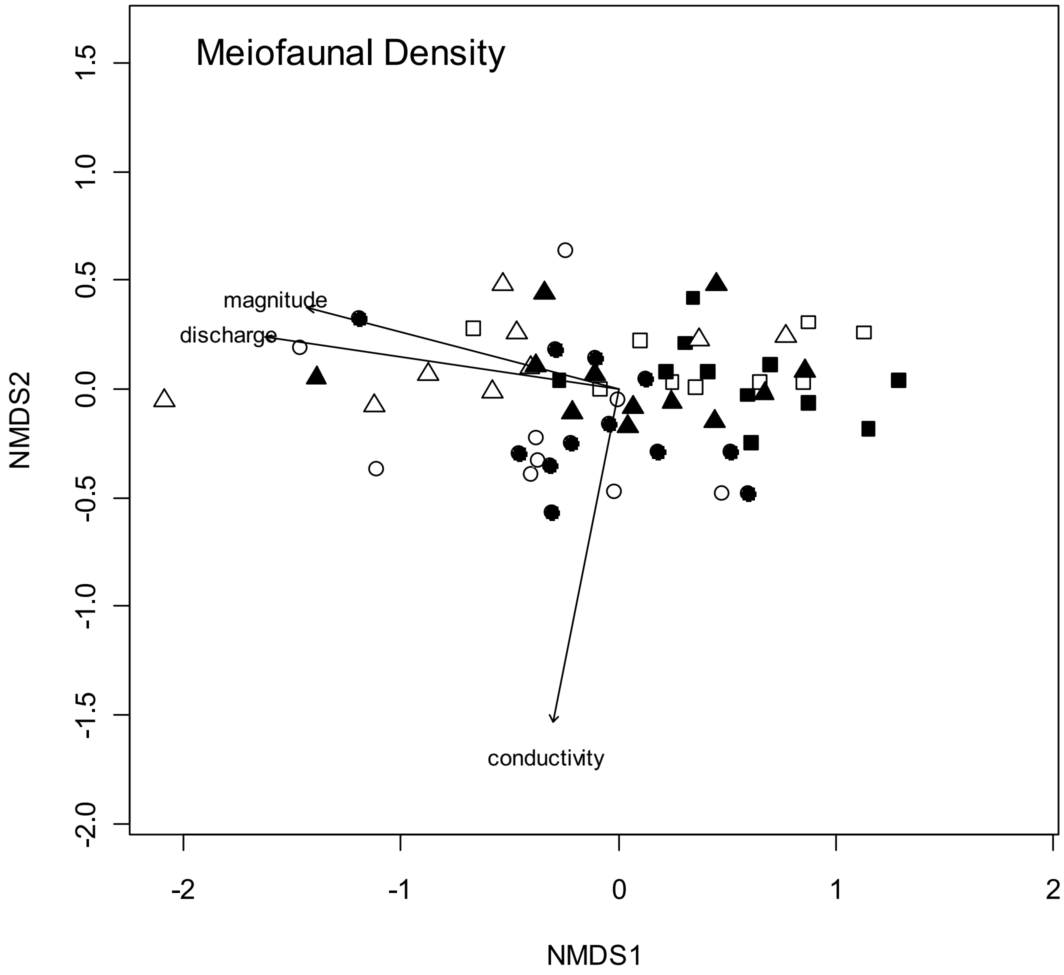

Fig. 1.4. Non-metric multidimensional scaling (NMDS) ordination plot of meiofaunal density (stress=0.09). Open symbols indicate 'wet years', solid symbols 'dry years'. The three reaches of a Mediterranean stream are indicated as follows: S1=squares, S2=triangles and S3=circles. Vectors of 'discharge', 'conductivity' and 'magnitude of the precipitation' are plotted (P<0.001).Effects of floods on meiofaunal communities

During the rainy year 2003–2004, three heavy rainfall events caused a dramatic increase in discharge. Immediately after the first and last floods (October 2003 and April 2004, respectively), the Fuirosos stream registered discharges greater than 3 $m^3 s^{-1}$ and total meiofaunal densities diminished to nearly zero in all reaches (Figs 1.2, 1.5). In December, 42 days after the first flood, when meiofauna had reached densities above previous values, a less severe second flood occurred. This event affected the recovery capacity of the meiofaunal population. At least 46 days were required to achieve normal meiofaunal densities in all reaches after the second spate. The meiofaunal community in the S1 reach was the fastest to recover (steeper regression slope). In spring 2004, the effect of flooding on density was more evident and the community needed more time (~60 days) to recover (Fig. 1.5). In this spring, discharge remained high (fed by a continuous drizzle), thereby hindering meiofaunal recovery.

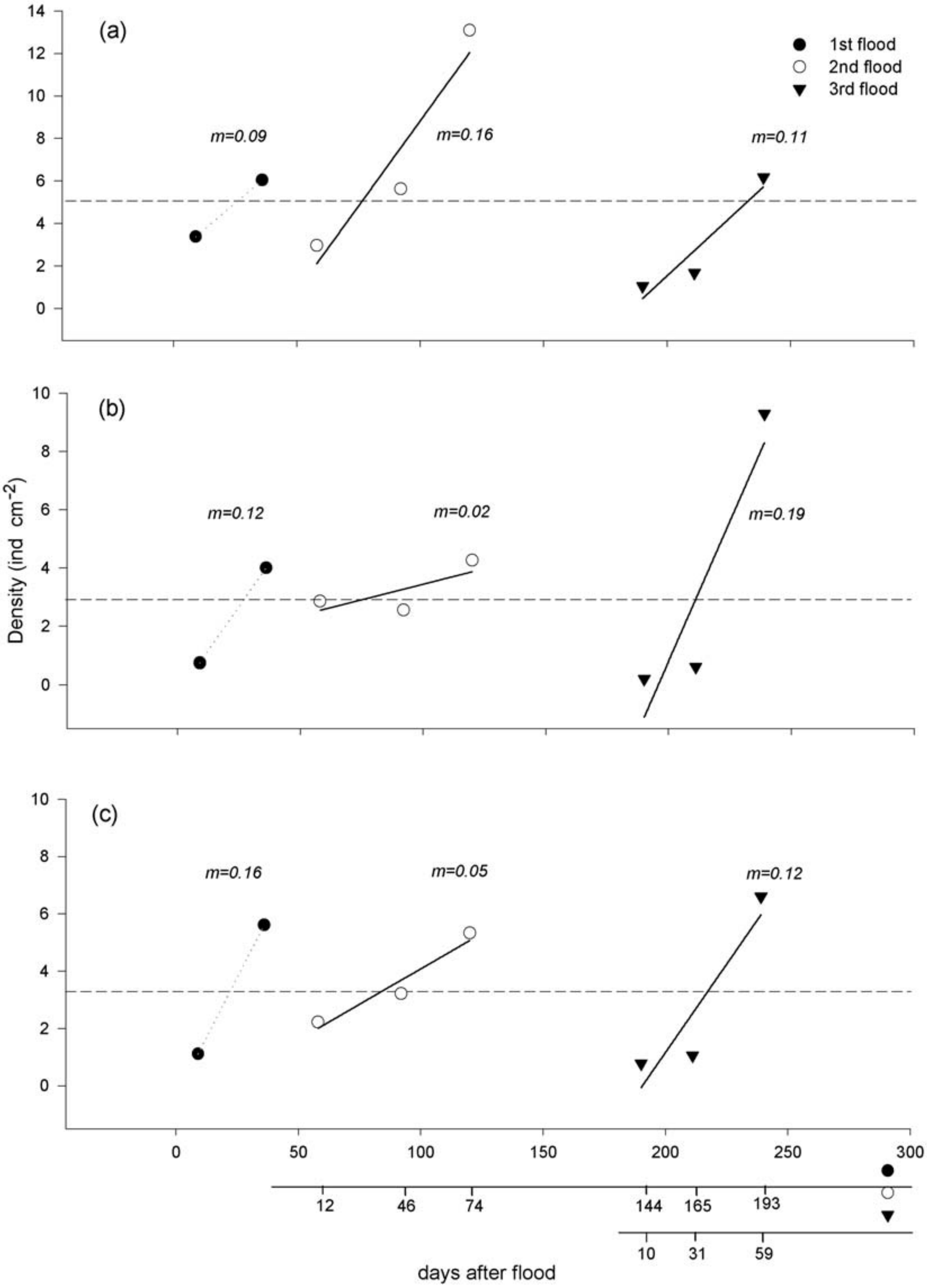

Fig. 1.5. Regression plots of meiofaunal density in the following three reaches of a Mediterranean stream: (a) S1 (pre-flood value=5.15 individuals cm^{-2}), (b) S2 (pre-flood value=3.14 individuals cm^{-2}) and (c) S3 (pre-flood value=3.3 individuals cm^{-2}). The dotted line between the two first flood points corresponds to a hypothetical trend line, reflecting insufficient sampling dates. The horizontal dashed line corresponds to the pre-flood value for each sampling point. The x-axis corresponds to the number of days after every flood event. Slopes (m) of the meiofaunal density regression plots are also shown.

1.5. Discussion

Meiofaunal responses to hydrology and environmental variables

The present study sought to analyse the structural and functional response of meiofaunal communities to hydrological variability in a Mediterranean headwater stream where summer dry periods and floods were common. Density and biomass found in this stream were in the range described by other authors (Palmer, 1990; Swan and Palmer, 2000; Reiss and Schmid-Araya, 2008), with maximum values occurring between late autumn and spring, when an optimal balance between discharge, temperature and organic matter availability was achieved. Oligochaetes, chironomids and nematodes were the foremost contributors to the total meiofauna in all three reaches. Small-sized organisms of these groups are often considered to be good colonisers because of their worm-like bodies. Worm-like morphology may be an advantageous trait when the hyporheic zone must be used as a refuge from scouring floods (Townsend, 1989; Dole-Olivier et al., 1997). Microcrustaceans also showed higher densities and biomasses. These were specifically related to retention and processing of streambed detritus by microorganisms during periods of flow stability (Gaudes et al., 2009). Traits associated with possessing active locomotory appendages (present in microcrustaceans to move from patch to patch) or with worm-shaped bodies to burrow within the sediment were positively correlated with hydrological stability parameters in Fuirosos (Table 1.3) and negatively correlated with flow discharge. These relationships were more evident in the first- and second-order reaches, where the density of taxonomic groups with the presence of these traits was also higher (Table 1.2). The absence of a positive correlation with flow discharge could be due to the lack of resistance strategies to floods by the meiofaunal organisms (although some temporary meiofauna can develop resistance structures in late larval stages).

Changes in discharge can indirectly affect quantity and quality of resources. In Fuirosos, during late winter, continuous floods cause the drift of the detrital layer on top of the biofilm, as well as the drift of accumulated autumn leaves. This drift leads to an increase in primary production until the first leaves of the riparian forest reduce incident light (Sabater et al., 2006; von Schiller et al., 2008; Artigas et al., 2009) and produce detritus that accumulates in the sandy riverbed. This fresh, high-quality resource could explain the positive correlation between the meiofauna and chlorophyll in the biofilm. Several authors have stressed the relevance of primary production in detritus-based systems (Thorp and Delong, 2002; Álvarez, 2004). However, the meiofauna did not show a direct relationship with accumulation of organic matter in the system (in terms of carbon and nitrogen per cm^2). After massive leaf abscission in autumn, the streambed was completely covered with leaf litter and some sandy patches became anoxic (A. Gaudes, pers. obs.), thereby diminishing meiofaunal abundance. Moreover, microcrustaceans and other meiofauna prefer to feed on the biofilms growing on the highly conditioned organic matter (Perlmutter and Meyer, 1991; Gaudes et al., 2009) that accumulates in stream depositional zones after several months of processing.

Temporary meiofauna had higher biomass throughout the study period and higher densities during flow disruption. Similarly, Acuña et al. (2005) observed higher macroinvertebrate densities and biomasses in wet summers when streams had partially disconnected pools. Organisms with emergent adults could benefit from these conditions because of the lower risk during extreme events such as floods or droughts. Sandy pools would furnish a refuge for their juveniles.

Previous hydrological history seems to modulate invertebrate response to floods or to influence community development after drying of the streambed (Boulton and Lake, 1992). The three reaches studied had a similar constrained morphology and were subject to continuous changes in discharge. From this perspective, these reaches could be considered to be hydrogeomorphic patches (Thorp et al., 2006). These hydrogeomorphic patches potentially promote development of a similar meiofaunal community as observed in macroinvertebrates (Downes et al., 1993). However, the conditions in one particular patch of the streambed depend on processes taking place on larger scales in the river network (Frissell et al., 1986; Minshall, 1988). Our results indicate that small differences in the watershed directly affected discharge and habitat stability, in turn affecting the structure of meiofaunal communities in the Fuirosos stream. Higher hydrological stability was related to higher density and biomass of almost all the meiofaunal groups in headwater reaches. These reaches will act as a refugium and thereby contribute to repopulation of downstream reaches.

Flood effects and resilience

Resilience of an organism to extreme flood events depends on its degree of mobility and is also linked to the mode of flood adaptation (i.e. life-history, behavioural or morphological adaptation). In turn, the adaptational mode of an organism determines the organism's vulnerability, depending on timing, predictability or duration of the perturbation (Ilg et al., 2008). Meiofauna in our stream showed relatively high resilience to floods. Approximately 50 days were necessary to restore pre-flood density values. This response differed among the reaches and as a function of flood frequency. Headwaters responded faster after repeated increases in flow, as was observed during the autumn–winter period. In contrast, flows in headwaters with higher slopes often increased when precipitation was relatively modest, as a result of direct runoff. These harsher conditions contrast with the higher density, biomass and resilient response observed in the S1 reach. Although upstream tributaries do not have an upstream faunal repository network (Townsend, 1989), these reaches can provide relative stability to these populations (Gooderham et al., 2007). The lower capacity of tributaries to carry large pieces of wood and rocks creates a stochastic distribution of dams and patches (e.g. dead zones) in which organic matter (leaves and detritus) can accumulate and be colonised. This streambed heterogeneity may be modified by an individual flood; however, given that extensive disturbances of the substratum are infrequent in typical headwater streams (e.g. Lepori and Hjerdt, 2006), their impact on populations may be less severe than in downstream reaches. In the Fuirosos stream, meiofaunal generalists with small body size, often related to shorter life cycles (Griswold et al., 2008), are adapted to move among patches (e.g. drift and crawling; Williams and Hynes, 1976) and to resist floods (e.g. worm-shape trait; Townsend and Hildrew, 1994) or desiccation (e.g. cocoons; Williams, 1987, 2006; Davies et al. ,2005).

The higher abundance of these generalists may also increase the plasticity of communities in such systems.

Overall, the results from the present study emphasise the significance of the natural variability of meiofaunal assemblages in low-order reaches, their adaptive response to discharge disturbances and the functional importance of their communities as sources of dispersers that can repopulate downstream reaches. Possession of a small body size, a worm-shaped body and locomotor appendages appear to be successful species traits in response to hydrological disturbance in the studied reaches. These phenotypic traits are widespread in aquatic organisms (Doledec et al., 1999). Adaptations of the meiofaunal community to sandy-pool refuges assure fast recuperation after floods. However, frequency of floods and the stream order can modulate this recovery.

CHAPTER 2

Contribution of microbial and invertebrate communities to leaf litter colonization in a Mediterranean stream

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Journal of the North American Benthological Society, 2009, 28:34-43

SUMMARY

Leaf litter inputs and retention play an important role in ecosystem functioning in forested streams. We examined colonization of leaves by microbes (bacteria, fungi, and protozoa) and fauna in Fuirosos, an intermittent forested Mediterranean stream. Black poplar (*Populus nigra*) and plane (*Platanus acerifolia*) leaf packs were placed in the stream for four months. We measured the biomasses and calculated the densities of bacteria, fungi, protozoa, meiofauna, and macroinvertebrates to determine their dynamics and potential interactions throughout the colonization process. Colonization was strongly correlated with hydrological variability (defined mainly by water temperature and discharge). The first week of colonization was characterized by hydrological stability and warm water temperatures, and allocation of C from microbial to invertebrate compartments on the leaf packs was rapid. Clumps of fine particulate organic matter (FPOM) were retained by the leaf packs, and enhanced rapid colonization by microfauna and meiofaunal collector-gatherers (ostracods and copepods). After two weeks, an autumnal flood caused a 20-fold increase in water flow. Higher discharge and lower water temperature caused FPOM-related fauna to drift away from the packs and modified the subsequent colonization sequence. Fungi showed the highest biomass, with similar values to those recorded at the beginning of the experiment. After 70 days of post-flood colonization, fungi decreased to nearly 40% of the total C in the leaf packs, whereas invertebrates became more abundant and accounted for 60% of the C. Natural flood occurrence in Mediterranean streams could be a key factor in the colonization and processing of organic matter.

2.1. Introduction

Leaf litter is an important energy source for food webs in forested stream ecosystems (Fisher and Likens, 1973; Webster and Benfield, 1986), especially in low-order systems (Minshall et al., 1985). Input and retention of organic matter in Mediterranean forested streams are strongly affected by climate (Sabater et al., 2001; Acuña et al., 2005) because the annual peak of leaf fall often coincides with heavy flooding in autumn (Acuña et al., 2007; Gasith and Resh, 1999). Thus, colonization and decomposition of leaf litter might be determined by the availability of organic matter and hydrology in Mediterranean forested streams (Gasith and Resh, 1999). Floods are a major organizing factor of organic matter dynamics because they determine cycles of accumulation and removal. Consequently, they provoke changes in consumer communities.

Leaf structure and chemistry and streamwater chemistry are the main determinants of microbial colonization and metabolism (Bärlocher et al., 1995; Gulis and Suberkropp, 2003). Fungi are the main colonizers during initial phases, whereas bacteria dominate during late stages (Baldy et al., 1995) and probably benefit from fungus-induced changes in leaf surfaces or from the release of labile compounds (Allan, 1995). The dominant role of fungi during colonization of large particulate organic matter (Gessner, 1997; Findlay et al., 2002) and their contribution to overall loss of C from leaves (Gulis and Suberkropp, 2003; Pascoal and Cassio, 2004; Hieber and Gessner, 2002) mean that net C transformation mostly reflects the capacity of the fungal community to metabolize leaf matter.

The shredding activity of macroinvertebrates on decaying leaves contributes to the breakdown process. Shredders feed on coarse particulate organic matter (CPOM) and can accelerate decomposition by increasing leaf tissue fragmentation (Graça, 2001; Graça and Canhoto, 2006). Macroinvertebrates feed preferentially on conditioned leaves, thereby ingesting nutrients from leaf tissue and from the attached microbial community (Slansky and Scriber, 1985). However, little information is available on the contribution of protozoa and smaller invertebrates to colonization and decomposition (Franco et al., 1998; Robertson and Milner, 2001). Micro- and meiofauna are mostly bacterial specialists (Meyer, 1994; Borchardt and Bott, 1995), and they can exert significant grazing pressure on bacteria and fungi that colonize detritus (Perlmutter and Meyer, 1991). Given the interaction between distinct groups of consumers, leaf litter breakdown is not a simple linear cause-and-effect relationship, but a simultaneous complex interaction between groups that could lead to several outcomes (Gessner et al., 1999).

We examined the complex interaction between decomposers and detritivores with regard to colonization of organic matter. We analyzed the successional colonization dynamics of microbes (including bacteria, fungi, and protozoa) and meio- and macrofauna on decaying leaves during a leaf-pack experiment in a forested Mediterranean stream. We addressed the following: 1) the most important factors that affect colonization dynamics, 2) the settlement sequence of leaf litter colonizers, and 3) the contribution of microbial organisms and meio- and macrofauna to the leaf litter biofilm and to the total C budget of the leaf packs. We hypothesized that the sequence of colonization would be done by microbes, meio-, and macrofauna in this order. If this hypothesis is correct, C allocation should change between compartments over time. We also speculated that co-occurrence of organic matter processing and high climatic instability could introduce disruptions or accelerations into this sequence in Mediterranean streams.

2.2. Study site

We worked in an intermittent 3rd-order stream (Fuirosos) in northeastern Spain (lat 41°42’N, long 2°34’W). Fuirosos drains a 10.9-km^2 forested catchment that lies in the Natural Park of the Montnegre-Corredor range. Precipitation occurs mostly in autumn and spring, which are periods of frequent flooding, with occasional storms in summer. High variation in rainfall is characteristic of this typical Mediterranean system, which has large deviations in mean monthly precipitation and considerable interannual differences. The stream usually has a mean discharge of 30 L/s, width of 3 to 4 m, and depths ranging from 0.1 to 0.5 m (see details in Acuña et al., 2005).

We did the colonization study in a 50-m long reach. The nearby riparian area (10–15 m width) formed a closed canopy from May to October. Dominant vegetation was alder (*Alnus glutinosa*, L.), black poplar (*Populus nigra*, L.), hazelnut (*Corylus avellana*, L.), and plane (*Platanus acerifolia*, Aiton – Willd.). Direct and lateral inputs of organic material (OM) ranged from 0.1 to 4.45 g C m^{-2} d^{-1} (Acuña et al. 2007). The canopy reduced incident light in summer, and total light increased in winter (Romaní et al. 2004).

2.3. Material and methods

Sampling strategy

We used the litter bag technique to monitor the microbial and faunal colonization of leaf litter. We collected recently abscised leaves of *Platanus acerifolia* and *Populus nigra* from the riparian floor in September 2003. We dried the leaves (room temperature, 48 h) and sterilized them in an autoclave (121°C, 30 min) before placing them in plastic mesh bags (1 mm mesh). The sterilization procedure can affect processing rates (Godshalk and Wetzel, 1978), but we used it to eliminate previous colonizers. We used the relatively small mesh to facilitate analysis of the C pathway throughout the biofilm where the microbial loop occurs and to prevent ingestion of the leaf tissue by larger external macroinvertebrates. We placed 11 thin ropes supporting 3 bags each (a total of 33 bags, each containing 4 *Platanus* and 6 *Populus* leaves) every 3 to 5 m (depending on the presence of natural leaf accumulation zones) along the 50-m reach. We retrieved 3 randomly selected bags on days 1, 2, 4, 7, 17, 28, 44, 58, 73, 93 and 112. Day 0 was 6 October 2003 when litter bags were immersed.

We reduced the loss of nonattached micro- and meiofauna from the leaf packs during retrieval by placing the still-immersed bags in a plastic container, which was then removed from the stream. We removed macroscopic colonizers from the outside of the bags if they were present. We took a leaf subsample and its surrounding water from the container and counted attached and nonattached protozoa. We used a metal borer to cut 1.1-cm diameter disks from the leaves and biofilm to sample fungi and bacteria. We placed samples for measurement of ergosterol (to estimate fungal biomass) in plastic vials and froze them (–20°C) until analysis. We preserved samples for bacterial density in formalin (2%). We dried additional leaf disks (70°C) to constant mass to obtain dry mass (DM). We passed the rest of the contents of the mesh bag through 500-µm and 32-µm nested sieves. We scraped the leaf fraction retained by the 500-µm sieve to detach macroinvertebrates and stored the leaves for later measurement of surface area with a scanner and ImageJ software (v. 1.40, http://rsbweb.nih.gov/ij/index.html), biomass, and C content. We fixed macroinvertebrates retained in the 500-µm sieve immediately in 4% formalin, and stored them for further counting and identification. We retained the fraction between the 500-µm and 32-µm sieves and stored it at 4°C for counting live meiofauna.

Physicochemical measurements

We measured O_2, pH, conductivity, and temperature with handheld meters (MultiLine F/SET-3, WTW, Weilheim, Germany) in the field on each sampling date. We measured current velocity (MiniAir2; Schiltknecht, Zurich, Switzerland) beside the litter bags before they were collected. We calculated stream discharge with using the slug-injection method with NaCl as the tracer (Gordon et al., 1992).

Bacterial density and biomass

We estimated bacterial density in triplicate in each litter bag after sonicating the samples (2 + 2 min, 40 W power, 40 kHz frequency; Ultrasons, Selecta, Abrera, Spain). After appropriate dilution, we stained fixed samples for 5 min with 4',6-diamidino-2-phenylindole (DAPI; final concentration = 2 µg m/L) and passed them through 0.2-µm irgalan black-stained polycarbonate filters (Nuclepore, Whatman, Maidstone, England). We counted bacteria in 15 fields/filter (400–800 organisms) with a fluorescence microscope (Eclipse E-600, Nikon, Tokio, Japan) at 1250× magnification. We measured the volume of cells with a Soft Imaging System (analySIS®,Olympus, Münster, Germany). We used empirical bacterial biovolumes of 0.147 μm^3 (*Populus*) and 0.163 μm^3 (*Platanus*) and a conversion factor of 2.2 × 10–13 g C/μm^3 to estimate bacterial C (Bratbak and Dundas, 1984).

Ergosterol content

We lyophilized frozen leaf samples and used 3 subsamples from each litter bag for ergosterol extraction by saponification with methanol (80°C, 60 min) in a shaking bath. We purified the resulting extracts by solid-phase extraction (Gessner and Schmitt, 1996) and measured ergosterol by high-performance liquid chromatography (HPLC) (Waters Inc., Milford, Massachusetts). We detected ergosterol at 282 nm and quantified it by comparison with ergosterol standards (0–200 µg m/L, Fluka Chemical Co.) (Gessner and Schmitt, 1996). We estimated fungal C biomass on the basis of an ergosterol content of 5.5 mg/g fungal biomass (Gessner and Chauvet, 1993) and 43% C content in fungal dry mass (Baldy and Gessner, 1997).

Determination of microfauna, meiofauna and macroinvertebrates

We counted live microfauna (protozoan) samples on the day of collection with a microscope at 400× magnification (Polivar, Reichert-Jung, Wien, Austria). Meiofauna were counted within the next 2 d (occasionally 3 d) under a dissecting microscope (MZ9.5, Leica, Wetzlar, Germany). Community composition and density of meiofauna in live samples were similar regardless of whether they were processed within 1 or 3 d (data not shown).

We determined microfaunal densities from counts in ten 100-µL drops from each litter bag subsample. We also examined leaf tissue in the subsamples under a microscope to observe sessile ciliates. We identified individuals to order and assigned them to feeding-type groups (after Fenchel, 1986, 1987; Franco et al., 1998): upstream filter feeders (UFF; with a ciliary upstream-collecting mechanism capable of capturing particles >2 µm), downstream filter feeders (DFF; with an opposed band or downstream-collecting mechanism capable of capturing particles <2 µm), and raptorial feeders (RF; with immobilization mechanisms, such as extrusomes). We estimated wet mass with values reported by Foissner and Berger (1996), and converted wet mass to C biomass with published power equations (Bottrell et al., 1976).

We identified and measured soft-bodied meiofauna live, but we counted, identified, and preserved hard-bodied meiofauna in formalin for further length and width measurements. We estimated biomass from exponential equations when they were available, or otherwise from biovolumes. We found published power equations or values for Ephemeroptera, Plecoptera, Coleoptera, Diptera (Meyer, 1989; Benke et al., 1999), Rotifera (Bott and Borchardt, 1999), Copepoda, and Cladocera (Bottrell et al., 1976). We used biovolume estimates for Nematoda (after Andrassy, 1956), Oligochaeta (Smit et al., 1993), Ostracoda, Hydracarina, Tardigrada, and Microturbellaria (Ramsay et al., 1997). We sorted fixed macroinvertebrates with a dissecting microscope, dried them (70°C) to a constant mass, and burned them (450°C, 4 h) to obtain biomass as ash-free dry mass (AFDM). We expressed all densities (meiofauna and macroinvertebrates) /µg leaf DM. However, we obtained biomass (µg organism C) through standard conversions (Waters, 1977) and expressed it /µg leaf C in each litter bag.

Data analyses

We used nonparametric Spearman's rank coefficient to identify possible correlations between environmental and biological variables. We applied a False Discovery Rate correction (FDR, Benjamini and Hochberg, 1995) because of the large number of comparisons made.

We did these statistical analyses with STATISTICA (version 8.0; StatSoft, Tulsa, Oklahoma). We used ANOSIM on 4th-root(x)-transformed data to compare average ranked Bray–Curtis similarities between sampled days with average ranked similarities within a sampling date (between replicates). We quantified dissimilarity between community assemblages through the colonization period with similarity percentages (SIMPER). We did these analyses with PRIMER (version 6.1.6; PRIMER-E, Plymouth, UK).

2.4. Results

Physicochemical

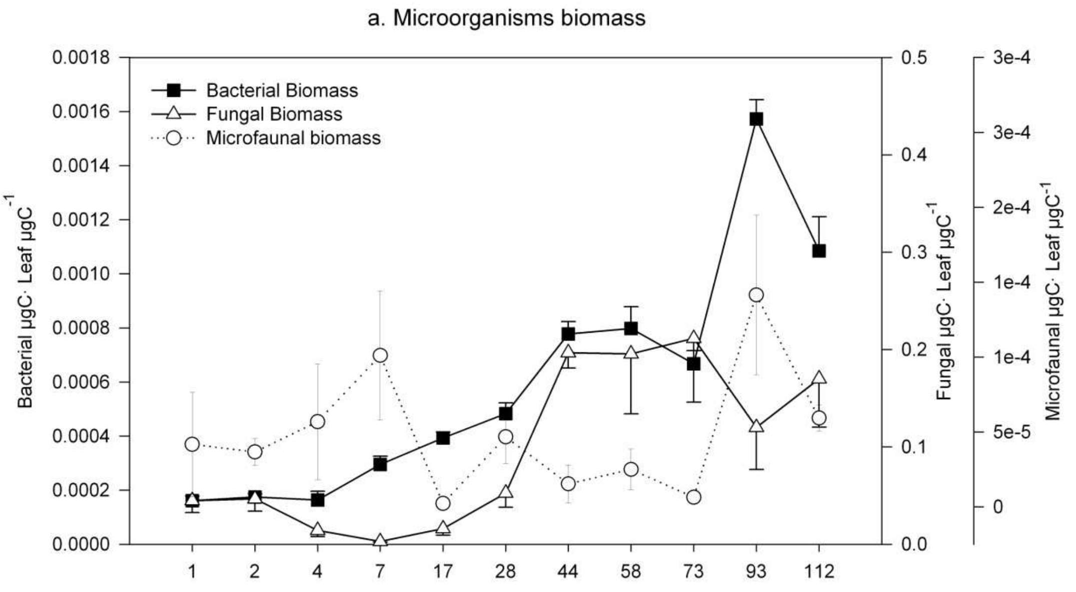
Water temperature decreased throughout the study period. It ranged from ~15°C in October to ~4°C in January (Table 2.1). On day 11, water flow suddenly increased from 1.2 to 90.4 L/s as a result of rain, and dissolved O_2 content increased and conductivity decreased. Water velocity next to litter bags ranged from 0.01 to 0.64 m/s. The flood caused significant differences between bags retrieved on consecutive sampling dates (Kruskal–Wallis statistic = 25.86, $p < 0.005$), but not between bags retrieved on the same day.

Table 2.1. Physical and chemical characteristics of Fuirosos stream water during the 4-mo colonization experiment. Values are individual measures of variables on the 11 sampling days.

Days	Temperature (°C)	Oxygen (mg/L)	Conductivity (µS/cm)	pH	Discharge (L/s)
1	11.61	6.41	307	6.67	4.62
2	12.5	6.29	305	6.75	4.62
4	13.67	5.83	314	6.6	1.46
7	15.3	5.2	302	6.54	1.19
17	12.8	10	181.8	7	90.41
28	11.8	18.1	188	6.64	63.97
44	9.4	9.66	196	7.44	14.66
58	10.3	10.61	173	7.24	60.05
73	8.5	11.2	174.3	7.79	60.10
93	4.3	12.64	188.6	7.73	30.23
112	8.2	9.55	198.2	6.82	14.00
Mean	**10.76**	**9.59**	**229.81**	**7.02**	**31.39**
s.e.	**0.92**	**1.13**	**18.61**	**0.46**	**31.64**

Bacteria and fungi

Fungi and bacteria accumulated on the leaf material following a logistic pattern (Fig. 2.1A). However, bacteria began a 2nd increase after day 73 of the experiment, whereas fungi remained stable until the end of the experiment. Fungal biomass was ~200× higher than bacterial biomass except during week 2 (Fig. 2.1A).



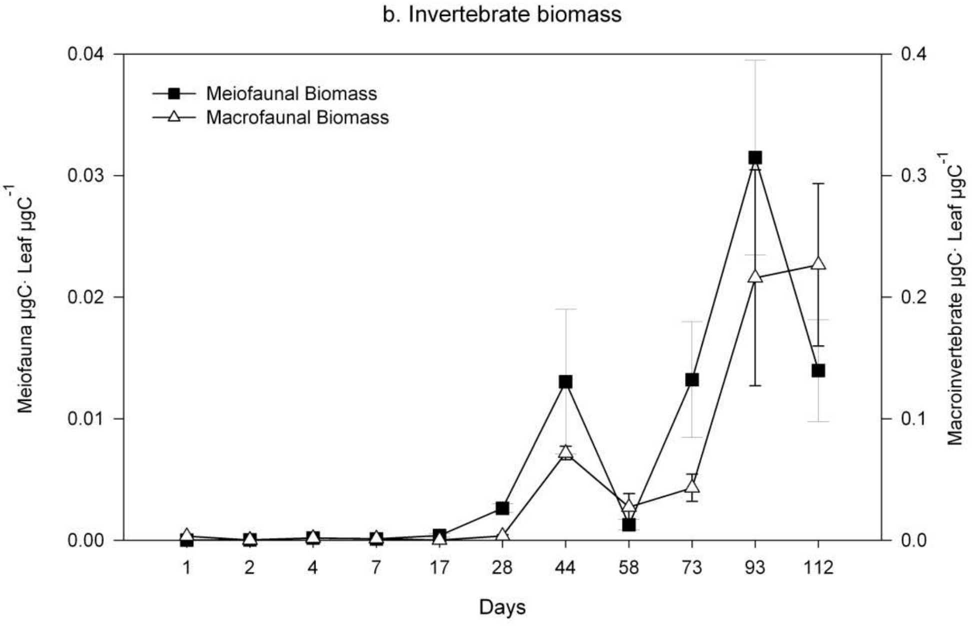
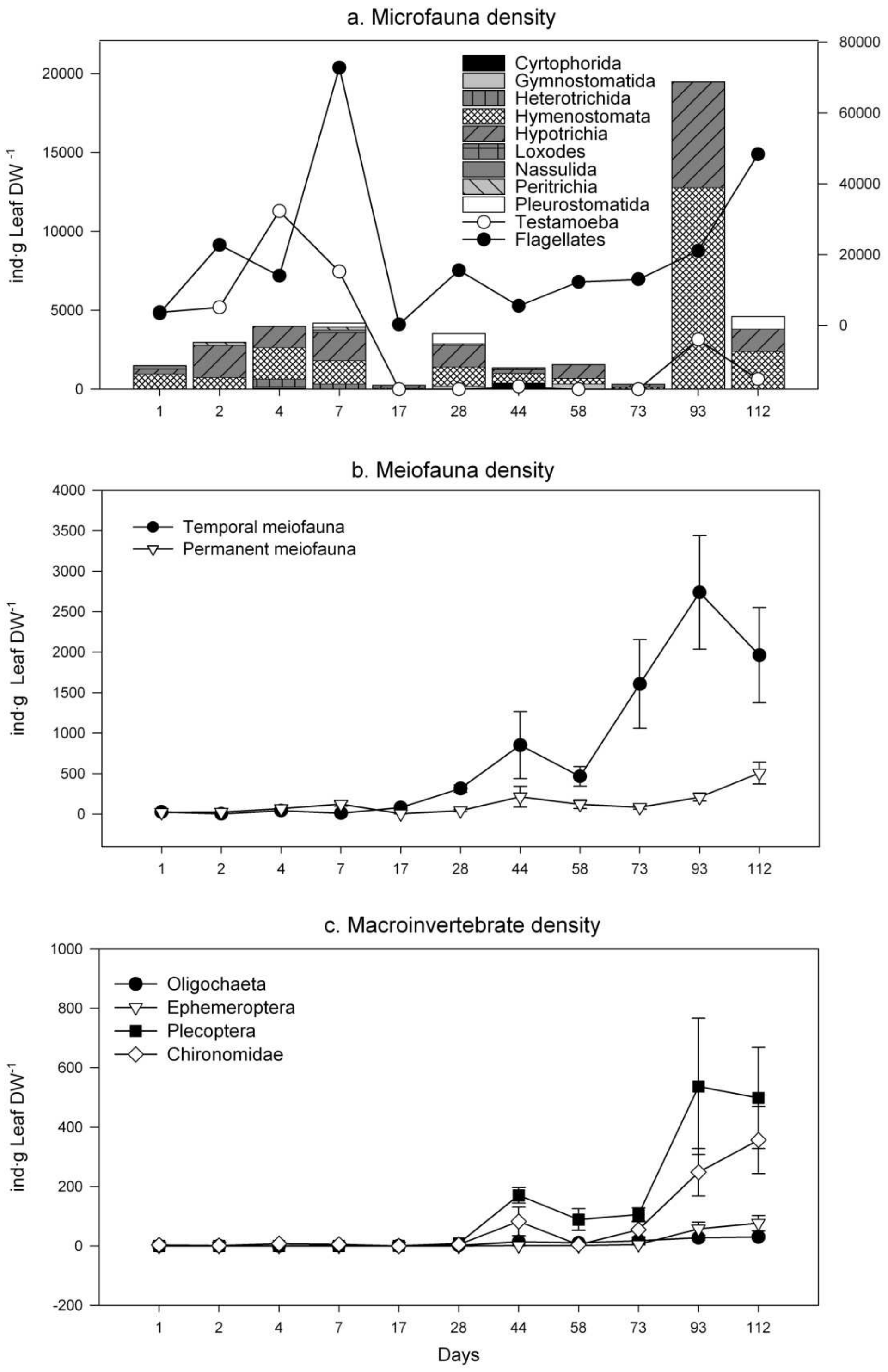


Fig 2.1. Mean (± SE) bacterial, fungal, and microfaunal biomass (A) and meiofaunal and macroinvertebrate biomass (B) during a 4-mo study of colonization of leaf litter in Fuirosos. Error bars are as follows: bacteria (positive thick line and long cap), fungi (negative thick line and short cap), microfauna (thin line and short cap), meiofauna (thin line and long cap), and macroinvertebrates (thick line and short cap).

Microfauna

The most abundant groups of microfauna were flagellates, testamoebae, and ciliates (Fig. 2.2A). Microfauna density and biomass increased during week 1 (Fig. 2.1A) to 1.12×10^4 individuals (ind.)/g leaf DM (testamoebae) and 7.3×10^4 ind./g leaf DM (flagellates) (Fig. 2.2A). Ciliate density was moderate (240–~5000 ind/g leaf DM), except on day 93 when it peaked at 1.95×10^4 ind./g leaf DM. Microfauna community composition differed among days (ANOSIM, $R = 0.52$, $p = 0.001$), and these differences were greatest (with dissimilarities >70%) immediately after the flood (day 17; SIMPER), when microfauna density was almost 0 (Fig 2.2A). Microfauna densities remained low until day 93, when they increased again. On day 93, ciliate density was high, but taxon richness was lower than before day 17. Hypotrichia (UFF) and Hymenostomata (DFF) were the most abundant ciliates from day 93 to day 112, when Pleurostomatida (RF) also were present.


a. Microfauna density

b. Meiofauna density

c. Macroinvertebrate density

Fig.2.2. Mean density of microfauna (A), meiofauna (±1 SE) (B), and macroinvertebrates (±1 SE) (C) during a 4-mo study of colonization of leaf litter in Fuirosos. In panel A, densities of ciliates and Testamoebae are shown on y-axis 1 and densities of flagellates are shown on y-axis 2. In panels B and C error bars are as follows: temporary and permanent meiofauna, Oligochaeta, and Ephemeroptera (normal line and cap); Plecoptera (positive cap); and Chironomidade (negative cap). DM = dry mass.

Meiofauna

Densities of Chironomidae, Rotifera, and Oligochaeta were higher than those of other groups of meiofauna. Community composition differed significantly among sampling days (R = 0.835, p = 0.001) and between samples collected before and after the flood (R = 0.73, p = 0.001). Microcrustaceans were the most common group of meiofauna only during the 1st week. This group consisted mainly of ostracods and copepods (with more nauplii than copepodites and adults) and, to a lesser extent, cladocerans. Two patterns were observed during colonization (Fig. 2.2B), corresponding to temporary and permanent meiofauna. Temporary meiofauna, including early larval stages of Chironomidae, Oligochaeta, Plecoptera, and Ephemeroptera (Robertson et al., 2000b) followed the dynamics of their macroinvertebrate representatives (Table 2.2, Fig. 2.1B) and increased during the late phases of colonization. Densities of permanent meiofauna (Rotifera, Nematoda, Microcrustacea, Microturbellaria and Tardigrada) were lower than those of temporary meiofauna (Fig. 2.2B). Permanent meiofauna contributed to total meiofauna density, but most meiofaunal biomass was made up by temporary meiofauna (Fig. 2.3).

TABLE 2.2. Spearman's rank correlation coefficients (r) between biomasses of macroinvertebrates and their meiofaunal- sized representatives (temporary meiofauna) across all sampling days. ^{a}p<0.05 (with false discovery rate correction).

TAXON	
COLEOPTERA	*0.92* ^{a}
OLIGOCHAETA	*0.95* ^{a}
CHIRONOMIDAE	*0.75* ^{a}
EPHEMEROPTERA	*0.94* ^{a}
MICROCRUSTACEA	0.30
PLECOPTERA	*0.80* ^{a}

Macroinvertebrates

Macroinvertebrate densites were low and ranged between 0 and 800 ind./g leaf DM (Fig. 2.2C). Large Ostracoda (>250-µm) were the earliest colonizers, followed by Plecoptera (mainly Nemouridae) and Chironomidae (abundant on day 44; Fig. 2.2C). Ephemeroptera, Oligochaeta, and Coleoptera increased late in the study. Macroinvertebrate biomass increased during colonization, and made up most of the total invertebrate biomass at the end of the study (Fig. 2.1B).

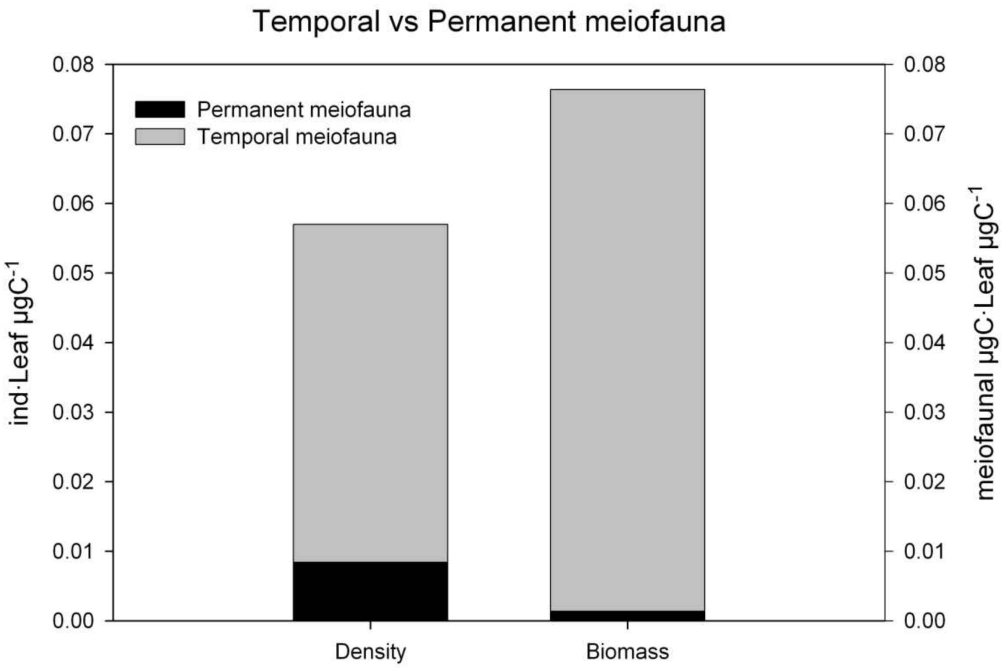


FIG. 2.3. Contribution of temporary and permanent meiofauna to total meiofaunal density and biomass. Both meiofaunal density and biomass are relative to leaf C biomass.

Relationships between organisms and environmental characteristics

Microcrustacean biomass and conductivity were significantly negatively correlated with discharge (Table 2.3). Some ciliates also tended to be negatively correlated with discharge, but these relationships were not statistically significant after FRD correction. Oligochaeta, Chironomidae, Plecoptera, and Ephemeroptera biomasses were significantly positively correlated with bacterial biomass and negatively correlated with water temperature. Fungal biomass was significantly positively correlated to macroinvertebrate biomass and negatively correlated with water temperature (Table 2.3).

TABLE 2.3. Spearman's rank correlation coefficients (r) test among protozoans (feeding types, see text), groups of metazoans, fungi, bacteria, and environmental variables. TM = temporary meiofauna size representatives of a macroinvertebrate group, PM = permanent meiofauna, DO ¼ dissolved O2, UFF = upstream filter feeders (ciliate feeding group). [a] p<0.05 but does not meet the False Discovery Rate (FDR) cut-off; [b]p<0.05 (corrected by FDR).

Biomass Spearman rank correlations	Fungi	Bacteria	Temperature	Discharge
Chironomidae	0.56	*0.83*[b]	*-0.73*[b]	0.47
CHIRONOMIDAE	*0.62*[a]	*0.81*[b]	*-0.75*[b]	-0.03
Ephemeroptera	*0.66*[a]	*0.86*[b]	*-0.79*[b]	0.34
EPHEMEROPTERA	*0.76*[b]	*0.88*[b]	*-0.90*[b]	0.24
GASTEROPODA	*0.74*[b]	*0.83*[b]	*-0.87*[b]	0.13
MICROCRUSTACEA	*-0.62*[a]	-0.58	0.49	*-0.87*[b]
Oligochaeta	*0.78*[b]	*0.79*[b]	*-0.75*[b]	0.26
OLIGOCHAETA	*0.69*[a]	*0.88*[b]	*-0.81*[b]	0.22
Plecoptera	*0.90*[b]	*0.77*[b]	*-0.72*[b]	0.53
PLECOPTERA	*0.75*[b]	*0.92*[b]	*-0.88*[b]	0.49
Rotifera	*0.66*[a]	*0.83*[b]	*-0.73*[b]	0.02
D.O.	*0.62*[a]	*0.62*[a]	-0.60	*0.88*[b]
Temperature	*-0.83*[b]	*-0.76*[b]	-	-0.36
Conductivity	-0.60	*-0.62*[a]	0.39	*-0.85*[b]
Fungi	-	*0.67*[a]	*-0.83*[b]	0.48
Bacteria	*0.67*[a]	-	*-0.76*[b]	0.44
UFF ciliates	-0.38	0.04	0.09	*-0.62*[a]

[a] *p-value<0.05 but it does not meet the FDR requirements;* [b] *p*-value<0.05 (corrected by FDR)

Allocation of C during the colonization

Fungal biomass accounted for ~32 to ~93% of the total organismal (nonleaf) C in the litter bags during the experiment, bacterial biomass accounted for ~0.2% to ~6%, and faunal biomass accounted for ~0.03% to ~8.4% (meiofauna) and ~1.7% to ~58% (macroinvertebrates) (Table 2.4). Between days 1 and 7, C exchange between compartments was rapid and shifted from fungal (~93%) to bacterial (~6%) and faunal (mainly macroinvertebrate) compartments (~30%). The flood reset the system and shifted the highest percentage of C back to the fungal compartment. Fungi continued to have the highest percentage of total C until late in the experiment when macroinvertebrates and, to a lesser extent temporary meiofauna, made up most of the nonleaf C in the litter bags. Between days 28 and 58, C in the temporary meiofaunal compartment was transferred to the macroinvertebrate compartment. After the flood, when water temperature was lower and discharge was higher than before the flood, almost 70 d of colonization were required to attain invertebrate C biomasses similar to those observed on day 7. On day 93, 60 to 70% of the total nonleaf C in the litter bags consisted of invertebrate biomass and 30 to 40% corresponded to fungal biomass.

TABLE 2.4. Percentages of total organismal C in each group of organisms that colonized leaf litter bags during a 4-mo experiment. Total absolute C is expressed as mg organismal C/litter bag.

C-BUDGET

% Carbon	Fungi	Bacteria	Protozoans	Perm. Meio	Temp. Meio	Macroinvert.	Total absolute Carbon
Day 1	92.68	0.33	0.09	0.03	0.03	6.84	142951.61
Day 7	59.80	6.18	2.11	1.87	0.58	29.46	13288.93
Day 17	93.65	2.29	0.01	0.02	2.35	1.68	65614.21
Day 28	88.64	0.81	0.08	0.10	4.33	6.03	184635.64
Day 58	86.95	0.35	0.01	0.06	0.51	12.11	404558.17
Day 93	32.51	0.43	0.04	0.08	8.45	58.49	71416.58
Day 112	41.28	0.26	0.01	0.11	3.28	55.05	127706.26

2.5. Discussion

Factors affecting colonization dynamics

Flow and temperature influence leaf litter breakdown. In desert streams, the duration of flood-free periods is an important factor regulating community biomass and efficiency (Grimm and Fisher, 1989). In Mediterranean streams, low rainfall and high temperatures in summer cause early leaf fall in riparian forests (Acuña et al., 2004). Vazquez et al. (2007) reported an increase of dissolved organic carbon when flow resumes due to the partly decomposed leaves accumulated in the streambed. This accumulated organic C is therefore available as a source of energy and shelter to aquatic organisms. Mechanical breakdown caused by the turbulent waters when flows resume provides more surface area for colonization, accelerates decomposition of leaf litter, and enhances its conditioning (Graça, 2001; Heard et al., 1999). A similar process occurs in semiarid Australian rivers (Francis and Sheldon, 2002).

In Fuirosos, biological succession on decaying leaf litter was strongly correlated with discharge and water temperature. Our study began shortly before a flood associated with the end of the summer drought. FPOM accumulation and rapid primary colonization occurred during the initial period of low discharge and moderate water temperature. During the flood, the FPOM drifted downstream. After the flood, Populus leaves broke apart and were almost entirely washed out of the mesh bags by day 58; Platanus leaves, though broken, remained in the bags. The flood reset the colonization process. Subsequent recolonization occurred under higher flow, cooler temperatures, and different leaf characteristics, and these changes were associated with shifts in the composition of the colonizing community and slower transfer of C between compartments.

Settlement sequence of colonizers

Our choice of mesh size for the litter bags allowed us to study colonization of leaf litter by micro- and meiofauna, but also might have favored accumulation of detritus and its associated fauna. A procedural control might have permitted us to correct for litter-bag effects (Boulton and Boon, 1991). However, Acuña et al. (2005) surveyed naturally deposited leaves in Fuirosos and reported similar invertebrate composition and densities of the same order of magnitude as those in our litter bags on days 2 and 4.

Hydrological stability (discharge between 1–5 L/s, water velocities <12 cm/s) and high water temperatures allowed initial colonization of litter bags by microfauna, which achieved maximum diversity in this period. UFF and DFF feeding types appeared during this early stage of colonization. Both groups have membranelles that allow them to capture small particles (DFF: <2 µm, UFF: ~2 µm), such as bacteria and flagellates, which were abundant in this period. However, microfaunal densities were much lower than those reported on decomposing leaves in temperate streams (Bott and Kaplan, 1989; Schönborn, 1982) or in sandy sediments in Fuirosos (Domènech et al., 2006).

During week 1, FPOM retained by the litter bags was quickly colonized by meiofauna. Microcrustaceans thrived in these conditions because of their feeding preference for FPOM-associated microflora (Perlmutter and Meyer, 1991) and the shelter provided by the clumps of FPOM (Robertson and Milner, 2001; Gaudes et al., 2006). Clumps of FPOM also facilitate recolonization of downstream substrata during high flow events because they are retained in woody debris, leaf packs, and shores of the stream following high flow events.

After the flood, litter bags were progressively colonized by shredders and collector-gatherers (achieving the 60% of total C on day 93). Leaves trap FPOM (Richardson, 1992), which increases their nutritional value to collector-gatherers (Gjerlov and Richardson, 2004; Scealy et al., 2007). After 1 mo of colonization, the density of temporary meiofauna also increased, probably as a result of the greater microbial biomass in the remaining leaf material (Palmer et al., 2000).

Contribution to C budget and microbial-loop

This study shows changes in C allocation between compartments during the leaf colonization related to hydrology, temperature and organismal dynamics. Before the flood, nearly 40% of the total organismal C in the litter bags was in the bacterial and faunal compartments. After the flood, C in the faunal compartments was carried downstream, and fungi had the highest biomass. Late in the colonization, fauna became more abundant again and transference from the temporary meiofauna to the macroinvertebrate compartment was observed as a consequence of organismal growth.

Meiofauna can consume 22% of daily bacterial C production (Perlmutter and Meyer, 1991). Meiofauna and macroinvertebrates differ in their consumption of bacterial C (Perlmutter and Meyer, 1991). Meiofauna apparently prefer detritus-associated microflora, whereas macroinvertebrates nonselectively ingest large amounts of detritus. Competition between macroinvertebrates and meiofauna for bacterial C (Swan and Palmer, 2000) and competition between the 2 major decomposers, macroinvertebrates and fungi, for leaf-tissue compounds (Gessner et al., 1999) are important, but underresearched, phenomena. Knowledge of the manipulation, processing, and consumption of organic matter by all groups involved will contribute to a better understanding of the decomposition process.

Our results indicate that colonization of decaying leaf material is highly dependent on hydrology and temperature. In Mediterranean forested streams, the occurrence of floods during organic matter accumulation in the stream bed could shape the colonization (and subsequent decomposition) process and be essential to ecosystem functioning. After the flood, almost 70 d were necessary to restore the C budget values in the different compartments to those achieved during the first 10 d of colonization. The Mediterranean region is expected to endure higher temperatures and lower precipitation, especially in summer, under predicted climatic changes (IPCC, 2007). Under these conditions, the quantity of leaves in the streambed could increase, as happens now in the driest years (Sabater et al., 2001; Acuña et al., 2005), and colonization and decomposition dynamics might be accelerated with the first autumn rains. Changes in patterns of precipitation could alter the duration, frequency, and magnitude of flow and flood pulses. Modification of the inundation regime is expected to decelerate breakdown rates and reduce breakdown heterogeneity—and both factors influence decomposition process (Langhans and Tockner, 2006). The influence of these changes (temperature and flow) on the C budget during leaf-litter processing in the Mediterranean headwater streams remains to be seen.

CHAPTER 3

The meiofaunal response to nutrient addition in a Mediterranean stream

Ainhoa Gaudes, Jordi Ocaña, and Isabel Muñoz

(submitted to Limnology and Oceanography)

SUMMARY

The effects of a moderate addition of nutrients were examined during a 2-year period to determine the response to nutrient addition in a meiofaunal community inhabiting sandy patches in a Mediterranean stream. The pattern of meiofaunal assemblages exhibits a high degree of intra- and interannual variability. This pattern alternates between periods of hydrological stability and disturbances, such as floods and droughts, which is a characteristic of Mediterranean systems. A before-after-control-impact (BACI) design was used to determine the outcome of the addition by comparing an upstream non-enriched reach with an enriched downstream reach. Analysis of the study data by means of a nonparametric permutational procedure (PERMANOVA) showed that the fertilisation had a positive effect. Differences in density and biomass were significant in the most abundant meiofaunal groups, including microcrustaceans, oligochaetes and chironomids. Microcrustaceans were the dominant group in the permanent meiofauna. We also examined differences in secondary production in both reaches. Ostracods and cyclopoid copepods increased their secondary production in the impacted reach as a result of the nutrient addition. In our stream, the meiofaunal compartment could be enriched either directly, by the release of nutrients to water, or indirectly, by the enrichment of the detritus in which the meiofauna feed and live.

3.1. Introduction

Eutrophication of freshwater and coastal ecosystems is one of the major environmental problems worldwide (Smith and Schindler, 2009). Human activities (e.g., logging, agricultural practices and industrial waste) are the main sources of organic or inorganic nitrogen (N) and phosphorus (P) entering aquatic ecosystems. Alterations in the nutrient and organic matter available to organisms may affect many aspects of river ecosystems, including algal standing crop and consumer abundances (see Smith et al., 1999 and references therein). In this sense, nutrient additions may not simply alter the energy of basal sources. They can also affect the community composition and the spatial distribution of aquatic organisms (Pringle, 1990). Many previous studies have examined the effects of these inputs on primary producers in autotrophic streams. These studies have found an increase in the biomass at the base of the food web (e.g., Peterson et al., 1993; Slavik et al., 2004). Conversely, in oligotrophic detritus-based systems, nutrient enrichment may cause an increase in the decomposition of organic matter, thereby stimulating associated microbial activity and reducing the amount of litter fall present in the stream (Gulis and Suberkropp, 2003; Gulis et al., 2004; Greenwood et al., 2007; Suberkropp et al., 2010).

The effects of enhanced nutrients on detritus may extend to the consumers as a consequence of the composition of the community (Cross et al., 2005, 2006). Reiss and Schmid-Araya (2008) have found higher species diversity in a nutrient-rich meiofaunal community than in an oligotrophic system. However, the effects of nutrient enrichment on bacterial, ciliate and meiofaunal biomass in a Swedish lake were unclear (Hillebrand et al., 2002). An observed lack of clear enrichment effects could result partly from natural variability in community temporal patterns. In some cases, this variability may conceal the effects of nutrient addition. In that sense, analysis of functional processes may represent a more robust approach to the assessment of these effects.

Secondary production is a functional measure of population dynamics that provides insights into individual-, population-, and ecosystem-level processes because it provides an estimate of the energy available for transmission from one trophic level to the next (Waters, 1977; Benke, 1993). Analyses of secondary production have provided insight into population and food web dynamics (Benke, 1993; Hall et al., 2000), but their application in studies of anthropogenic stress is still rare (Benke, 2010). However, some studies have included the use of secondary production to assess the effects of pesticide manipulation (Lugthart and Wallace, 1992; Whiles and Wallace, 1995) and urbanisation (Shieh et al., 2002) on stream communities. The former studies found that production measures were more effective than either abundance or diversity in increasing the ecological relevance of the observed stressor responses.

Nutrient-induced changes in the detrital/microbial food base can provide a resource subsidy, thereby increasing production. This process demonstrates the importance of food quality to fast-growing detritivores (Cross et al., 2005, 2006). Similarly, growth and production of chironomids have been found to be very sensitive to phosphorus increases in Costa Rican streams (Ramírez et al., 2006). However, De Lange et al. (2004) have suggested that invertebrate production at moderate levels of pollution in floodplain creeks affected assemblage structure but not production.

Studies conducted in aquatic habitats seldom include meiofauna groups. They focus instead on the macrofauna. Studies having this emphasis give the impression that total secondary production equals that of insects (freshwaters: Benke et al., 1999; Poepperl, 2000; Meyer and Poepperl, 2004) or crustaceans (marine environments: e.g., Cartes et al., 2002).

Stead et al. (2005) have estimated the production of benthic fauna in an English acid stream. They found that 15% of the production was attributable to permanent meiofauna (e.g., taxa always small enough to pass through 500-µm mesh). An additional 36% was attributable to temporary meiofauna (early stages of taxa usually small enough to pass through 500-µm mesh). Although the annual P/B for taxa with relatively small body size may range over several orders of magnitude, these taxa are capable of generating some of the highest rates of biomass turnover reported for eukaryotes (Stead et al., 2005; Huryn and Benke, 2007).

Meiofauna in Mediterranean systems adapt to hydrological disturbances by using either behavioural or structural traits (Gaudes et al., 2010). Most papers that have addressed the effects of perturbations on stream communities have focused on the deterministic control that these perturbations exert on communities (e.g., Lepori and Malmqvist, 2009). Mediterranean regions are characterised by a high amount of seasonal and interannual variability that involves the frequency and timing of droughts and floods (Gasith and Resh, 1999; Lake, 2003; Acuña et al., 2005). Stream communities in Mediterranean systems are highly diverse. The organisms that make up these communities have adapted their life cycles to predictable seasonal disturbances in the hydrological regime. However, hydrologic alterations affect the functional organisation of streams and rivers, and they lead to a simplification and impoverishment of the biota within these ecosystems (Sabater and Tockner, 2010).

We consider that nutrient enrichment interacts with many site-specific conditions, especially with the ecological stability of the system. Accordingly, our objective was to determine the direct effect of a moderate nutrient addition on a Mediterranean meiofaunal community. In eutrophic conditions, we would expect that the species pool of these communities would be reduced. Then, if a pulsed disturbance occurred, the community would be less able to withstand the effects of the disturbance. With this consideration in mind, the whole meiofaunal community was studied both before and after fertilisation occurred. Specifically, this experiment addressed the influence of the nutrient addition on the temporal pattern of the community and on secondary production. To some extent, we would expect that organisms such as meiofauna with short life cycles would respond rapidly to perturbations (i.e., an increase in nutrient availability). We would expect that this response would be progressively transferred to the higher trophic levels.

3.2. Study site

The study was performed in Fuirosos, a third-order stream that drains a 15.6 km^2 forested granitic basin. Nearly the whole catchment is included in the Montnegre–Corredor Natural Park, located in Northeastern Spain (lat 41° 42’N, long 2° 34’E; 150-

400 m asl). Holm oak, coniferous, and deciduous forest cover 90% of the total catchment, which also has small areas of agricultural land.

The two stream reaches selected were contiguously placed and 50 m in length. Both had similar slope, light irradiance, water flow and streambed composition (Sabater et al., 2011). Surveys were focused on sandy pools in which the meiofaunal organisms are dominant.

3.3. Material and methods

The two reaches were monitored monthly for water chemistry and environmental characteristics during the pre-enrichment (1 year) and enrichment (1 year) periods. From 14 June 2004 until 13 June 2005, nutrients were added continuously to the enriched reach (E) by a flow system consisting of a 200-litre reservoir connected to a tap that dripped dissolved nutrients at a constant rate. The tap was placed over a small waterfall and thereby ensured complete mixing of the effluent with stream water. The average nitrogen concentration was increased 2-fold, and phosphorus was increased 3-fold with respect to the background concentrations in the stream (Sabater et al., 2011). This increase was chosen to produce a moderate enhancement of the concentration of dissolved nutrients, particularly of phosphorus, which is the most limiting nutrient in the stream (Butturini et al., 2003). This outcome was achieved by making weekly adjustments to the solution concentration and the rate of dripping according to the changes occurring in stream flow.

Physical and chemical measurements

Conductivity, temperature, dissolved oxygen and pH were measured with handheld field meters (MultiLine F/SET-3; WTW, Weilheim, Germany) on each sampling date. Current velocity (MiniAir2; Schiltknecht, Zurich, Switzerland) was measured in the sandy pools before sample collection. Stream discharge was calculated using the slug-injection method with sodium chloride as the tracer (Gordon et al., 1992).

Water samples for nutrient content analyses were collected in triplicate and passed through pre-combusted glass fibre filters (Whatman GF/F) prior to analysis. Ammonium, nitrate, and dissolved phosphorus were analysed following standard methods (APHA, 1992).

Invertebrate sampling

Five cores were randomly taken from the sandy substrate (Perspex cylinders 3.15 cm in diameter, first 7-8 cm depth) of the two reaches. Sandy cores were immediately passed though 500- and 32-µm nested sieves. The fraction retained between these sieves was immediately stored and chilled to count live meiofauna.

Fresh meiofaunal samples were sorted under a dissecting microscope (MZ9.5; Leica, Wetzlar, Germany) and identified to the level of order within two or three days after sampling. High taxonomic resolution was not necessary in our study because we wanted to assess general patterns in both reaches that would emerge in terms of the relative contribution of groups to overall abundance, biomass and species richness (Reiss and Schmid-Araya, 2008). The community composition and density of meiofauna in live samples were similar whether they were processed within 1 or 3 d (data not shown).

Meiofaunal soft-bodied organisms were identified and measured alive, whereas hard- bodied organisms were simply counted and identified and then kept fixed in formalin for further length and width measurements (nearest 0.01 mm). Biomass was estimated by using power equations if available in the literature. Otherwise, biovolumes were used (Gaudes et al., 2009).

Density data were expressed as individuals per habitat cm^2 and biomass data as micrograms of carbon per habitat cm^2.

Microcrustacean secondary production

Most meiofaunal populations lack discrete cohorts because their generation times may be short and because reproduction is nearly continuous for much of the year. Production was calculated by the size–frequency method of Hynes and Coleman (1968), applying the modifications recommended by Hamilton and Hynes (1969) and

Benke (1979). This method assumes that the mean size–frequency distribution of a taxon across sampling dates is an approximation of the survivorship curve of a hypothetical average cohort.

Because the individual body sizes of all the taxa were known, mass classes were used (Hamilton and Hynes, 1969) rather than the length classes used in the original method of Hynes and Coleman (1968). We divided all taxa into 10 equal mass classes and excluded any apparent negative production in the initial size classes from the summed production across size classes (Benke and Wallace, 1980). The result of the size–frequency calculation is an estimate of area-specific production (g dry mass m^{-2} y^{-1}) for a taxon.

We estimated the uncertainty in our production estimates using a bootstrap procedure (Efron and Tibshirani, 1993). Bootstrap techniques have improved the estimation of uncertainty in secondary production studies by avoiding problematic assumptions about the sampling distribution of production estimates (Giberson and Galloway, 1985; Morin et al., 1987; Brey, 1990). Bootstrapping is now applied commonly in secondary production studies, particularly in lotic systems (Huryn, 1996; Carlisle and Clements, 2003; Woodcock and Huryn, 2007). In our analysis, 10,000 bootstrapped production estimates were assembled by randomly selecting *n* scores, with replacement, from the *n* biomass estimates collected throughout the year. For each bootstrapped data set, we used the procedure described above to calculate the production of each taxon at each site (control and impacted) and year (before and after fertilisation). We calculated approximate 95% confidence intervals for the production of individual taxon–site and year combinations as the upper and lower 2.5% percentiles of the 10,000 bootstrapped production estimates for that taxon–site and year combination.

In fact, the bootstrap resampling that we used was a combination of the nonparametric bootstrap (each resample based on *n* scores randomly selected, with replacement) and the parametric bootstrap because to compute each bootstrapped aggregated production value, CPI was also resampled from an estimated normal distribution (Huryn, 1996) obtained from the literature (Dole-Oliver et al., 2000).

To compare production rates (e.g., before and after fertilisation), we interpreted nonoverlapping confidence intervals as strong evidence (in fact, this is an overly conservative decision criterion) for a difference in rates (Carlisle and Clements, 2003; Woodcock and Huryn, 2007).

Analyses of the data on secondary production were performed using R (R Development Core Team, 2010; for commands and functions see the Appendix).

Statistical design

A prior Before-After-Control-Impacted (BACI) design (Underwood, 1994) was used to reduce the effects of temporal and spatial variability on our experiment. Square-root transformed data were analysed using permutational multivariate ANOVA (PERMANOVA) on the basis of Bray–Curtis distances among the respective communities. PERMANOVA allows multivariate information to be partitioned according to the full experimental design (BA and CI were considered fixed factors, whereas time was a random BA nested factor). It makes no assumptions regarding the distributions of the original variables. All P-values are obtained by permutation. All tests were done using the type III sum of squares and 999 permutations under the reduced model (Freedman and Lane, 1983; Anderson and Ter Braak, 2003). Given the high number of permutations run, additional Monte Carlo tests were not necessary to reinforce the permutation P-values obtained (Anderson et al., 2008).

All these analyses were performed with the software package PRIMER (version 6.1.6; PRIMER-E, Plymouth, UK) and the PERMANOVA+ module (version 1.0.1. PRIMER-E, Plymouth, UK).

3.4. Results

Physical and chemical characteristics

Both reaches had similar physico-chemical characteristics during the study period.

The water temperature ranged between 3 and 7°C in winter. It increased to values as high as 21°C in early summer.

Nutrient concentrations in both reaches during the pre-enrichment period fluctuated seasonally. This fluctuation was primarily a response to water flow and organic matter dynamics (Table 3.1). Both reaches also had a higher averaged nitrate concentration. Evidence for nutrient addition was mostly represented by differences in ammonia and phosphate. The impacted reach showed a high variability in phosphate owing to flow cessation and pool concentration during summer 2004. Nitrate was higher during episodes of high flow, particularly during autumn-winter (Sabater et al., 2011). The NP ratio showed high variability in the control reach during the study period. However, the lowest value observed was achieved after the addition of nutrients in the impacted reach. This addition produced a shift in the NP ratio from 57 to 17 (Table 3.1).

Table 3.1. Physical and chemical characteristics of the control and impacted reaches before and after the nutrient addition. Means and standard deviations (s.d.) are shown.

	Control				**Impacted**			
	Before		*After*		*Before*		*After*	
pH	7.18	±0.65	7.24	±0.46	7.28	±0.49	7.39	±0.45
T (°C)	11.52	±4.61	12.31	±5.88	11.12	±4.66	12.23	±6.27
Oxygen (mg/L)	9.75	±2.62	9.91	±2.79	9.96	±1.61	9.86	±3.48
Oxygen (%)	88.65	±17.63	91.03	±16.20	90.36	±9.54	88.69	±25.52
CE (µS/cm)	189.71	±17.90	231.64	±31.58	186.97	±17.87	234.2	±33.28
Ammonia (µgN-NH_4^+/L)	39.22	±39.03	17.19	±16.89	26.83	±24.29	197.32	±197.13
Phosphate (µg P-PO_4^{3-}/L)	24.44	±32.01	10.88	±7.25	23.88	±31.88	590.01	±1717.04
Nitrate (µg N-NO_3^-/L)	674.74	±653.85	349.71	±512.23	525.42	±457.99	599.87	±567.37
N:P	104		56		57		17	

Effect of addition (PERMANOVA)

Temporal variability, shown as “time” (sampling dates) and “BA” (Before-After periods) interactions, was significant in the PERMANOVA test for meiofaunal density (Table 3.2a). Meiofaunal biomass was also significantly different in both reaches (CI, Control-Impacted) (Table 3.2b). However, the same analysis also yielded a significant interaction in the complete model (CIxtime (BA)). Thus, despite the variability observed, fertilisation may also be affecting meiofaunal density and biomass in both reaches (Table 3.2a, b).

Table 3.2. PERMANOVA results for (a) total density and (b) total biomass of meiofaunal community. CI: control-impacted; BA: before-after.

	Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**
(A) Total density	CI	1	3133.5	3133.5	1.0091	0.386	999
	BA	1	8291.8	8291.8	2.9068	0.014*	999
	time(BA)	18	51736	2874.2	2.2226	0.001*	995
	CIxBA	1	6807.7	6807.7	2.1924	0.054	999
	CIxtime(BA)	18	56347	3130.4	2.4207	0.001*	999
	Res	138	1.78E+05	1293.2			
	Total	177	3.04E+05				
(B) Total biomass	CI	1	1.33E+05	1.33E+05	49.213	0.001*	998
	BA	1	8436.3	8436.3	2.0191	0.056	999
	time(BA)	18	77458	4303.2	4.3804	0.001*	998
	CIxBA	1	2585	2585	0.95983	0.452	998
	CIxtime(BA)	18	49681	2760	2.8095	0.001*	997
	Res	147	1.44E+05	982.4			
	Total	186	4.19E+05				

The primary constituents of the temporary meiofauna (Chironomidae and Oligochaeta) and of the permanent meiofauna (microcrustaceans and nematodes) were also affected by nutrient addition (PERMANOVA, Table 3.3).

Table 3.3. PERMANOVA results for the CIxti(BA) model interaction in the most abundant meiofaunal groups.

	Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**
Ostracoda	CIxtime(BA)	18	5442.1	302.34	2.7077	0.0004*	9915
Cyclopoida	CIxtime(BA)	18	4524.5	251.36	1.9877	0.0103*	9927
Harpacticoidea	CIxtime(BA)	18	2526.2	140.34	1.323	0.1719	9911
Nematoda	CIxtime(BA)	18	5592	310.67	1.9521	0.0123*	9914
Chironomidae	CIxtime(BA)	18	6586.8	365.93	2.5588	0.0007*	9901
Oligochaeta	CIxtime(BA)	18	10703	594.63	3.9546	0.0001*	9900

Effect on the community dynamics

The meiofaunal communities from the control and the impacted reaches showed a high level of variability over time (Fig. 3.1). The Impacted reach tended to show higher values of meiofaunal density and biomass during watercourse desiccation. During the two-three months following the addition, the increase of biomass in the enriched reach was higher. This increase could have been a direct effect of the nutrient addition and its massive concentration in summer pools. However, the control reach had a higher meiofaunal density and biomass during periods in which highly processed organic matter and detritus accumulated in the streambed (autumn 2003, autumn 2004 and late winter 2005) (Fig. 3.1). These periods were related to high hydrological stability. In contrast, abundance and biomass decreased in both reaches in November 2003, January 2004, and May of 2004 after floods (Fig. 3.1).

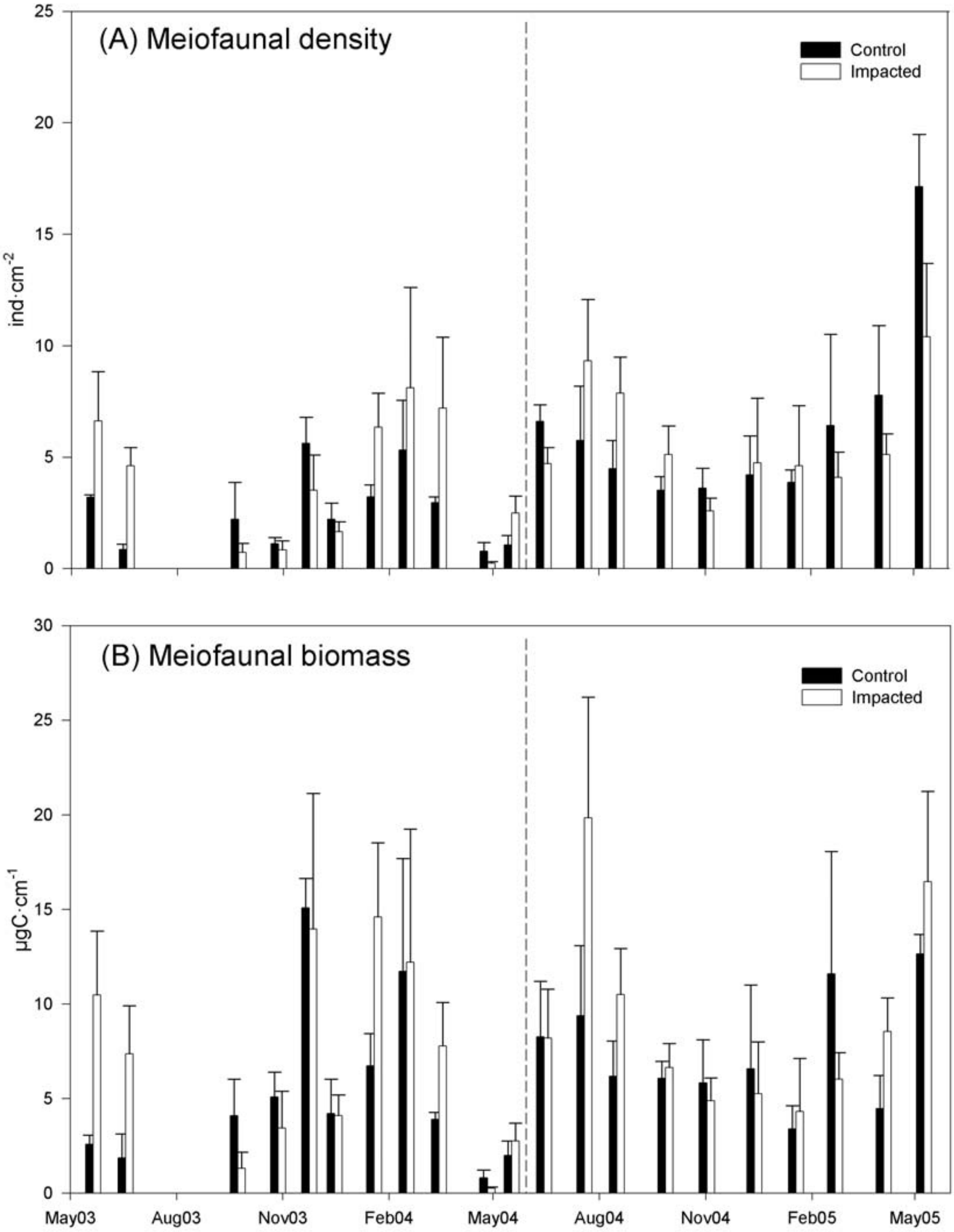


Fig. 3.1. Mean (±s.e.) density (A) and biomass (B) of meiofauna during the study period. The dotted line represents the starting point of fertilisation.

Microcrustacean dynamics and secondary production

Among other meiofaunal groups (PERMANOVA, Table 3.3), microcrustaceans showed a positive increase after fertilisation. The chief taxa in that group were the cyclopoid copepods and ostracods (Fig. 3.2). The secondary production of cyclopoids (dominated by *Eucyclops*) and ostracods (primarily Candoninae and Cypridopsinae) increased after the fertilisation in the impacted reach and also increased in the control reach (Table 3.4, Fig. 3.3). The higher values of productivity observed after the addition may have represented positive responses to the higher levels of hydrologic stability in both reaches.

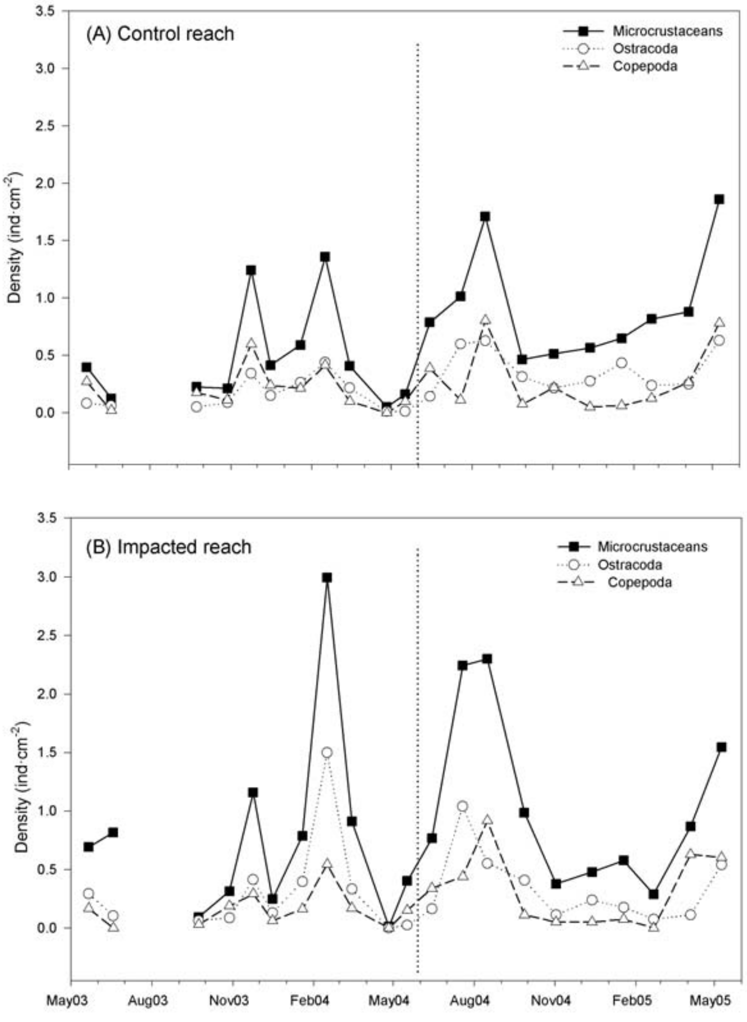


Fig. 3.2. Mean (±s.e.) density of ostracods and copepods and total microcrustaceans during the study period in control (A) and impacted (B) reach. The dotted line represents the starting point of fertilisation.

Prior to fertilisation, the mean annual production value of cyclopoids in the control reach was higher, but the P/B ratio was higher in the impacted reach (Table 3.4). Following fertilisation, the increase in annual production mean values was higher in the impacted reach. The value for ostracods was double that observed in the control reach (Table 3.4).

The P/B ratio for ostracods in the control reach diminished after the fertilisation, whereas it increased in the impacted reach (Table 3.4).

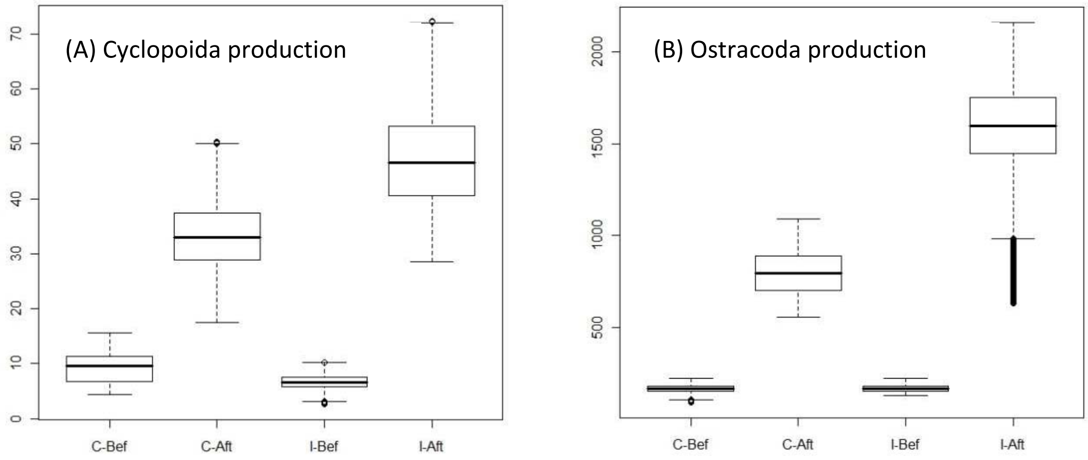


Fig.3.3. Boxplot of the 95% confidence intervals of the bootstrapped data for cyclopoid (A) and ostracod (B) secondary production (only points falling within the 95% Confidence Interval are included). Control (C) and impacted (I) points are represented by before (Bef) and after (Aft).

Table 3.4. Annual secondary production (mg DM·m^{-2} year^{-1}) and Production to Biomass ratio for Cyclopoida and Ostracoda. Values represent mean values of the 95% confidence intervals of the 10,000-times bootstrapped dataset.

		CONTROL REACH		**IMPACTED REACH**	
		Before	After	Before	After
Cyclopoida	*Annual production (mg DM·m^{-2} year^{-1})*	9.32	33.11	6.59	47.26
	P/B ratio	10.93	14.79	12.61	16.47
Ostracoda	*Annual production (mg DM·m^{-2} year^{-1})*	163.69	795.03	167.1	1578.02
	P/B ratio	81.79	68.53	62.37	115.25

3.5. Discussion

Our results indicate that meiobenthic communities in Mediterranean forested streams can react to moderate nutrient inputs. Previous studies conducted in our stream have revealed that the effects of transient nutrient additions were easily observed in epilithic biofilms. However, psammic communities inhabiting pools had a different pattern (Sabater et al., 2005). These habitats act as sinks for detritus and organic matter. This process tends to slow the diffusion of nutrients released in water, but it can also favour nutrient concentration during summer periods. In a long-term study of these communities, Sabater et al. (2011) have recently found significant effects of nutrient addition on the stoichiometry of macroinvertebrate individuals and biofilms. In naturally variable systems like Mediterranean streams, longer periods of experimentation are evidently required to elucidate clear patterns. Similarly, studies performed in detritus-based headwaters found that nutrient addition caused large increases in secondary production during the first two years, but changes in community composition were not evident until the third year after the fertilisation (Cross et al., 2005b, 2006, 2007).

Although the nitrate concentration was similar in both reaches, ammonia and phosphate increased after the addition and ensured a 3-fold nutrient increase. These two reaches were placed contiguously and have similar habitat composition, but hydrology in Fuirosos is probably the major factor that modulates habitat heterogeneity. Small benthic communities are patchily distributed, and even small differences in substrate can produce significant differences in abundance and biomass. Differences in the timing and frequency of hydrologic disturbances during the two years of the experiment have also been an important determinant of meiofaunal community structure and microcrustacean secondary production in both reaches. In a previous study that included the control reach, differences in community composition were attributable to differences between the two hydrologic years, a dry year and a wet year (Gaudes et al., 2010).

The temporal pattern of the meiofauna in both reaches has been demonstrated to be tightly linked to the hydrology of the reaches and to autotrophic pulses that naturally occur in Fuirosos (Artigas et al., 2009) by the end of spring and the beginning of summer. However, these factors do not seem to be the only influence on the meiofaunal community inhabiting sandy pools. Detritus and FPOM accumulated in sands could be directly enriched by these autotrophic pulses (through remineralisation or exudates) (Gaudes et al., 2010) and further reinforced and enhanced by the increased nutrient supply (Sabater et al., 2011). In Fuirosos, nutrient addition did not affect the natural accumulation or the temporal pattern of microbial and detrital resources (Artigas et al., 2009; Sabater et al., 2011), as in other studies in heterotrophic streams did (Suberkropp et al., 2010). This outcome probably reflects the primacy of hydrological factors. Intraannual variability plays an important role in modulating the effects of our nutrient addition to the stream. Such variability can either exacerbate or diminish the effects of nutrients on the meiofaunal community.

In forested streams like Fuirosos, the presence of allochthonous inputs means that carbon is often the major element that is readily available to consumers (Artigas et al., 2009). Thus, in ecosystems with large pools of refractory detrital C, increased availability of N and P may speed up the rate at which C is passed on to and processed by higher trophic levels. These effects may ultimately alter patterns of system-wide C accumulation (Cross et al., 2007). However, high carbon: phosphorus ratios can have negative effects by reducing production: biomass ratios in consumer populations. Indeed, growth and reproduction require a diet that balances the individual’s demands for energy, elements and macromolecules (Elser and Hessen, 2005). An excess of carbon may be sufficient to maintain the basic metabolic rates of individuals that are nearly non-reproducing. In its most refractory forms, carbon can even reduce the fertility of some metazoans like nematodes (Höss et al., 2001).

Because phosphorus is a conservative element, the availability and supply of P is a key determinant of the binding, flux and fate of C in freshwater food webs (Elser and Hessen, 2005). Changes in the P supply can alter trophic transfer efficiency by modulating the nutritional match between producers and consumers (Elser, 2001). Consumers with elevated RNA allocations (e.g., some cyclopoids; Standiford, 1988) will enjoy a growth advantage during good food conditions (owing to variation in their ribosomal genome) but will be unusually susceptible to stoichiometrically unbalanced food (Elser, 2000).

The relative values of secondary production found in this study revealed the same rank order reported for similar species in the literature (Lemke and Benke, 2009). However, the annual production and the P/B ratio of ostracods in the impacted reach after the fertilisation were both considerably higher than the results of previous studies. In both groups, cyclopoids and ostracods, interannual variability may have affected annual production and P/B ratios in both reaches (control and impacted). However, differences in the impacted reach were considerably higher than in the control reach owing to the nutrient addition. This result was especially noteworthy for the ostracods. Compared with the P/B ratios found for ostracods, the P/B ratios of cyclopoids were remarkably low. CPI values used for these measurements (from 3 to 9 months of the lifespan; Dole-Olivier et al., 2000) were determined by the high abundance of *Eucyclops serrulatus* individuals in the cyclopoid community. The lifespan of this cyclopoid is much greater than the 3-4 week lifespan of some Candonidae (Dole-Olivier et al., 2000).

The factors controlling the rates of biomass turnover are myriad. However, aside from food quality and supply, the two most influential factors are probably temperature and body size (Huryn and Benke, 2007). Thus, in highly variable systems like the Mediterranean, these abiotic factors may increase the variability of biomass turnover in meiofaunal communities.

Streams having high levels of hydrological disturbance are an example of a type of habitat in which the risk of mortality is high during development. In such habitats, growth rate should be maximised and size minimised. Conversely, where the risk of mortality is low, size should be maximised. Such maximisation of size generally results in slower growth rates and longer developmental periods (Jackson and Sweeney, 1995, Feminella and Resh, 1990). However, disturbance may facilitate high food quality and may also remove biomass. This process would affect growth rates via density-dependent competition for food. These varied effects on growth rate make it more difficult to predict the effects of disturbance on production.

Nutrient-induced changes in organic matter quality have been thought to be responsible for increased community secondary production (Cross et al., 2006). Our study provides evidence that bottom-up effects of nutrient enrichment in a Mediterranean detritus-based ecosystem can stimulate meiofaunal community production through detrital- microbial pathways. However, these effects are strongly modulated by hydrology and by intra- and interannual variability. Periods of water scarcity are predicted to increase in Mediterranean systems as a consequence of global change (Giorgi and Lionello, 2008). Our findings suggest that the response of meiofauna to nutrient inputs would probably be intensified by the periods of water scarcity associated with global change.

CHAPTER 4

The nematode community in cyanobacterial biofilms in the river Llobregat, Spain

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Nematology, 2006, 8, 909-919

SUMMARY

The aim of our study was to understand the role of the nematode community in a cyanobacterial-dominated biofilm located in the Llobregat River (NE Spain). This biofilm was mainly composed by oscillatorial species and diatoms. Significant fractions of these mats can become unattached from the substrata and free-floating, dispersing downstream and acquiring different structural and physiological properties. Both cyanobacterial biofilms, attached and free-floating, were contrasted to another benthic biofilm co-occuring in the studied reach. We found rather poor nematode diversity, dominated by *Chromadorita leuckarti* (De Man), *Diplogaster rivalis* (Leydig), *Plectus parvus* Bastian, *Neotobrilus diversipapillatus* (Daday), *Monhystera* spp., *Dorylaimus* sp. and *Mononchus* sp. Nematode density and biomass were significantly higher in the free-floating biofilm (maximum values of 752 ind/cm^2 and 171,3 µgC/cm^2). Different trophic and sexual strategies were observed. The mobile free-floating biofilm showed higher abundances of juveniles and a high proportion of gravid females. A positive correlation was observed between cyanobacterial density and the abundance of juveniles or adults of the commonest nematode species indicating their potentiality of being food resources.

4.1. Introduction

Nematodes and other groups of the meiofaunal community live and feed on biofilms, affecting film structure and function through bioturbation, grazing (Farmer, 1992; Traunspurger, 2000), faeces and mucus secretions (Traunspurger et al., 1997; Riemann and Helmke, 2002; Moens et al., 2005). Nematodes are an important invertebrate group in all benthic habitats. In comparison with soil and marine habitats, relatively little is known about the ecology of freshwater nematodes, especially those in running freshwater systems (Traunspurger, 2002; Hodda, 2006).

In shallow littoral zones of running waters, cyanobacteria often form densely entangled mats on the substrate. These thick biofilms have been observed in the mid reach of the Llobregat River (NE Spain) from early winter to late spring in years characterised by low river discharge (Vilalta, 2004). The mats were found to be composed of *Oscillatoria limosa* C. Agardh ex Gomont and *Oscillatoria affinis tenuis* C. Agardh ex Gomont (Vilalta et al., 2003). The masses slough off and drift downstream in a characteristic process that has been previously described for *Oscillatoria* mats (Komárek, 1992). This detachment and further transport may have major consequences for the fate of the metabolite geosmin produced by these mats, thereby affecting water quality (Sabater et al., 2003).

Maximum growth of filaments in the Llobregat River coincides with an increase in the number and biomass of nematodes and other meiofaunal groups (oligochaetes, chironomids and tardigrades) in cyanobacterial biofilms (Sabater et al., 2003). However, the potential interactions between the nematode community and the microbial biofilms remain unclear.

The study here presented aimed to characterise the nematode community (species composition, richness, biomass and functional feeding groups) in a cyanobacterial biofilm during its occurrence in the Llobregat River as well as its use as a potential food resource. We also wanted to assess if the previously described biofilm detachment had major consequences in the nematode community. For that purpose, the attached and the free-floating biofilms were studied separately and contrasted to the benthic biofilm growing in the riffle zones of the river that co-ocurred in the studied reach.

4.2. Study site

The study was carried out in the Llobregat River in Catalonia, NE Spain. This watercourse is characterised by a calcareous geology and a typical Mediterranean regime, which causes frequent floods in spring and autumn and minimum flow in summer. The main channel of the river is continuously interrupted by a series of small dams and derivation channels, which leads to further instability of the water regime, especially during periods of low flow. The river receives waste waters of industrial and urban origin in its mid and lower stretches, leading to a severe deterioration in water quality.

Sampling was conducted in Pont de Vilomara (U.T.M. 406502, 4604544), a site in the middle stretch of the river, from January to May 2002. The physical and chemical properties of the water during the study period have been described in Vilalta et al. (2003).

Characterisation of the Biofilms

Attached benthic cyanobacterial biofilms grow in the shallow littoral zones of the study site. Due to changes in water flow, bioturbation or increases in oxygen bubbling during the photosynthesis maximum (Vilalta and Sabater, 2005), attached forms detach and drift downstream, forming a free-floating mat. Both the attached and the free-floating forms are primarily composed of *Oscillatoria limosa* and *Oscillatoria tenui*s. The free-floating form has a higher density of cyanobacterial cells, higher concentrations of chlorophyll-*a*, nitrogen, phosphorus, glucose, geosmin and organic matter, and a lower C:N ratio, while the attached form has a higher proportion of diatoms and green algae (Vilalta et al., 2003). In riffle zones, biofilms are dominated by diatoms (mainly *Navicula* sp. and *Nitzschia* sp.) and filamentous algae (especially *Vaucheria* sp. and *Cladophora glomerata* (L.) Kützing (Vilalta et al., 2003). This is referred to hereafter as the riffle community.

4.3. Material and Methods

Nine samples from each biofilm were taken monthly during spring time in 2002 (3 samples for bacteria, 3 for algae and 3 for fauna). Samples were taken with an end-cut syringe device (3.14 cm^{-2} and with the plunger fixed in order to obtain equivalent volumes) and standardized for surface.

Bacterial number and biomass

Samples for measuring bacterial densities were collected on only four sampling dates (from February to April 2002). Only samples from the attached and the free-floating biofilms were processed as described by Romaní and Sabater (2001). They were sonicated (90s, Selecta [J. P. Selecta S.A., Abrera, Spain], 40 W power, 40 kHz frequency) to achieve cell dispersion. After appropriate dilution (dilution factor of 10–40), fixed samples were stained for 5 min with DAPI (4,6-diamidino-2-phenylindole; 2 µg/mL), filtered through 0.2-µm irgalan black-stained polycarbonate filters (Nuclepore, Newton, Massachusetts, USA) and bacteria were counted under a fluorescence microscope (Reichert-Jung [Depew, New York], Polyvar) at 1250× magnification (Porter and Feig, 1980). Twenty fields were counted per filter for a total of 400–1500 organisms. A mean biovolume of 0.1µm3 was assumed for freshwater bacteria (Theil-Nielsen and Sondergaard, 1998). Bacterial biomass was calculated using a conversion factor of 2.2 × 10−13 gC μm^{-3} (Bratbak, 1985; Kemp, 1990), which lies in the mid range of the values reported in the literature (Psenner, 1990).

Cyanobacterial and algal composition and abundance

Cyanobacterial and algal biofilm samples were collected from the distinct biofilms (attached, free-floating and riffle community) on the same sampling dates described for bacteria. Samples were fixed in 4% formaldehyde to study community composition and abundance. They were then observed under a light microscope (Reichert-Jung [Depew, New York], Polyvar). Cell volumes for the most abundant diatom species were taken from the literature (Snoeijs et al., 2002). Cyanobacterial biovolumes were obtained by measuring filaments and approximating their shape to a cylindrical body (Hillebrand et al., 1999). Biomass was calculated using a conversion factor of 1.1 x 10-13 gC µm^{-3} (Mullin et al., 1966).

Nematode sample collection

Samples were collected monthly, from January to May, and were immediately fixed in 4% formaldehyde. In the laboratory, in order to facilitate sample sorting, they were passed consecutively through differential mesh size sieves (250, 100 and 50µm) to obtain corresponding size fractions. All fractions were counted under a stereomicroscope (Leica, Germany, MZ9.5), except for the 50µm fraction, which was observed and counted under an inverted microscope (Olympus, Japan, IMT2). Estimates are given as the sum of the distinct fractions.

Nematode identification

On each sampling date, one hundred nematode individuals from each biofilm type were dehydrated following Seinhorst's ethanol-glycerol method (1959) and mounted on slides with glycerol and paraffin. The length and width of individuals were measured to calculate biovolume (Andrássy, 1956). Specific gravity of 1.13 (Wieser, 1960), dry-to-wet-weight ratio of 0.25 (Feller and Warwick, 1988) and Carbon content-to-Dry weight ratio of 2.4 (Margalef, 1983) were assumed. Nematodes were identified to the species level, except for the Dorylaimida and Monhysteridae families, due to the difficulty in doing so, and constant reclassification of the species. Nematodes were classified by "sex" (juveniles, males, females and gravid females) and were assigned to different feeding-type groups depending on their buccal cavity (Traunspurger, 1997 revised by Moens et al., 2006).

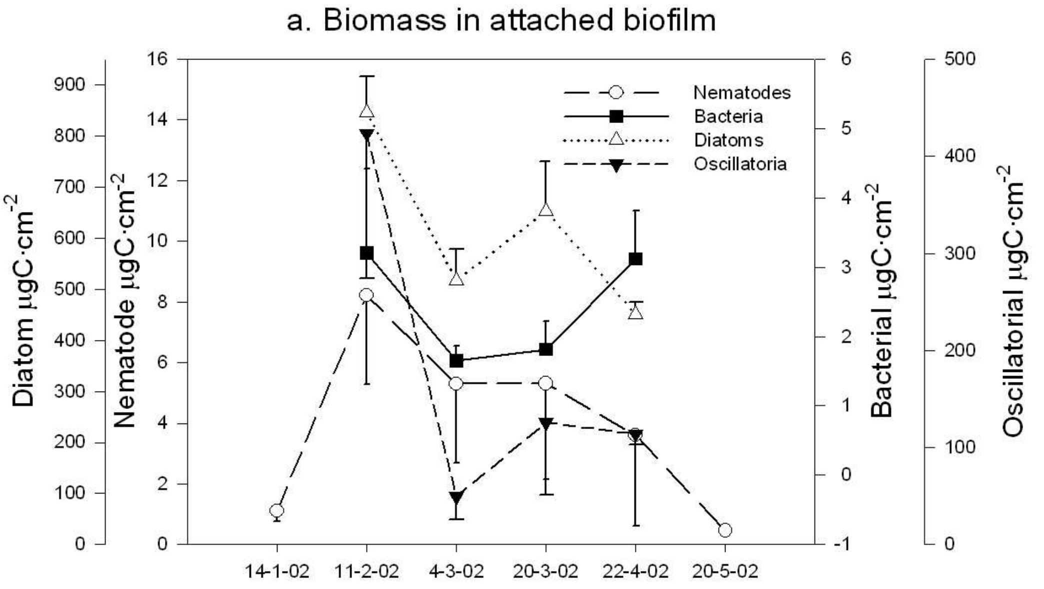
Statistical analysis

The normality of the sample distribution and homogeneity of variances were assessed to determine whether data transformation (log (x+1)) was required. Three 2-way ANOVA (using time and bacterial, diatom or cyanobacterial biomass in each test as factors) and a MANOVA (using time, biofilm, nematode species and "sex" as factors) were used to detect significant differences between biofilms over time. Post-hoc comparisons between means were analysed with a Tukey Honest Significant differences (HSD) test. Pearson's multiple correlations test was used when sampling dates were coincident (from February to April) to find relationships between densities of nematode species and their potential food sources: diatoms, bacteria, oscillatorial cyanobacteria, other cyanobacteria and green algae (Vilalta, 2003). A Multidimensional scaling representation was performed to observe the spatial distribution of biofilm samples based on Bray-Curtis similarity matrix. MDS was performed using the PRIMER 5.2.4 package while ANOVA analyses and Pearson's correlations were performed using the STATISTICA 5.5 package.

4.4. Results

Biofilm characteristics

Bacterial biomass in the free floating biofilm was significantly higher than in the attached (2-Way ANOVA, F= 4.66, $p<0.05$). Mean diatom biomass in the free-floating and the attached biofilms were similar (828.4 and 611 µgC cm^{-2} respectively) and showed no significant differences. In contrast, cyanobacteria and nematode biomass were almost ten times greater in the free-floating than in the attached biofilm (Fig. 4.1 a, b) (For cyanobacteria 2-Way ANOVA, F= 10.28, $p<0.05$) .



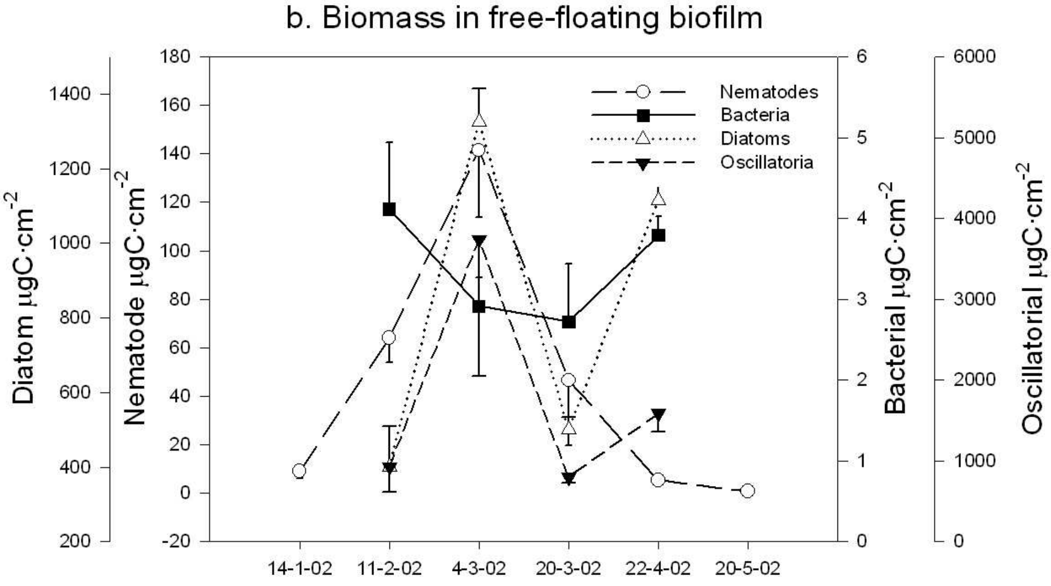
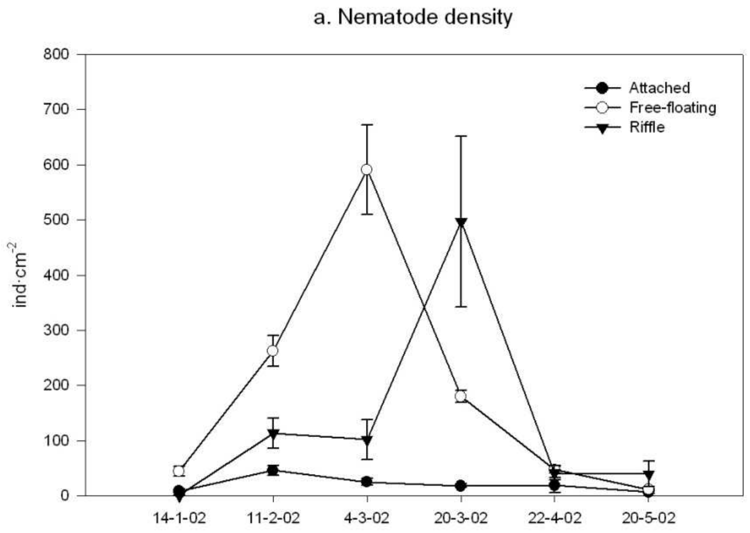


Fig. 4.1. Nematode, diatom, oscillatorial and bacterial biomass in the attached (A) and in the free-floating biofilm (B). Data in µg C cm^{-2} (mean and standard error are shown). Dates are sampling times (day, month, year).

In the attached biofilm, biomass of the various components (diatoms, bacteria, cyanobacteria and nematodes) tended to decrease at the beginning of March and only the bacterial biomass increased in the last sampling date (Fig.4.1a). In the free-floating biofilm, biomass temporal pattern was divergent for the bacterial and nematode (Fig.

4.1b). A remarkable decrease in nematode density and biomass was observed in the three analysed biofilms at the end of the study period (Fig 4.2 a, b).



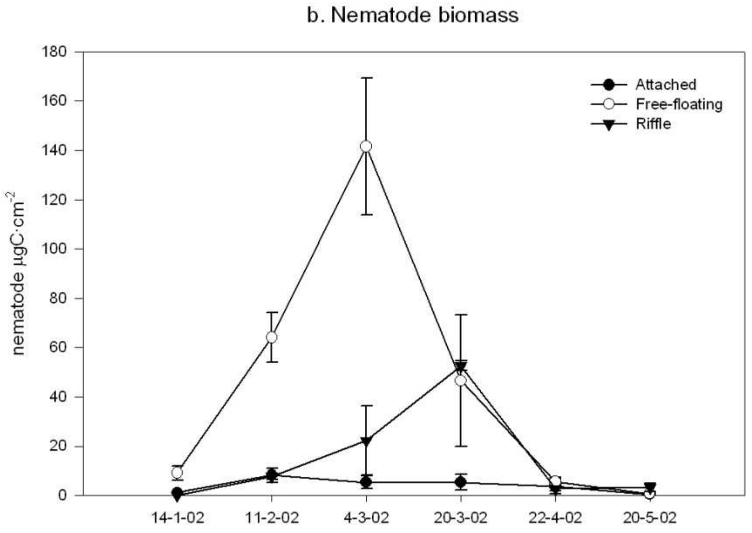


Fig. 4.2. Nematode density (A) and biomass (B) for the three types of biofilm (mean and standard error are shown). Dates are sampling times (day, month, year).

Nematode community characteristics

The free-floating biofilm had a significantly higher nematode density (mean value of 190 indiv. cm^{-2} and maximum of 752 indiv. cm^{-2}) (Fig. 4.2a) and biomass (mean value of 44.6 µgC cm^{-2}) (Fig. 4.2b) than the attached biofilm (MANOVA Post Hoc comparisons, $p<0.05$). Nematode density in the latter did not exceed 42 indiv. cm^{-2}.

The diatom-dominated riffle biofilm also showed statistical differences with the free-floating biofilm (MANOVA Post Hoc comparisons, $p<0.05$) since it had an intermediate density range (mean of 133 indiv. cm^{-2}) but peaked in late March (maximum of 680 indiv. cm^{-2}). This peak showed weaker correspondence with biomass (Fig. 4.2b). The MANOVA results showed no significant differences between both benthic biofilms (attached and riffle biofilm).

The nematode community was similarly low in species number in all three types of biofilm. The following species were found: *Chromadorita leuckarti* (De Man), *Diplogaster rivalis* (Leydig), *Plectus parvus* Bastian, *Neotobrilus diversipapillatus* (Daday), *Monhystera* spp., *Dorylaimus* sp. and *Mononchus* sp.

The attached biofilm was firstly dominated by C. leuckarti (Fig. 4.3), and later on by juveniles of *N. diversipapillatus*. This substitution occurred coinciding with the maximum growth of the cyanobacterial filaments in February (Fig. 4.4). The nematode community inhabiting the free-floating biofilm was dominated by *D. rivalis* (Fig. 4.3), except for May, when the three biofilms showed a similar Chromadorita-dominated community. In February, males and gravid females of *D. rivalis* were the most abundant. Ovigerous females increased in March with maximum numbers of around 213 indiv. cm^{-2} (Fig. 4.4). *C. leuckarti* also showed a high number of males and gravid females during the maximum cyanobacterial growth period (Fig. 4.4).

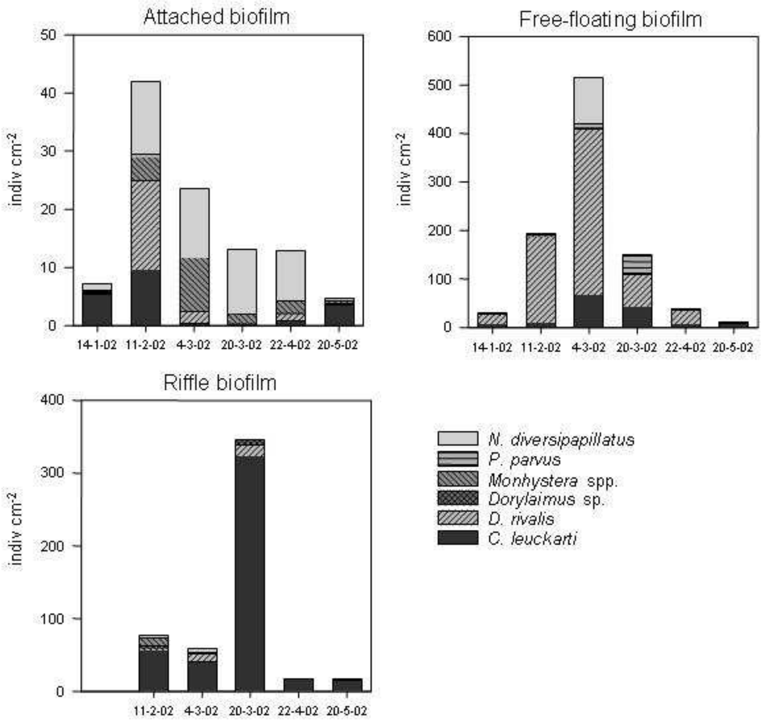


Fig. 4.3. Mean nematode composition and density (ind. cm^{-2}) of biofilms. Dates are sampling times (day, month, year).

There was a clear predominance of *C. leuckarti* (Fig. 4.3) in the diatom-dominated riffle community. The number of males and gravid females of *C. leuckarti* increased at the end of March (Fig. 4.4).

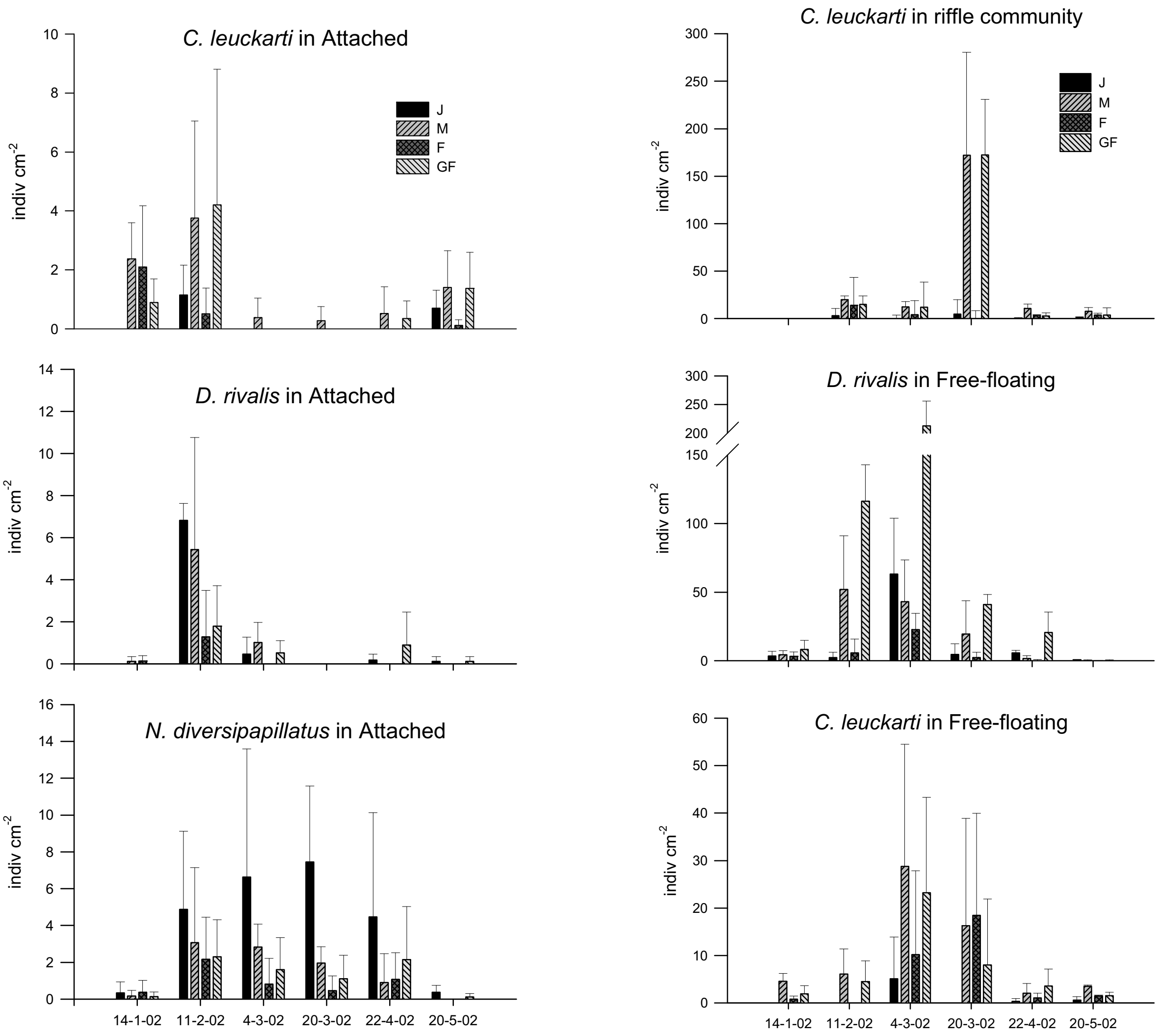


Fig. 4.4. Sex composition of most abundant nematode genera in biofilms (mean and standard error are shown). Juveniles (J), males (M), females (F), GF (gravid females). Dates are sampling times (day, month, year).

The MANOVA, applied to all our samples, revealed significant differences for the four factors used (Table 4.1). Post-hoc tests showed differences in species abundance between the two commonest species, *C. leuckarti* and *D. rivalis,* and *N. diversipapillatus* (the least abundant) ($p<0.05$ in both cases). A high abundance of gravid females and males was related. The density of *C. leuckarti* juveniles was lower than expected after numbers of pregnant females were observed to increase. These first stages may be underrepresented due to the mesh sizes used (juveniles of some species may be smaller or narrower than 50 µm). Significantly higher nematode abundance was seen in the free-floating biofilm on the 4th March sampling date than on any other day ($p<0.05$).

Table 4.1. MANOVA test; degrees of freedom (d.f.), significant F and P values are given ($P < 0.05$). Species used: *Diplogaster rivalis, Chromadorita leuckarti* and *Neotobrilus diversipapillatus.*

MANOVA			
factors	d.f.	F	*p-value*
Date	4	13.83	0.000001
Biofilm	2	13.58	0.000002
species	2	13.52	0.000002
“sex”	3	6.05	0.000468

In concordance with the MANOVA results, the MDS plot of nematode species composition (Fig. 4.5) also showed differences between the attached and the free-floating community over time. The two biofilms showed opposite hysteresis-like patterns, with similar species composition at the beginning and at the end of the cyanobacterial growth period. When the cyanobacterial mats reached their maximum density, the free-floating biofilm was characterised by high density and dominance of *D. rivalis*, while the attached biofilm had lower total density and high nematode species composition (due to the presence of *N. diversipapillatus* and *Monhystera spp*). The F6 point of the figure corresponds to the last sampling date, when the cyanobacterial mats began to decline. The riffle community did not show such a cyclic pattern.

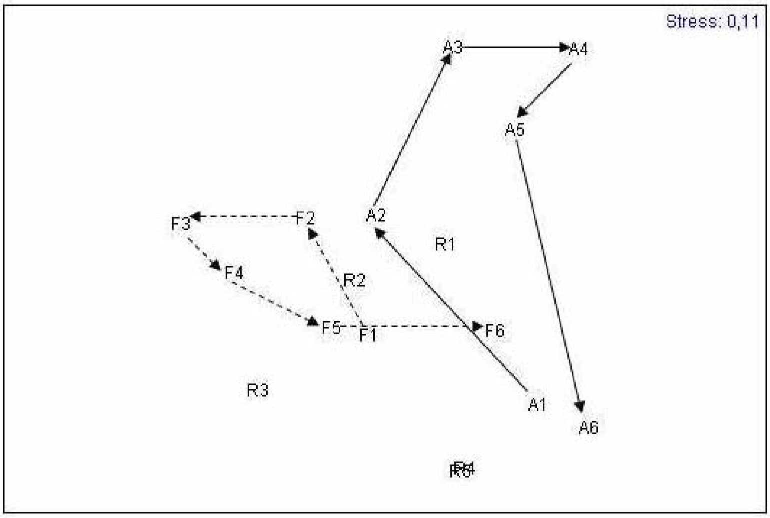


Fig. 4.5. *Results of the Multidimensional Scaling Dimension analysis of nematode density in each sampling date (from 1-6) and biofilm: attached (A), free-floating (F) and riffle (R).*

Pearson’s multiple correlations (Table 4.2) showed a significant positive correlation of densities of *C. leuckarti* juveniles and adults, *D. rivalis* juveniles, and *P. parvus* adults with both groups of cyanobacterial density in the attached biofilm. D. rivalis adults only showed a significant correlation with non-Oscillatoria cyanobacteria. In the free-floating biofilm, the positive correlation with Oscillatoria and non-Oscillatoria cyanobacteria was only observed for the juvenile forms of *C. leuckarti* and *D. rivalis* and for *N. diversipapillatus*. None of the nematode groups was correlated to diatom and bacterial abundance.

Table 4.2. *Pearson correlation. Values correspond to r2. Asterisk means significance with* $P < 0.05$. *J: juveniles; Ad: adults.*

		Oscillatoria	Non-oscillatoria cyanobacteria
Attached	*C.leuckarti* J	0.98*	0.99*
	C.leuckarti Ad	0.98*	1.00*
	D. rivalis J	0.97*	0.98*
	D. rivalis Ad	0.94	0.98*
	P. parvus Ad	0.98*	0.99*
Free-floating	*C.leuckarti* J	0.98*	0.98*
	C.leuckarti Ad	0.66	0.80
	D. rivalis J	0.97*	0.98*
	D. rivalis Ad	0.72	0.90
	N. diversipapillatus J	0.98*	0.98*
	N. diversipapillatus Ad	0.97*	0.99*

4.5. Discussion

Nematode range density (10^5-10^6 indiv. m^{-2}) in cyanobacterial biofilms in the Llobregat River supports the density reported by Farmer (1992) in cyanobacterial biofilms. These densities are similar to those found in riverine soft substrata (e.g. Beier and Traunspurger, 2003 a, b). However, the highest values (7.5 10^6 indiv. m^{-2}) found in the free-floating biofilm were above of those reported in other lotic systems (e.g. 1.6 10^5 indiv. m^{-2} in Palmer, 1990 or 2.3-7.9 10^4 indiv. m^{-2} in Barbuto and Zullini, 2005; for reviews see Traunspurger, 2000 and Hodda, 2006). The corresponding biomass range (2.5-171.3 µgC cm^{-2}) confirms the relevance of nematodes in the community structure in river systems.

Attached cyanobacterial biofilm was characterised by low cyanobacteria, diatom and nematode biomass when compared to the free-floating form. Bacteria biomass in free-floating biofilm ranged from 1.4 to 5.7 µgC cm^{-2} while diatoms accounted for 2.4 10^2-1.5 10^3 µgC cm^{-2} and cyanobacteria for 6.6 10^2-6.9 10^3 µgC cm^{-2}. The prokaryotic compartment in this biofilm was consequently dominated by cyanobacteria. According to data, cyanobacteria and diatoms stand out as the main potential food resources for nematode in this biofilm.

Although high species diversity is common in marine and freshwater nematode communities (e.g. Traunspurger, 1996 a, b; Beier and Traunspurger, 2003 a, b), low diversity was found in the Llobregat River. Differences in sampling strategies, organic contamination and human alteration of the main channel could help to understand this low diversity (Hodda, 2006). In that sense, the dominant species, *C. leuckarti, D. rivalis* and *N. diversipapillatus* could be acting as opportunistic species, with high density and biomass. *C. leuckarti* dominated in the biofilm made up of diatoms and green algae (riffle biofilm), although it was also present in other biofilms. This dominance in riffle biofilm might suggest that the diet of *C. leuckarti* was mainly comprised of diatoms. *C. leuckarti* possess a sclerotised tooth in its mouth, which allows them to pierce diatom valves and use their muscular pharynx to suck out valve content (Jensen, 1982). Although considered an epistrate feeder, the sclerotised tooth in *C. leuckarti* may also allow individuals to puncture cyanobacterial cells (Moens and Vincx, 1997), thereby diversifying their diet. Positive correlations were observed in the attached biofilm between this species and cyanobacteria in general, even though only juveniles showed positive correlation with the high cyanobacterial biomass characterising the free-floating biofilm.

Cyanobacteria may be an energetic base for the benthic food web in some environments, such as tropical rocky shores, because of their high protein content, which could compensate their lack of polyunsaturated fatty acids in grazer diet (Nagarkar et al., 2004). Individuals of *N. diversipapillatus* and juveniles of *D. rivalis* were correlated with cyanobacteria biomass in the free-floating biofilm. Although D. rivalis has well developed teeth and is a predatory species (Zullini, 1982), Yeates et al. (1993) mentioned bacterivory as the predominant trophic strategy in the Diplogasteridae family and therefore cyanobacteria could be a food complement for these nematodes. Low C:N ratio and high organic matter content characterising the free-floating biofilm (Vilalta, 2004) could favour greater development of nematode, mainly juveniles, both directly, via cyanobacterial ingestion, or via bacterial growth. Meiofauna abundance is related to high microbial biomass or low C:N content (Swan and Palmer, 2000). Höckelmann et al. (2004) reported the ingestion of cyanobacterial filaments by predacious/omnivorous species like *Mononchus truncatus* and *Dorylaimus* sp. A high protein content inside the filaments could explain the low availability of inorganic nitrogen found in water and inside the biofilm matrix (Sabater et al., 2003). Dissolved inorganic nitrogen would be assimilated by cyanobacteria to store proteic substances inside. These authors also reported an increase of the exoenzymatic activity leucine-aminopeptidase (AMA) in free-floating biofilms of the Llobregat, indicating that bacteria and cyanobacteria could also obtain the inorganic form of nitrogen from organic sources.

Nematodes had higher density and biomass in the cyanobacterial mats. Cyanobacteria and nematodes could benefit mutually since nematode grazing and faeces and mucus excreta may enhance bacterial and cyanobacterial growth (Traunspurger et al., 1997; Riemann and Helmke, 2002; Moens et al., 2005). Bioturbation may also increase biofilm permeability (Boulton et al., 2002) and therefore enhance the dispersion of the mat and animals downstream. In our biofilms, maximum density of nematodes in the attached form observed in February could have favoured the detachment process. The free-floating biofilm studied here was full of gravid females with well developed embryos inside thin egg-walls, suggesting that females disperse downstream via the free-floating mat in order to colonise new habitats. From that point of view, the arrival of gravid females plenty of nearly formed juveniles could allow faster colonisation. In the Llobregat, the presence of free-floating cyanobacterial biofilm was coincident with maximum geosmin concentration in the water and inside the cyanobacterial biofilms (Sabater et al., 2003). Höckelmann et al. (2004) demonstrated the capacity of nematodes to detect geosmin and multicomponent odours. It is therefore possible that geosmin in our benthic biofilms could be an attractant (either alone or combined with other undetected bacterial or cyanobacterial volatile organic compounds) which indicates the presence of food, habitat or shelter to nematodes. Some authors pointed out that some aquatic insects, attracted by cyanobacterial biofilm odours, also deposit their eggs in similar biofilms (Evans, 1982; Rejmankova et al., 2000) in order to guarantee the individual dispersion.

The hysteresis-like pattern of nematode community in the attached and the free-floating biofilm would confirm a coupling between the cycle of the nematode community and the cyanobacterial growth. Highest cyanobacterial growth in free-floating biofilm corresponds to the highest *D. rivalis* density (mainly gravid females). It arises that several factors contribute to the complexity of links between cyanobacterial biofilm dynamics and the nematode community, may be indicating not only a food resource but a shelter to live and disperse.

CHAPTER 5

Bottom-up effects on freshwater bacterivorous nematode populations: a microcosm approach

Ainhoa Gaudes, Isabel Muñoz, and Tom Moens

(In prep.)

SUMMARY

The aim of this study was to test the effect of different nutrient concentrations on the population development of two bacterivorous freshwater nematodes, *Bursilla monhystera* and *Plectus aquatilis*. A microcosm experiment was designed to complement data obtained from two *in situ* nutrient addition experiments in a pristine Mediterranean forested stream, the Fuirosos, located in the NE of Spain. Phosphate, nitrate and ammonia were added to both the Fuirosos stream and the microcosms. In addition, in the microcosm experiment, glucose as a biodegradable dissolved organic carbon (BDOC) source was tested at a basal concentration (low carbon) and at 20 times the average concentration found in the stream (high carbon). Different N and P concentrations were combined with each of the carbon conditions. The microcosms were filled with autoclaved natural sand from the stream and milliQ water with the corresponding nutrient solution. Bacteria isolated from the stream were inoculated at the beginning of the experiment, and nematodes were added 3 days later. The experiment lasted 2 months. On each sampling date, four replicates of each treatment were analysed for dissolved nutrients in water, bacteria abundance, and nematode abundance and biomass. *Bursilla monhystera* reacted to changes in their environment, with a significant increase in numbers after the inoculation. However, if nutrients were less abundant, *Bursilla* numbers tended to decrease, probably because they did not invest in reproduction and thus the population was maintained by small–medium juveniles. On the other hand, under enriched conditions, *B. monhystera* tended to increase in number over time, although there was no significant increase in reproductive forms. *Plectus aquatilis* only increased under high nutrient concentrations although their efficiency was much lower than that of *Bursilla* individuals. Our results indicate that episodic nutrient enrichment may affect populations of bacterial-feeding nematodes in the short term, but that longer-term population dynamics are largely determined by leaching of nutrients and labile carbon from sediment-bound organic matter and nutrient re-cycling rather than by the nutrient concentrations of the overlying water.

5.1. Introduction

Nematodes are often the most abundant metazoan phylum in the soft sediments of freshwater bodies. Their ecological significance in terms of productivity, food-chain relationships and the remineralization of organic substances may be substantial (Ingham, 1999). Compared with soil and marine habitats, relatively little is known about the ecology of freshwater nematodes, especially those in lotic systems (Traunspurger et al., 2002; Hodda, 2006).

Bacterial-feeding nematodes can affect bacterial communities at different levels, influencing bacterial activity, either positively (Findlay and Tenore, 1982; Alkemade et al., 1992a, b; Traunspurger et al., 1997; Ingham, 1985, 1999) or negatively (De Mesel et al., 2003). This may be a direct effect of grazing (especially at low nematode numbers; Ingham, 1999), but bioturbation (Abrams and Mitchell, 1980; Alkemade et al., 1992b), the production of faeces and mucus secretions (Traunspurger et al., 1997; Riemann and Helmke, 2002; Moens et al., 2005) by nematodes are also important. In addition to affecting microbial activity, nematode activity may alter the composition and diversity of bacterial communities (De Mesel et al., 2004, 2006).

Bottom-up effects such as nutrient availability on bacterial and nematode populations have been extensively studied in soil environments due to the importance of these animals in nutrient mineralization and consequently in soil fertility (e.g. Ferris et al., 1995; Yeates, 2003). Again, however, there is a paucity of similar studies in freshwater systems. There is a large amount of literature on the direct effects of nutrient enrichment on the periphyton, focusing on the autotrophic compartment, while less attention has been paid to the heterotrophic bacteria, fungi, protozoa and meiofauna inhabiting these complex biofilms (Hillebrand et al., 2002). Recently, studies have shown that nutrient enrichment in oligotrophic systems may increase the rate of organic matter decomposition, stimulating associated microbial activity and reducing the amount of litterfall present in streams (Gulis and Suberkropp, 2003; Gulis et al., 2004; Greenwood et al., 2007). The effects of enhanced nutrients on detritus may extend to consumers, both to their community composition and productivity (Cross et al., 2005, 2007; Chapter 4).

Rhabditidae and Plectidae are two nematode families that are common in a variety of freshwater and moist soil habitats (Eyualem et al., 2008). They are both mainly bacterivorous, yet they exhibit substantial differences in life history and autecology. Rhabditidae are generally characterized by a very short generation time, high fecundity, high tolerance to various kinds of disturbance and pollution, and high food availability thresholds for feeding and reproduction (Zullini, 1982; Hodda et al., 2006). As a result, they are often considered enrichment opportunists: they rapidly develop under nutrient-enriched conditions since these favour high bacterial populations, and form metabolically dormant dauer larvae under conditions of crowding and food depletion (Bongers, 1999). Their population dynamics are characterized by successive colonizations and extinctions in a patchy environment (Derycke et al., 2007). Although many Plectidae may also behave as colonizers, they are characterized by comparatively longer generation times (3–4 times longer than Rhabditidae), lower food thresholds and a lower tolerance to pollution and disturbance (Schiemer, 1983; Kammenga et al., 1994; Martinez et al., submitted). They also lack a dauer stage. They are classified as general opportunists *sensu* Bongers (1999), and often replace Rhabditidae in succession when the food supply (i.e. bacterial abundance) decreases (Schiemer, 1983).

Our main objectives were to study the bottom-up effects of nutrient enrichment on nematode populations in terms of density and biomass and the resulting effect on bacterial abundance (top-down vs. bottom up). We compared two species (*Bursilla monhystera* and *Plectus aquatilis*) with different life histories under enriched and non-enriched conditions (with the addition of N, P and glucose). We hypothesized that *Bursilla* populations would show a more rapid increase in density and biomass and would perform better in the most enriched microcosms than *Plectus* populations.

The most opportunistic species, *B. monhystera* (Postma-Blaauw et al., 2005), was therefore deeply investigated using intermediate nutrient levels in different combinations to detect possible nutrient thresholds. Additional functional measures, such as nematode respiration and excretion, were calculated to assess the impact of the experimental release of nutrients and the potential nutrient leach from the bulk detritus and the evolution of the population over time.

5.2. Material and methods

Experimental design

A factorial design was prepared using two biodegradable dissolved organic carbon (BDOC) conditions (basal and 20-fold higher). Each BDOC treatment comprised three levels of nutrient availability (basal, 3-fold, and 10-fold) for nitrogen, phosphorus or both. The nutrient concentrations in our experiment were based on long-term bio-monitoring in the Fuirosos, a third-order forested Mediterranean stream located in the NE of Spain (Artigas et al., 2009; Sabater et al., 2011). The reagents used for nutrient enrichment were common standards for D-glucose, $N\text{-}NO_3^+$ and $P\text{-}PO_4^{3-}$. Each treatment was sampled five times (9th, 17th and 22nd of December 2005 and 7th and 20th of January 2006) for *Bursilla* and three times (21st of December 2005, 6th and 19th of January 2006) for *Plectus*. Each treatment had four replicates, with the exception of the basal nutrient treatment, which had six replicates (Table 5.1). The model thus contained ×3 (N, P and N+P nutrient combinations) ×2 (nutrient levels: x3 or x10) ×2 (BDOC conditions) ×5 (sampling dates) ×4 (replicates) + ×1 (basal nutrient level) ×2 (BDOC conditions) ×5 (sampling dates) ×6 (replicates) = 300 microcosms (Table 5.1).

Table 5.1. Experimental design. Replicates of each treatment.

		N	**P**	**N+P**
LOW BDOC	Basal	6 REPLICATES		
	3-Fold	4 REP.	4 REP.	4 REP.
	10-Fold	4 REP.	4 REP.	4 REP.
HIGH BDOC	Basal	6 REPLICATES		
	3-Fold	4 REP.	4 REP.	4 REP.
	10-Fold	4 REP.	4 REP.	4 REP.

Sediment characterization

In autumn 2005, natural sandy sediments were taken from the Fuirosos riverbed. The granulometry of these sediments (mean grain size of 740 µm, Table 5.2) was assessed using a Malvern Hydro 2000G particle size analyser. The organic matter content was ca. 1%. Natural detritus in Fuirosos contains an annual average of 11% carbon and 0.61% nitrogen (Gaudes, unpublished data). Sediments were autoclaved prior to the start of the experiment in order to eliminate natural fauna and microbiota.

Table 5.2 Sediment charaterization

Particle size	% (n=5)
<4µm	0.14
≥4<38µm	2.22
≥38<63µm	0.89
≥63<125µm	1.29
≥125<250µm	5.49
≥250<500µm	24.46
≥500<800µm	28.11
≥800<1000µm	13.42
≥1000<1600µm	20.29
≥1600µm	3.69

Isolation and culturing of organisms

Natural stream water was filtered through a sterile GF/F Whatman filter to remove all particles >1.2 µm. The filtrate was used as the bacterial inoculum, a few drops of which were inoculated into Erlenmeyer flasks containing 50 mL liquid broth medium including pepton and beef extract. These cultures were further inoculated with bacteria growing in the nematode stock cultures (see below), to ensure that nematode inoculation would not introduce any bacterial strains not present in the control or other nematode treatments. The cultures were renewed every 4–5 days; no fungal or protozoan proliferation was observed.

The freshwater nematode *Bursilla monhystera* (Rhabditidae) was isolated from a small pond in the botanical garden at Ghent University, Belgium, in 1999. *Plectus aquatilis* (Plectidae) was isolated from the shores of Lake Zürich, Switzerland, in spring 2001. Following isolation both species were kept in permanent agnotobiotic culture on 1% agar media composed of bacto and nutrient agar in a 4:1 ratio. Unidentified bacteria from the natural habitat served as food (Moens and Vincx, 1998). The pH of the agar medium was buffered at 7.5 with TRIS-HCl at a final concentration of 5 mM. Stock cultures were maintained at 18 °C in the dark. Nematode cultures were boosted prior to harvesting by the addition of a frozen-and-thawed *E. coli* suspension (Moens and Vincx, 1998). Nematodes were then harvested by rinsing them off the surface of the agar layers with sterile distilled water (*B. monhystera*), or extracting them from the agar through density centrifugation in sucrose (*P. aquatilis*) (Moens and Vincx, 1998).

Microcosm design and sampling procedure

The microcosms were prepared as follows. A thin layer of sterile (autoclaved) sediment was spread on the bottom of 9 cm diameter Petri dishes, and hydrated with 10 mL of the corresponding nutrient solution (see above). They were then inoculated with 20 µL of bacterial inoculum and kept at 20 °C in the dark for one week. Bacteria were allowed to grow in the microcosms for 5 days prior to nematode inoculation. The microcosms were then inoculated with 126.6 ± 20.0 individuals + 17.7 ± 4.9 eggs of *B. monhystera*, and 186.5 ± 28.9 individuals + 71.8 ± 11.5 eggs (n=10 in both cases) of *P. aquatilis*.

Sampling started 7 days after nematode colonisation and was performed at weekly intervals until day 21 and biweekly until day 49 for *B. monhystera* or biweekly from day 7 to 56 for *P. aquatilis*.

A subsample of sediment was taken from each microcosm with a metal borer and fixed in formalin (2%) for bacterial counting. Pore water was carefully extracted from the sediments to perform nutrient analyses, and the remaining sediment was transferred to plastic pots and fixed in 4% formalin for later extraction and quantification of nematodes.

Sample processing for nutrient concentration and bacterial abundance

Water from each microcosm was filtered through pre-combusted glass-fibre filters (Whatman GF/F) before analysis. Ammonium, nitrate and dissolved phosphorus were analysed using standard methods (APHA, 1992) on a SKALAR San Plus (Breda) automatic chain.

The bacterial abundance in each microcosm was estimated after sonicating the samples (2+2 min, 40-W power, 40-kHz frequency; Ultrasons, Selecta, Abrera, Spain). After appropriate dilution, fixed samples were stained for 5 min with 40,6-diamidino-2-phenylindole (final concentration 2 µg/mL) and passed through 0.2-ml irgalan black-stained polycarbonate filters (Nuclepore; Whatman, Maidstone, UK). Bacteria were counted in 25 fields/filter under a fluorescence microscope (Eclipse E-600; Nikon, Tokyo, Japan) at 1250x magnification.

Nematode counts and measurements

Samples for nematode quantification were washed through a 32-µm sieve and the nematodes extracted by flotation using Ludox TM50 (Heip et al., 1985). After staining with Rose Bengal, all nematodes were counted and classified as ‘Large’ (J3, J4 and adults) or ‘Small’ (J1 and J2 individuals) (Large/Small ratio). For detailed measurements, a minimum of 100 individuals per sample were picked out, dehydrated, included into anhydrous glycerol (De Grisse, 1969) and mounted on slides. The body length and maximal width of all nematodes were measured under a microscope (Eclipse E-600, Nikon, Tokio, Japan) using an image analyser (analySIS®, Olympus, Münster, Germany). Nematode volume was calculated according to the following formula (Warwick and Price, 1979):

$$\text{Biovolume (nL)} = 530 \times \text{Length } (\mu\text{m}) \times \text{Width}^2\ (\mu\text{m}) / 16 \times 10^5,$$

and this was converted to dry weight using a specific gravity of 1.13 (Wieser, 1960) and a dry-to-wet weight ratio of 0.25 (Feller and Warwick, 1988).

Nematode bioenergetics

Nematode samples were divided into five size classes, roughly corresponding to the four juvenile (J1, J2, J3 and J4) stages and clearly reproductive adults; some young, not yet reproductive adults may have ended up in the J4-class (modified from Ferris et al., 1995). The numbers in the different size classes were used to estimate respiration according to the power equation $R = a \cdot W^{b}$ (Schiemer, 1982a, 1983). Values for “a” and “b” were based on data for the rhabditid *Caenorhabditis briggsae* fed *Escherichia coli* at 20 °C (Schiemer, 1982a). Production and assimilation were calculated according to efficiencies described in the literature for *Bursilla labiata* and other rhabditids (Ferris et al., 1997; Marchant and Nicholas, 1974).

To estimate the potential contribution of nematodes to nitrogen cycling in our microcosms, we used a mean C-to-N ratio of 5.89 for nematodes (as described for eight nematode species cultured on *E. coli* on agar; Ferris et al., 1997) and a mean C-to-N ratio of 4.12 for bacteria (Ferris et al., 1997). In order to consume sufficient bacteria to provide the C required for their body structure and respiration, nematodes assimilate more N than necessary, and this excess is excreted mainly as ammonia, which freely permeates the nematode cuticle (Lee and Atkinson, 1977). We calculated the amount of nitrogen excreted based on the assumption of an assimilation efficiency of 20–40% (e.g. Marchant and Nicholas, 1974). The difference between this excretion rate and the N consumption rate of nematodes was then extrapolated between two consecutive sampling dates and compared to the difference in total inorganic N measured in the microcosm pore water.

Statistical analysis

In the *Bursilla* experiment, the normality of the sample distribution and homogeneity of variances were assessed to determine whether data transformation (log(x + 1)) was required. A three-way ANOVA (using time, NP treatment and glucose treatment) was used to detect significant differences between treatments over time. *Post-hoc* comparisons between means were analysed using a Tukey Honest Significant Differences (HSD) test.

Spearman multiple correlation was applied to real nutrient concentrations (ionic N forms in water) and bacterial and nematode density and biomass (Large/Small ratio of nematodes). We applied a false discovery rate correction (FDR; Benjamini and Hochberg, 1995) because of the large number of comparisons made.

5.3. Results

Under basal nutrient treatments at both glucose levels, *Bursilla* populations showed exponential growth until they reached a plateau and then decreased, while *Plectus* populations showed no significant increase over the course of the experiment except for a slight increase in the glucose amended treatment (Fig. 5.1).

However, in the high nutrient treatment, especially that enriched with glucose, the *Plectus* population did increase, showing a similar pattern to that described for *Bursilla*. In these enriched conditions, however, the increase in *Bursilla* numbers started much earlier than that of *Plectus* and then showed a variable pattern towards the end of the experiment, rather than a clear decrease as in *Plectus* populations (Fig. 5.1).

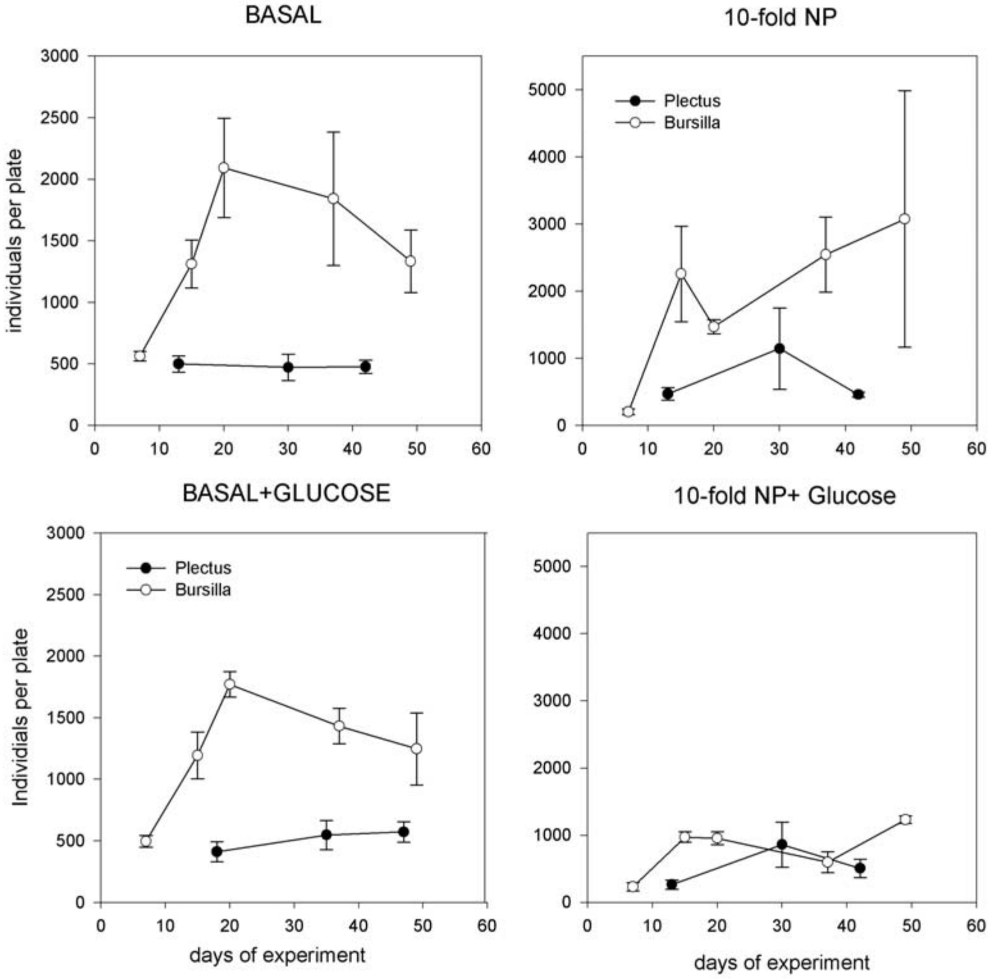


Fig 5.1. *Plectus* (open dot) and *Bursilla* (empty dot) abundances. X-axis represents the days of experiment after nematode inoculation.

Effect of nutrient levels on Bursilla and bacterial abundances

The density of bacteria was similar in all treatments (Fig. 5.2). However the pattern of bacterial abundance differed significantly between nutrient treatments ($p<0.05$) and also responded significantly to a Time x Treatment effect (Table 5.3). Tukey *post-hoc* comparisons showed differences between the first three sampling dates and the last two, and between the basal treatments and the 3-fold P-enriched treatments at low glucose. Strikingly, bacterial abundance did not show a similar response at high glucose levels.

Table 5.3. Results of the 3-way ANOVA for bacteria (using time, NP treatment and glucose treatment)

Univariate Tests of Significance for bacteria	SS	D.F.	MS	F	p
Intercept	1.65E+20	1	1.65E+20	11839.56	*0.0000*
Date	3.05E+18	4	7.63E+17	54.68	*0.0000*
Glucose	9.90E+15	1	9.90E+15	0.71	0.4013
nutrient	1.90E+17	6	3.17E+16	2.27	*0.0404*
Date*glucose	3.62E+16	4	9.04E+15	0.65	0.6293
Date*nutrient	1.26E+18	24	5.23E+16	3.75	*0.0000*
Glucose*nutrient	5.22E+16	6	8.70E+15	0.62	0.7116
Date*glucose*nutrient	4.85E+17	24	2.02E+16	1.45	0.0968
Error	1.87E+18	134	1.40E+16		

Bacteria followed a similar trend in each treatment to that observed for nematodes, except in the 10-fold N treatment in which bacteria, unlike nematodes, showed continuous growth (Fig. 5.2).

Bursilla abundance responded significantly to time, glucose and NP treatment (Table 5.4). Tukey *post-hoc* comparisons also revealed a major difference between the first sampling date and the other dates, and between the basal treatment and the 3-fold NP and 10-fold P treatments. An effect of glucose was only observed on the first sampling date.

Table 5.4. Results of the 3-way ANOVA for nematodes (using time, NP treatment and glucose treatment)

Univariate Tests of Significance for nematodes	SS	D.F.	MS	F	p
Intercept	1727.663	1	1727.663	39955.34	*0.0000*
Date	8.143	4	2.036	47.08	*0.0000*
Glucose	0.217	1	0.217	5.03	*0.0265*
nutrient	1.296	6	0.216	4.99	*0.0001*
Date*glucose	0.302	4	0.076	1.75	0.1432
Date*nutrient	2.521	24	0.105	2.43	*0.0007*
Glucose*nutrient	0.852	6	0.142	3.29	*0.0047*
Date*glucose*nutrient	1.438	24	0.06	1.39	0.1249
Error	5.967	138	0.043		

The pattern of *Bursilla* abundance varied between treatments. Nematode abundance in basal nutrient conditions tended to follow a unimodally shaped curve with initial exponential growth, a plateau and a slight decrease at the end of the experiment (Figs. 5.2, 5.3, 5.4). Nitrogen-enriched treatments showed a similar trend to basal treatments (Fig. 5.2). Conversely, phosphorus- and combined NP-enriched treatments showed highly variable, but continuous exponential growth (Figs. 5.3, 5.4).

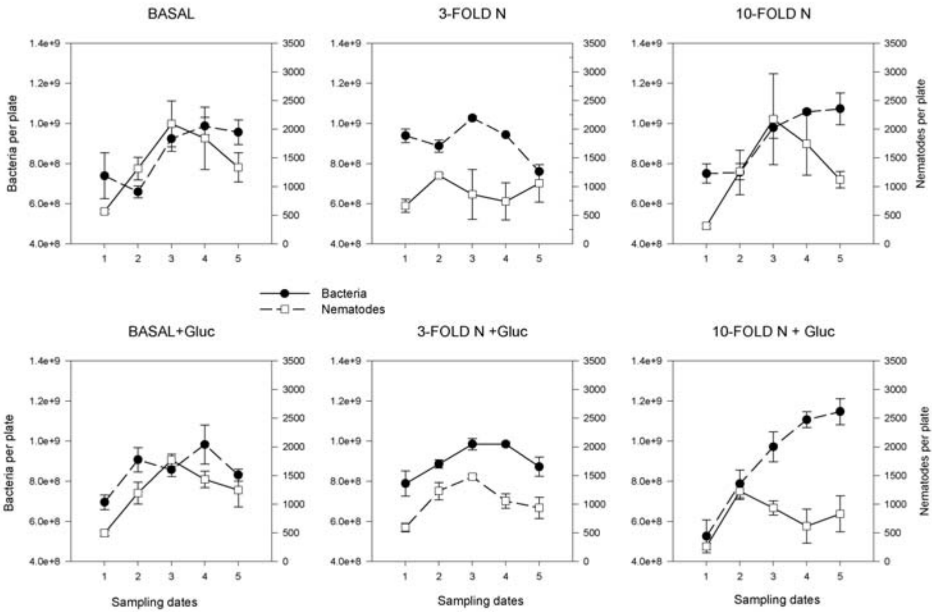


Fig. 5.2. Abundances of bacteria and nematode under N enriched levels (basal, 3- and 10-fold).

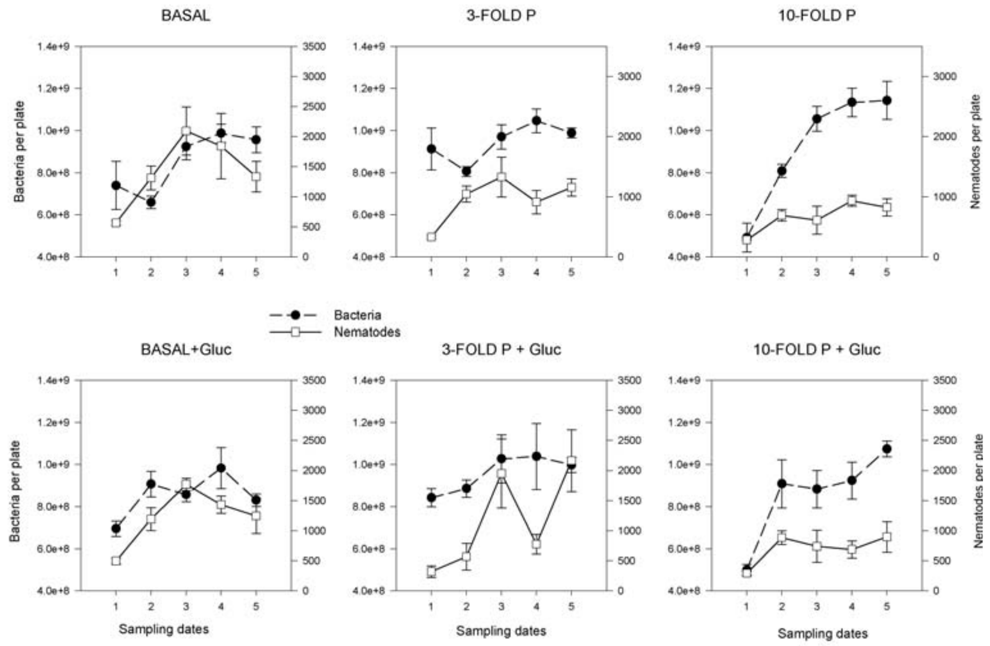


Fig. 5.3. Abundances of bacteria and nematode under P enriched levels (basal, 3- and 10-fold).

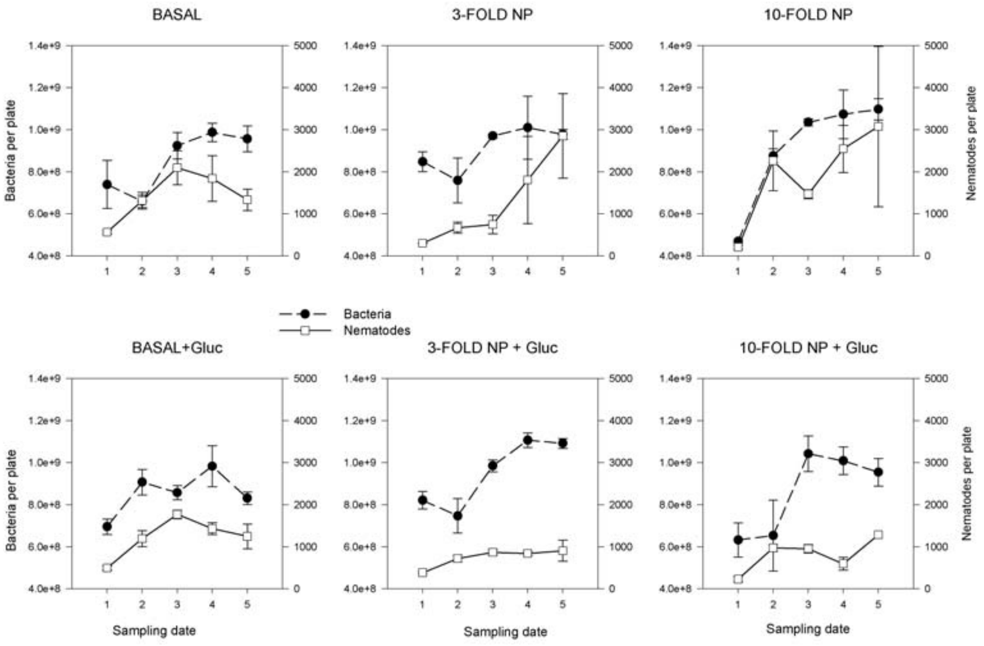


Fig. 5.4. Abundances of bacteria and nematode under NP enriched levels (basal, 3- and 10-fold).

In the 10-fold N treatment without glucose, the density of nematodes was almost double the maximum density achieved in the 3-fold N treatment (Fig. 5.2). In the phosphorus-enriched treatments the pattern was the opposite, with more nematodes in the 3-fold P treatment with glucose compared to the 10-fold P enrichment (Fig. 5.3). In the combined NP treatments, the highest *Bursilla* abundances were found in the 3-fold and 10-fold NP treatments without glucose (Fig. 5.4).

Spearman tests showed significantly positive correlations between ammonia and phosphate concentrations and nematode abundance as well as the ratio of large to small individuals (Table 5.5). Ammonia and phosphate concentrations were also positively correlated with bacterial density but only at a significance level that did not meet the FDR cut-off requirements. Nitrite and nitrate concentrations, in contrast, showed a negative correlation with bacteria and nematode biomass. There was also a positive correlation between densities of bacteria and nematode over the different treatments (Table 5.5).

Table 5.5. Results of Spearman correlation test. $p<0.01$; (+)= positive effect, (-)= negative effect, N.S.= Not significant. False Discovery Rate has been applied to the significant correlations (see text).

	Nematode density	Ammonia	Phosphate	Nitrate	Nitrite
Nematode density	N.S	(+)	(+)	(-)*	N.S
Large/small ratio	(+)	(+)	(+)	(-)	(-)
Bacteria density	(+)	(+)*	(+)*	(-)	(-)

$p<0.01$

* does not meet the FDR cutt-off

Nematode bioenergetics

Respiration in basal conditions was lowest on the first sampling date when the different life stages of *Bursilla* were present in similar proportions (Fig. 5.5). However, on the second and third sampling dates there was an increase in respiration concomitant with a larger proportion of J4 and adult nematodes. This increase was much lower in the glucose-enriched treatments (labeled “G”; Fig. 5.5). On the last two sampling dates the population was mainly dominated by small and medium sized nematodes (J1, J2 and J3) and there was a decrease in respiration.

On the first sampling date, the pattern of respiration in the 10-fold NP treatments was similar to that observed for the basal treatment (Fig. 5.5). From the second sampling date onwards, there was a variable but increasing pattern of respiration in the enriched treatment dominated by small to medium sized nematodes (J2, J3). The glucose-enriched treatments showed lower respiration, and on the last two sampling dates, the population was dominated by small and medium sized nematodes (J1, J2 and J3), as seen in the basal treatment.

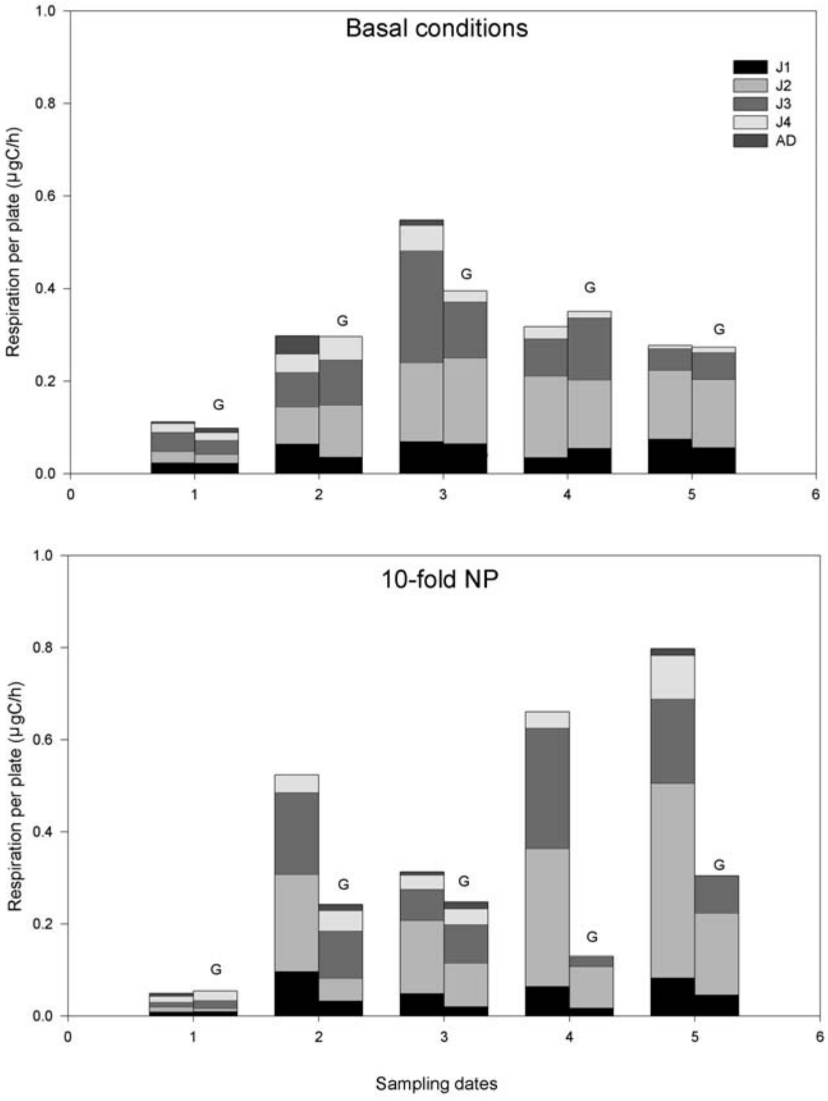


Fig. 5.5. Respiration ($\mu C \cdot h^{-1}$) of each developmental stage (J1, J2, J3, J4, Adult; see text) on each sampling date.

Availability of N

Based on the excretion–consumption values measured for *Bursilla* and the measured N-nutrient concentrations, the N concentration measured in water from the first to the second sampling date was higher in all treatments than the theoretical amount that could be attributable to an increase in the metabolic activity of the nematodes, which could indicate the possible involvement of detritus leaching (Table 5.6). On subsequent sampling dates, the nematode demand for nitrogen was higher than the N-forms measured in the water (Table 5.6).

Table 5.6. Availability of nitrogen in plates based upon the theoretical content (Excretion rate minus Consumption rate) and total inorganic N found in the microcosms water.

Sampling dates		**1-2**	**2-3**	**3-4**	**4-5**
time (between sampling dates)		*(192h)*	*(120h)*	*(408h)*	*(288h)*
BASAL	Theoretic (Exc-Prod)	2410.9	3921.5	28810.1	13882.2
	Real (measured in water)	2951.7	1817.3	4319.3	2195.3
	Difference	*540.7*	*-2104.2*	*-24490.8*	*-11686.9*
BASAL+Gluc	Theoretic (Exc-Prod)	2163.0	3902.8	20767.1	11068.5
	Real (measured in water)	2266.0	2149.0	1988.8	1444.3
	Difference	*103.0*	*-1753.8*	*-18778.4*	*-9624.3*
10NP	Theoretic (Exc-Prod)	1075.2	6897.9	16485.1	21505.8
	Real (measured in water)	2645.3	3928.0	4283.3	9792.0
	Difference	*1570.2*	*-2969.9*	*-12201.8*	*-11713.8*
10NP+Gluc	Theoretic (Exc-Prod)	1171.1	3190.9	13193.9	4184.0
	Real (measured in water)	3117.3	3180.0	3197.3	1798.3
	Difference	*1946.3*	*-10.9*	*-9996.6*	*-2385.8*

Units:µg/L

5.4. Discussion

Heterotrophic bacteria and fungi and their grazers are an important link in the functioning of many ecosystems, particularly for the mineralization of nutrients (Valiela, 1995). Organic matter provides an energy source for microbial growth, resulting in the release of inorganic nitrogen and other essential nutrients by the microbiota and thereby increasing their biomass (Freckman, 1988). While Coleman et al. (1977) found a significant increase in P and N immobilization in glucose-amended sterile sediments, we did not find a clear effect of glucose addition. Bacterial abundances were in the same order in all treatments, which could be partly due to a DOM release from bulk detritus as a result of the pre-treatment of the sediments. However, their abundance pattern differed, with a clear increase in the 10-fold N, P and NP treatments, which could indicate that the initial addition of inorganic nutrients may have favoured bacterial dynamics creating an optimal stoichometry (C:N:P) in these microcosms.

An increase in bacterial and *Bursilla monhystera* density and a predominance of larger nematodes were related, demonstrating a clear bottom-up control in the microcosms. Some Rhabditids have been reported to increase in length in well-fed conditions (Sudhaus, 1980). *B. monhystera* reacted to changes in their environmental conditions (e.g. the initial increase after the inoculation on plates), but if nutrient levels were not high enough, their numbers tended to decrease, probably because they did not invest in reproduction and thus the population was basically maintained by small–medium juveniles. On the other hand, under enriched conditions (10-fold N and P), *B. monhystera* tended to increase their numbers over time although there was no significant increase in reproductively active adults. In fact, some Rhabditids have found to increase their colonizing ability by an early reproductive effort instead of a constant resource allocation into reproduction (Schiemer et al., 1983; Woombs and Laybourn-Parry, 1985). Moreover, the unbalanced stoichometry (C:N:P) in microcosms (driven by the potentially high amount of carbon leached from the detritus) may be sufficient to maintain the basic metabolic rates of non-reproducing individuals (Elser et al., 2001). Dissolved organic matter in its most refractory form can even reduce nematode fertility (Höss et al., 2001).

Like *B. monhystera*, *Plectus aquatilis* populations only increased in abundance under high nitrogen and phosphorus concentrations, although their response was much slower than that of *Bursilla* individuals. This differential increase in abundances have been previously described for *B. monhystera* and another Plectidae, *P. parvus* (Postma-Blaauw et al., 2005). They attributed this difference mostly to the distinctive life history strategies (and cp values). *B. monhystera*, like other cp-1 nematodes, has a stronger competitive ability while *Plectus*, and other cp-2 nematodes, can endure lower food thresholds and feed on a wider variety of bacteria (Postma-Blaauw et al., 2005).

The amounts of nutrients found in the water were consistently higher than those added at the beginning of the experiment. This demonstrates again, the potential N release from bulk detritus in sediments. However, this effect may have been concealed by the increased nematode activity throughout the experiment. Abundances of bacteria and nematodes were positively correlated to increased nitrate and ammonia availability in the water. An increase in nutrient mineralization has been reported in the presence of bacterial grazers, mainly nematodes and protists (Johannes, 1965; Ferris et al., 1997; Bonkowski et al., 2000).In this sense, it has been widely demonstrated that bacterial-feeding nematodes contribute to N mineralization by feeding on and by dispersing bacteria (Anderson et al., 1981; Bouwman et al., 1994; Freckman, 1988; Griffiths, 1994). By grazing on bacteria, bacterial-feeding nematodes participate in decomposition food webs and N mineralization to an extent that is determined by their metabolic and behavioural attributes, by their demography and physiology, and by the relative C-to-N ratios of the nematodes and their bacterial prey (Ferris et al., 1995, 1996a, 1997; Ingham et al., 1985; Venette and Ferris, 1997). Through their grazing activity, rhabditid nematodes can attain among the highest N-mineralization values reported in the literature (Ferris et al., 1998). The excess N assimilated during growth and egg production or to meet the C needs of respiration is excreted in mineral form (NH_4^+) (Lee and Atkinson, 1977; Wright and Newall, 1976).

In our experiment, only the treatments to which phosphorus was added (mainly the 10-fold addition) maintained a constant increase in the nematode population over the entire experiment. Since phosphorus is a conservative element, the availability and supply of P is a key determinant of the binding, flux and fate of C in freshwater foodwebs (Elser and Hessen, 2005). Changes in P supply can alter the efficiency of trophic transfer by modulating the nutritional match between producers and consumers (Elser et al., 2001). Moreover, glucose- and nitrogen-amended treatments (3-fold NP and 10-fold N and NP) also showed lower nematode abundances than the same treatments without glucose addition, which could be due to a sub-optimal stoichiometry.

Human activities (e.g. logging, agricultural practices and industrial waste) are the main sources of organic or inorganic nitrogen (N) and phosphorus (P) that enter aquatic ecosystems. Climate change studies predict that in Mediterranean systems, slow-moving, shallower waters may persist for longer than usual (Sabater and Tockner, 2010), which may favour higher water temperatures and nutrient concentrations as well as a decrease in the efficiency of many biological processes (Nilsson and Renöfält, 2008). However, although a decrease of biodiversity is predicted under such conditions (Sabater and Tockner, 2010), the different traits, energy partitioning and life strategies observed in freshwater bacterivorous nematode species, could enable several species to coexist and influence ecosystem processes.

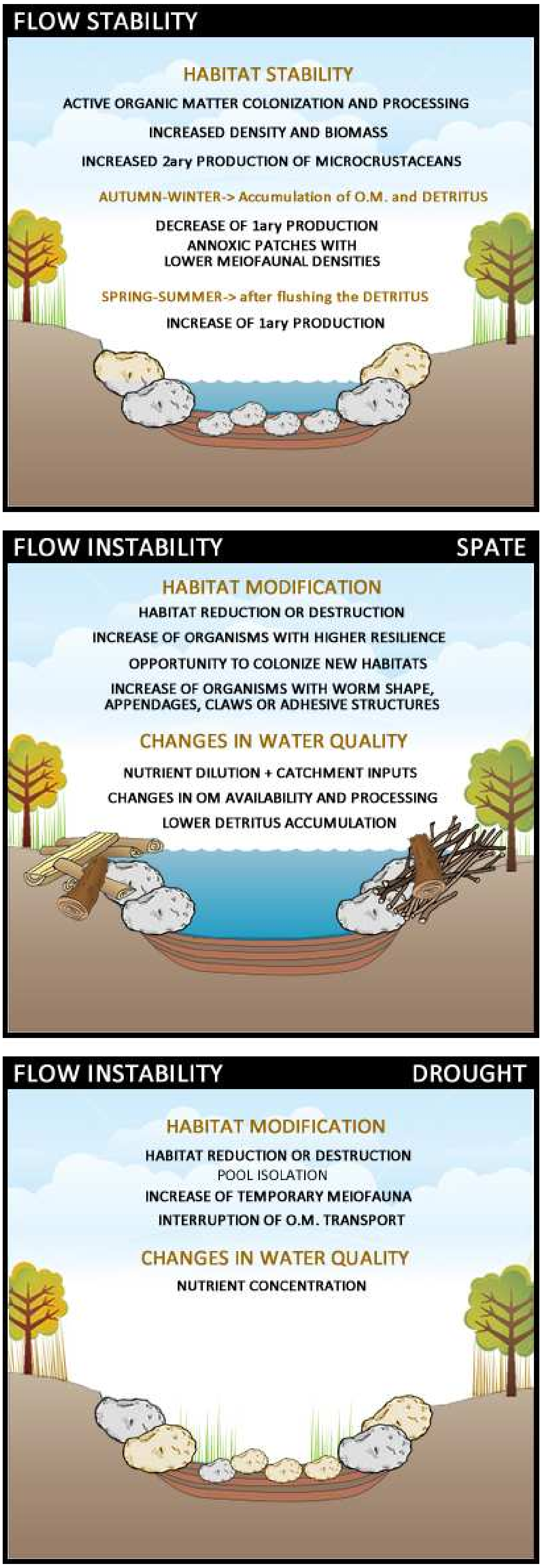
GENERAL DISCUSSION

GENERAL DISCUSSION

The general aim of this thesis was to characterise and integrate meiofauna into our current understanding of lotic functional processes. The previous set of chapters has focused on several aspects of lotic processes, taking into consideration their multiple interactions with the conceptual framework of Mediterranean systems. It implies the natural intra- and inter-annual variability exhibited by these systems (Gasith and Resh, 1999; Acuña et al., 2005). The annual regime of rainfall and temperature in Mediterranean areas directly determines fluvial hydrology and the sequences of floods and droughts. Indirectly, these effects also influence lotic processes like organic matter deposition and decomposition (Acuña et al., 2004; Artigas et al., 2009), DOC dynamics (Vázquez et al., 2007), nutrient in-take and re-cycling (von Schiller et al., 2010; Sabater et al., 2011), sediment transport, and habitat stability. Some of these lotic processes and their relationship with the meiofaunal community have been examined in this thesis using field and laboratory experiments. The results are are summarized in the Fig. D1, and discussed below.

Effects of hydrology on the meiofaunal assemblage.

Several meiobenthologists have defined flow and substrate as the main small-scale determinants of meiofaunal distribution (Swan and Palmer 2000, Silver et al. 2002). These two factors may have direct effects on meiofaunal organisms. For example, Palmer (1992) determined that water current velocities above 12 cm/s increase larval detachment and downstream dispersion for midges. But flow and substrate commonly act together, because at the same time that organisms enter the drift, substrate transported by the increased flow may act as a raft, thus transporting the inhabiting organisms to downstream reaches. During this thesis, this effect was observed in sandy sediments (**Chapter 1**), detritus (**Chapter 2**), and detached cyanobacterial mats (**Chapter 4**). The implications of these effects on dispersal are discussed later on.



FLOW STABILITY

HABITAT STABILITY

ACTIVE ORGANIC MATTER COLONIZATION AND PROCESSING

INCREASED DENSITY AND BIOMASS

INCREASED 2ary PRODUCTION OF MICROCRUSTACEANS

AUTUMN-WINTER-> Accumulation of O.M. and DETRITUS

DECREASE OF 1ary PRODUCTION
ANNOXIC PATCHES WITH
LOWER MEIOFAUNAL DENSITIES

SPRING-SUMMER-> after flushing the DETRITUS

INCREASE OF 1ary PRODUCTION

FLOW INSTABILITY **SPATE**

HABITAT MODIFICATION

HABITAT REDUCTION OR DESTRUCTION

INCREASE OF ORGANISMS WITH HIGHER RESILIENCE

OPPORTUNITY TO COLONIZE NEW HABITATS

INCREASE OF ORGANISMS WITH WORM SHAPE,
APPENDAGES, CLAWS OR ADHESIVE STRUCTURES

CHANGES IN WATER QUALITY

NUTRIENT DILUTION + CATCHMENT INPUTS

CHANGES IN OM AVAILABILITY AND PROCESSING

LOWER DETRITUS ACCUMULATION

FLOW INSTABILITY **DROUGHT**

HABITAT MODIFICATION

HABITAT REDUCTION OR DESTRUCTION
POOL ISOLATION
INCREASE OF TEMPORARY MEIOFAUNA
INTERRUPTION OF O.M. TRANSPORT

CHANGES IN WATER QUALITY

NUTRIENT CONCENTRATION

Fig. D1. Processes related to meiofauna and hydrology in Mediterranean lotic systems

Bed-moving spates may have dramatic consequences on organisms as a result of scouring effects. However, meiofaunal organisms have developed adaptive responses to hydrological disturbances. Thus, a small worm-shaped body and locomotor appendages appear to be successful species traits in the reaches of the Fuirosos stream. Bed-moving spates reset ecosystem metabolism, especially gross primary production (GPP). However, they are generally short and followed by rapid recovery (Uehlinger, 2006). In **Chapter 1**, the resilient response of meiofauna has been shown to act in a similar way, as a result of short pulses in headwater reaches. Conversely, in downstream reaches heavy rainfall events resulted in higher discharges (as a consequence of higher water run-off from catchments) and meiofaunal communities showed a slower recovery. In the Fuirosos stream, previous hydrological history also appeared to modulate invertebrate response to floods (Boulton and Lake, 1992), diminishing recovery response of the community in the most downstream reaches. In contrast, this effect was not observed in the most upstream reach (S1). The upstream reaches of the Fuirosos stream, many of them tributaries or first-order reaches, showed an intriguing complexity of habitat properties and heterogeneity. In these intermittent reaches, organic matter transported by water flow tended to aggregate and accumulate at every flow disruption point, creating stochastic wood debris dams between rocks, riparian trees and fallen logs (Gooderham et al., 2007). These dams provide relative stability to organisms by directly offering shelter and food inside or deviating water flow and creating “dead zones”, in which communities can develop. However, the notion that these reaches act as a organism sink for downstream recolonisation and maintain biodiversity often clashes with the common idea of the hyporheic refuge. For example, Olsen and Townsend (2005) provided little evidence of the hyporheic refuge during a flood event in a fourth-order gravel-bed stream. They found that flooding affected benthic (0-10 cm) and hyporheic (10-50 cm) invertebrate densities in a similar way. Likewise, Boulton et al. (2004) did not find a significant transfer of epigean water mites to downwelling zones or a decrease in hyporheic species after an *in situ* simulated spate. These results suggest that both responses occur depending on the suddenness of the process. Floods in Mediterranean systems generally arise abruptly and, while their severity is largely unpredictable, their return times and durations are often less so. However, droughts may occur progressively (allowing for some adaptability of the biological communities) and many ecological responses may exhibit a 'stepped' response: gradual change alternating with thresholds of swift transitions, e.g. when a habitat disappears or is fragmented (Boulton, 2003).

Droughts in Mediterranean climate regions are predictable and periodic and are typified as seasonal droughts (Lake, 2003). However, the intensity can vary from year to year as a result of interannual meteorological variability. These perturbations are a result of a sequential decline in precipitation, runoff, soil moisture, groundwater levels and stream flow (Dahm et al., 2003). Flow reduction involves a decrease in the transport capacity of the system and an increase in the detritus coverage of the streambed. Acuña et al. (2005) described the desiccation of the Fuirosos stream and the watercourse fragmentation into a series of isolated pools. During this process, spatial habitat heterogeneity increased and physicochemical conditions changed abruptly, turning into a critical threshold for the ecosystem (e.g. Boulton and Lake, 1990; Stanley et al., 1997). In this situation, the hyporheos of this stream could have been a way to escape from the complete desiccation of pools, especially for organisms with advantageous traits like a worm-like morphology. However, given the geologic characteristics of the Fuirosos catchment and the complexity of its hyporheos, this process deserves a special consideration in further research on this stream.

Intra-annual variability in the meiofaunal community

In **Chapters 1** and **3**, the meiofaunal community in the Fuirosos stream showed an increase in density and biomass during desiccation, in spring (2003 and 2005) and in summer (2004). In spring, these increments coincide with naturally occurring autotrophic pulses in this stream (Artigas et al., 2009; von Schiller et al., 2008). Algal standing crop increases when a determined light availability and flow stability threshold is achieved. Although this response is easily observed in epilithic algae, sandy substrate and the inhabiting communities could also be directly or indirectly affected by these environmental conditions. Benefits for the meiofaunal community could derive from increased food availability because of higher densities of diatoms and autotrophic flagellates or detritus enrichment from epilithic exudates, which in turn, could favour an increase in bacterial and fungal densities. However, these processes often coincide with a flow reduction and ecosystem contraction (Stanley et al., 1997). The latter implies a concentration of nutrients and further on, caused by flow disruption, an interruption of organic matter transport (Acuña et al., 2005). During pool isolation, many opportunistic meiofaunal species (like some chironomids, oligochaetes and nematodes) could be benefited. Many macroinvertebrate studies have reported that pool isolation leads to a decrease in biodiversity. However, as shown in **Chapter 5**, even in opportunistic nematode species, slight behavioural differences may lead to a short pattern of succession that would imply constant replacement of microorganisms and meiofauna. Notwithstanding, these processes will be overlooked by lotic researchers who focus only above 250µm.

In **Chapter 4**, the nematode community inhabiting the cyanobacterial mats also showed species succession, which was directly related to algal composition. The evolution of these mats was also tightly linked to the spring increase in water temperature and a decrease in flow.

Interannual variability in the meiofaunal community

Chapters 1 and **3** showed that the interannual variability greatly affected communities as a result of the succession of two hydrological years in which precipitation varied considerably. Acuña et al. (2004) found significant differences in the total amount of benthic organic matter in the streambed between the drier and wetter years. Thus, these differences also determined physicochemical properties and the ecosystem respiration values. The annual secondary production estimated for ostracods and cyclopoids in **Chapter 3** was also higher in the control reach. This observation indicates that this increase was caused mainly by flow stability and lower precipitation in the second year (**Chapter 1**). However, this increase in secondary production was exacerbated by the nutrient addition, as observed in the impacted reach (**Chapter 3**).

The Mediterranean region is expected to endure higher temperatures and lower precipitation, especially in summer, under predicted climatic changes (IPCC, 2007). Alterations in precipitation patterns could alter the duration, frequency, and magnitude of flow and flood pulses. Under these conditions, the alternation between wet and dry years may expand, affecting the response of meiofauna and other stream communities to these events. Long-term studies of flow variability in intermittent streams revealed that extreme floods or dry-periods have long-lasting consequences on community structure (Sponseller et al., 2010). Therefore the most pessimistic predictions of the climate change for Mediterranean systems are that aquatic communities could be greatly simplified. However, although this change may fully affect temporary meiofauna, permanent meiofauna may exhibit a faster response and more efficient adaptation to harsher conditions (through higher turnover rates, better access to the hyporheos, etc). Thus, these adaptations may lessen the hydrologic impact on microorganisms and meiofauna; however, the climatic effects on food availability would also re-shape these communities inasmuch as modification of the flooding regime is expected to decelerate decomposition (Langhans and Tockner, 2006).

Patterns of organic matter colonisation and dispersal.

Organic matter is possibly the major determinant of meiofaunal distribution, abundance and species composition because it provides food, structure, habitat and material for case-building (see Silver et al., 2002 and references therein). However, once again, flow is a key determinant of organic matter deposition and distribution in the streambed. Our results in **Chapter 2** support this effect since hydrology and temperature highly influenced the colonisation of decaying leaf material. The microbial successional pattern observed during the experiment provides insight into the colonisation and further decomposition of leaves. The carbon allocation and transference between compartments proved useful to describe the process. Moreover, a direct transference between temporary meiofauna and macroinvertebrates was observed, as well as a general transference from the bacterial and fungal compartments to the faunal ones over the experiment.

Flow generally outweighs other small-scale factors, and its effect has major consequences on meiofaunal organisms. Some temporary meiofauna may have body structures to increase resistance to flow (e.g. claws, adhesive structures, etc) but these structures are generally fully developed when they are macroinvertebrate-size. Therefore, in general, meiofauna and other organisms with high turnover rates may respond to hydrological disturbances with greater resilience and may benefit from the dispersal power of water flow. Silver et al. (2002) defined passive drift as one of the major processes behind meiofauna redistribution. In **Chapter 1**, a diminished resilient response in site S2 was observed by a slower slope in organism response. However, in at least one case, in situ observations revealed that immediately after flooding, instead of a scoured reach, a sand-depleted reach was detected with organisms inhabiting the detritus. Thus, high organism abundance immediately after the event influenced the resilient response slope.

In **Chapter 2** the natural colonisation pattern on leaves may have been accelerated by the arrival of densely inhabited clumps of FPOM from upstream reaches after a flood event. Therefore, detritus-associated flora and microcrustaceans (mainly Ostracods and Cyclopoids) were retained by our leaf-packs, natural wood-debris dams and sandy shores of the stream.

In **Chapter 4**, the increase in nematode individuals may have accelerated the detachment of the cyanobacterial mats, through increased bioturbation. Palmer et al. (1992) demonstrated that meiofauna have some control of the entry into and exit from the drift under very low flow conditions. In addition, the capacity of nematodes to actively move towards food patches (e.g. detecting bacterial CO_2; O'Halloran et al., 2006) or to detect volatile compounds, such as geosmin (Höeckelmann et al., 2004), could indicate that these communities remained in these detached mats because they offered better conditions than patches that were attached. Higher temperatures in the detached mats floating on the surface would have accelerated reproductive rates. However, interestingly, gravid females of *Diplogaster rivalis* dominated in these floating mats. They had viviparous behaviour since their embryos developed inside their bodies instead of hatching outside. This reproductive strategy could be considered an advantageous trait for the recolonisation of new substrata. Given that substrate detachment is not an isolated process, these nematodes could have adapted their dispersal mode to this mechanism.

Effects of nutrient inputs

The results reported in **Chapter 3** indicated that meiobenthic communities in Mediterranean forested streams react to moderate nutrient inputs. Nutrient release increased not only the density and biomass of the meiofaunal community but also the annual secondary production and production to biomass (P/B) ratios of some microcrustaceans. In the field, a 3-fold increase in nutrient concentration was sufficient to observe changes in the meiofaunal community; however, in laboratory conditions, the nematode populations mentioned in **Chapter 5** required greater enrichment, although there was a clear bottom-up effect. This finding could be attributable to the marked opportunistic nature of the nematode species studied. In addition, in the field, the overall response may conceal multiple interactions between organisms. At the same time, flow reduction, like in summer 2004, could have favoured the availability of higher concentrations of nutrients in some pools.

In this regard, hydrology is a crucial factor while nutrient enrichment was taking place. The differences observed between hydrological years in **Chapter 1** may have concealed the effects of nutrient addition. In the control site, greater hydrologic stability during the enrichment year also favoured greater development of the meiofaunal community. Nevertheless, changes in the enriched reach were more evident indicating that the combination of hydrology and nutrient release has a large impact on communities. This impact also may have further implications since nutrient effects on communities may be exacerbated when water is scarce.

In the Llobregat river, hydrology and nutrients also had a considerable influence on communities although their mechanisms acted on a different scale from that observed in the Fuirosos stream. The Llobregat catchment has been highly disturbed over the last 150 years (see López-Doval et al., 2010 and references therein). In addition to high nutrient and wastewater releases of this river, its morphology has been constrained into a series of reservoirs and dams. This development implies that in years with less discharge (as a result of reduced snow-melt or precipitation) primary producers proliferate because of the combination of high nutrient availability and hydrological stability. In **Chapter 4**, our cyanobacterial mats dominated the primary producer compartment during springtime, in the period comprised between the diatom-dominated community of winter and the green algae-dominated communities typical of summer. This cyanobacterial proliferation may have consequences for use of this water for human consumption. In those mats, *Oscillatoria* produced an organic volatile compound (VOC), harmless for humans, but a hindrance because it is too expensive to extract in water drinking plants and is thus perceived in tap water.

These cyanobacterial mats held nematode densities from 100- to 600-fold higher than the whole meiofaunal community in the Fuirosos sands. These two substrates showed a substantial difference in C:N content. While in the Llobregat, the low C:N ratio and high organic matter content that characterised the cyanobacterial biofilm (Vilalta et al., 2004) could have favored greater development of nematodes, in the Fuirosos stream the high amount of organic carbon from the riparian forest inputs (Acuña et al., 2004; Artigas et al., 2009) increased the C:N ratio. In **Chapter 5** this increase was also observed to be the result of the detritus in the Fuirosos sands added to the experimental plates. Thus, a high amount of refractory carbon and high C:N ratios would explain the lower densities and biomasses found in the field (**Chapter 1** and **Chapter 3**) and in the low nutrient concentration plates described in **Chapter 5,** thereby indicating a possible sub-optimal stoichometry.

In its most refractory forms, carbon affects nematode reproduction (Höss et al., 2001). In **Chapter 5**, under experimental conditions of low nutrient availability nematode populations tended to decrease. This reduction may have been due to lack of investment in reproduction and the observation that the population was basically maintained by small-medium juveniles. Conversely, in the 10-fold nitrogen and phosphorus conditions, nematodes exhibited exponential growth, although no significant increase in reproductive forms was observed. In the experimental plates, an increase in dissolved inorganic nitrogen compounds (mainly ammonia) was observed, thereby suggesting that nematode activity involves remineralising organic compounds from the detritus or through bacterial ingestion.

Bursilla monhystera and *Plectus aquatilis* were found to be efficient colonisers under enriched conditions. However, the response of these species differed since the growth of *B. monhystera* was much faster in all conditions while *P. aquatilis* increased only in the most enriched treatments. This differential response by these two opportunistic nematode species also indicated that small differences in behavioural attributes may shape the successional pattern of recolonisation after a disturbance.

The findings reported in this thesis show that meiofaunal communities show numerous adaptive traits to hydrological disturbances and nutrient enhancement.

CONCLUSIONS

CONCLUSIONS

Chapter 1

1. The density and biomass of meiofauna in the Fuirosos stream were in the range described in the literature, with maximum values occurring between late autumn and spring, when an optimal balance between discharge, temperature and organic matter availability was reached.
2. Temporary meiofauna had a higher biomass throughout the study period and higher densities during flow disruption.
3. Organisms with worm-like morphology like oligochaetes, chironomids and nematodes, were the foremost contributors to the total meiofauna in all three reaches. Microcrustaceans with active locomotory appendages were also abundant in the streambed during periods of detritus retention and processing.
4. Small differences in the watershed directly affected discharge and habitat stability, which in turn determined the structure of meiofaunal communities in the Fuirosos stream. Thus, the most upstream reach (S1) showed higher density, biomass and resilient response, thus being a potential recolonisation source for downstream reaches after floods.

Chapter 2

5. Biological succession on decaying leaf litter was strongly correlated with discharge and water temperature in the Fuirosos stream.
6. A direct transference between temporary meiofauna and macroinvertebrates was observed as well as a general transference from the bacterial and fungal compartments to the faunal ones during the experiment.
7. Fungal biomass accounted for ~32 to ~93% of the total organismal (non-leaf) C in the litter bags during the experiment, bacterial biomass ~0.2% to ~6%, and faunal biomass ~0.03% to ~8.4% (meiofauna) and ~1.7% to ~58% (macroinvertebrates)

Chapter 3

8. Meiobenthic communities in Fuirosos, a Mediterranean forested stream, reacted to moderate nutrient inputs.
9. The primary constituents of the temporary (Chironomidae and Oligochaeta) and permanent (microcrustaceans and nematodes) meiofauna were also affected by nutrient addition.
10. The secondary production of cyclopoids (dominated by *Eucyclops*) and ostracods (mainly Candoninae and Cypridopsinae) increased after nutrient addition in the impacted reach.
11. The P/B ratio for ostracods in the control reach diminished after the nutrient addition, whereas it increased in the impacted reach.
12. Differences in the timing and frequency of hydrologic disturbances during the two-year experiment were also determinants of meiofaunal community structure and microcrustacean secondary production in the control and impacted reaches. Thus, interannual variability can either exacerbate or diminish the effects of nutrients on the meiofaunal community.

Chapter 4

13. The nematode range density in cyanobacterial biofilms was similar to those reported in the literature. However, in the free-floating mats, densities and biomass exceeded those reported in other lotic systems.
14. Cyanobacterial mats showed low diversity but a high density and biomass of opportunistic species like *Cromadorita leuckarti*, *Diplogaster rivalis* and *Neotobrilus diversipapillatus.*
15. The hysteresis-like pattern of the nematode community in the attached and the free-floating biofilm confirms coupling between the cycle of the nematode community and cyanobacterial growth.
16. Cyanobacteria and nematodes could benefit mutually from each other since nematode grazing, and faeces and mucus excreta may enhance bacterial and

cyanobacterial growth. Nematodes could also influence biofilm permeability through bioturbation and thus favour their dispersal through the detachment.

Chapter 5

17. Under enriched conditions, the populations of *Bursilla monhystera* (cp-1) tended to increase over time, although no significant increase in reproductive forms was observed. In contrast, *Plectus aquatilis* (cp-2) increased only in response to high nutrient concentrations, although the efficiency of this species was much lower that of *Bursilla* individuals.
18. The significant correlation between bacterial and *Bursilla* abundances supported a clear Bottom-up control on the nematode population.
19. Enhanced bacteria and *Bursilla* density and a major presence of large nematodes were related to increased forms of nitrate and ammonia forms in water. This finding could be partly due to nitrogen release from the detritus bulk or excreted nitrogen as a result of the increased nematode activity.
20. Only treatments with phosphorus addition (mainly the 10-fold treatment) has the capacity to maintain a constant increase in nematode population.

INFORME DEL DIRECTOR DE LA TESI DOCTORAL

Isabel Muñoz Gracia, professora titular del Departament d'Ecologia (Universitat de Barcelona), i directora de la Tesi Doctoral elaborada per la Sra. Ainhoa Gaudes Saez, amb el títol "Freshwater meiofauna in Mediterranean lotic systems: community structure, adaptations and contribution to functional processes".

INFORMA

Que el treball de recerca fet per la Sra. Ainhoa Gaudes Saez, com a part de la seva formació com a estudiant de doctorat i inclòs en aquesta tesi doctoral, ha donat lloc a quatre publicacions i un manuscrit preparat per a la seva revisió. A continuació es detallen els articles i la informació corresponent dels índexs d'impacte (segons el SCI de la ISI web of Knowledge).

1) Gaudes, A., Artigas, J., and I. Muñoz (2010). *Species traits and resilience of meiofauna to floods and drought in a Mediterranean stream*. Marine and Freshwater Research, 61: 1336-1347.

L'índex d'impacte de la revista era de 1.56 a l'any 2009. Aquesta revista estava situada en el segon quartil de la categoria "Limnology" (6/18), i de la categoria "Marine and Freshwater Biology" (32/88).

2) Gaudes, A., Artigas, J., Romani, A.M., Sabater, S., and I. Muñoz (2009). *Contribution of microbial and invertebrate communities to leaf litter colonization in a Mediterranean stream.* Journal of the North American Benthological Society, 28 (1): 34-43

L'índex d'impacte de la revista és de 2.13 a l'any 2009. Aquesta revista està situada en el primer quartil de la categoria "Marine and Freshwater Biology" (16/88), i en el segon quartil a la categoria "Ecology"(52/127).

3) Gaudes A., Ocaña, J., and I. Muñoz *The meiofaunal response to nutrient addition in a Mediterranean stream*, enviat a "Limnology and Oceanography".

L'índex d'impacte d'aquesta revista a l'any 2009 era de 3.54. Està situada al primer quartil de la categoria "Limnology" (1/18).

4) Gaudes, A., Sabater, S., Vilalta, E., and I. Muñoz (2006). *The nematode community in river cyanobacterial biofilms*. Nematology 4 (6): 909-919

L'índex d'impacte de la revista és de 0.72 a l'any 2006. Aquesta revista està situada en el tercer quartil a la categoria "Zoology"(81/114).

5) Gaudes, A., Muñoz, I. and T. Moens. *Bottom-up effects on freshwater bacterivorous nematode populations: a microcosm approach* (en preparació per enviar).

A més, CERTIFICA

- Que la Sra. Ainhoa Gaudes Saez ha participat activament en el desenvolupament del treball de recerca associat a cadascun dels articles, així com en la seva elaboració. En tots els treballs ha participat en el planejament dels objectius i hipòtesi a testar, i en el disseny i desenvolupament del treball de camp i experimental. Ha realitzat tots els mostrejos de camp, recollint les mostres per a l'estudi de la composició biològica, amb la separació, identificació i processat de les mostres. Ha realitzat el tractament estadístic de les dades que apareixen en els diferents articles. Ha tingut el suport d'experts en alguns aspectes en que el grup no era especialista com en la comunitat microbiana i algun tractament estadístic, que apareixen com a coautors dels articles.

- Que els treballs experimentals s'han realitzat a les instal·lacions del Departament d'Ecologia de la Facultat de Biologia i en el laboratori de Biologia Marina de la Universitat de Gent (Bèlgica), on l'estudiant va fer una estada per a desenvolupar un experiment de laboratori. En aquests experiments també ha participat en totes les tasques relacionades amb el disseny, desenvolupament, anàlisi de les mostres, càlcul de resultats i anàlisi de les dades.

- Que ha participat en la redacció de tots els articles i en el procés de revisió dels mateixos.

Finalment, certifico que cap dels coautors dels articles abans esmentats i que formen part de la Tesi Doctoral de la Sra. Ainhoa Gaudes ha utilitzat o bé té previst utilitzar aquests treballs per a l'elaboració d'una altra Tesi Doctoral.

Atentament,

Barcelona, 15 d'abril de 2011

Isabel Muñoz Gracia

RESUM
EN
CATALÀ

Meiofauna

Una mica d'història sobre la definició de meiofauna

El prefix meio- (que en Grec vol dir més petit) va ser emprar per primera vegada per Mare (1942) mentre estudiava la fauna associada a fangs estuàrics a Plymouth. Per tant, la paraula meiofauna es va fer servir per descriure aquells metazous compresos entre 100-1000 µm, cobrint així la mida intermèdia entre la micro i la macrofauna (tal i com es mostra a la Fig. I.1). Els límits superiors i inferiors de la meiofauna no han estat del tot consensuats. Al principi van ser descrits des d'una òptica funcional com "aquells animals capaços de passar per una xarxa de 500- µm però que quedin retinguts en una xarxa de 42 µm" (Fenchel, 1978; Higgins i Thiel, 1988). Giere (1993) va canviar aquests límits a 1000 i 63 µm. Tant els grups com els hàbitats han estat modificats d'un autor a l'altre. Així, per exemple, alguns autors inclouen els protozous grans dintre de la seva definició de meiofauna (ex. Schmid-Araya, 1994). També hi ha hagut una confusió en quant a l'hàbitat, ja que en nombroses ocasions només s'ha tingut en compte la meiofauna de l'hiporreic dintre dels ecosistemes d'aigua dolça (ex. Giere, 1993). Al llarg d'aquesta tesi, hem emprat la definició proposada per Fenchel aplicat als metazous epibentònics.

Meiofauna temporal i permanent

Meiofauna inclou aquells metazous que romanen sempre dintre del rang de mida de la meiofauna durant tot el seu cicle vital (meiofauna permanent), i els estadis més petits de la macrofauna mentre són de la mida de la meiofauna (meiofauna temporal). En els ambients marins, la majoria de Phyla d' invertebrats hi tenen representants temporals o permanents (Taula I.1).

Per altra banda, els sistemes d'aigua dolça, posseeixen menys Phyla de la meiofauna permanent , però molts grups de macroinvertebrats contribueixen a la meiofauna temporal (Taula I.2).

Meiofauna vs. macroinvertebrats

Tot i que la meiofauna ha estat tot sovint oblidada en molts estudis de sistemes lòtics, la interacció entre ambdues comunitats pot ser notòria. Els mostrejos bentònics han sigut sovint enfocats al mostreig de macroinvertebrats ja sigui netejant pedres, fent servir tècniques de "kick sampling" (emprant xarxes més grans de 250 µm), etc. A la majoria d'aquests estudis, els sediments tous de la llera del riu s'ha mostrejat de forma deficient, donant la impressió que només l'habitava algunes espècies d'oligoquets i quironòmids. Enlloc d'això, en aquests hàbitats, un món minúscul roman encara desconegut. Al llarg d'aquesta tesi, s'ha mostrejat diferents hàbitats (flocs de cianobacteris, zones sorrenques i agregats de fullaraca) en els quals la meiofauna ha dominat en termes d'abundància (Fig. I.2).

En ambients marins, alguns predadors meiobentònics (ex. microturbel·laris) han demostrat ser molt voraços, alimentant-se de juvenils de macrofauna, influint en el seu reclutament al bentos i la seva posterior densitat poblacional (Watzin, 1983). Tot i això, algunes espècies han desenvolupat larves planctòniques que només s'estableixen al bentos un cop la seva mida és més gran que la del meiobentos. En aigües dolces, especialment en ambients lòtics, aquesta estratègia no és possible i la interacció entre meiofauna i macroinvertebrats és constant sobretot durant les fases més primerenques d'aquests darrers. Fins i tot els organismes més reòfils (ex. gasteròpodes) poden passar part dels seus primers estadis en zones del riu on el corrent és menor i es formen petits tolls. En aquestes zones de lents, la meiofauna hi sol tenir elevades poblacions i per tant poden competir o fins i tot depredar aquests macroinvertebrats.

Els organismes de la meiofauna són també una baula important a la cadena alimentaria, unint bacteris i metazous més grans com són els macroinvertebrats i vertebrats, a més de regular la descomposició de la matèria a través de la seva ingesta microbiana (veure Schmid-Araya i Schmid, 2000 i referències incloses). Donades aquestes interaccions tròfiques, alguns autors (Schmid i Schmid-Araya, 2002, Woodward et al., 2005) han remarcat la importància d'incloure aquests organismes dins els estudis de xarxes tròfiques ja que n'incrementen el nombre d'interaccions i el grau de complexitat (Fig. I.3.).

Recerca en meiofauna d'aigua dolça

Des del començament, els estudis fets en aigües dolces i marines s'han desenvolupat de manera totalment independent, emprant diferents estratègies de mostreig i nomenclatures. La recerca en comunitats meiobentòniques d'aigües dolces va començar a la primera meitat del segle XX amb diversos treballs en rius i llacs (per exemple els iniciats per Sassuchin, Wiszniewski, Wilson i Pennak) però van caure en l'oblit durant molts anys. Tot i que recentment (els darrers 20 anys) els esforços s'han intensificat novament, els conceptes bàsics sobre la biologia i l'ecologia de la meiofauna s'ha beneficiat de la recerca en ambients marins. És a dir, que al llarg dels anys foscos per la recerca de la meiofauna d'aigua dolça, els treballs en ambients marins va anar avançant donant com a fruit nombrosos estudis que han permès una millor comprensió dels patrons i els processos dins de les comunitats meiobèntiques (per exemple els treballs de Coull i Bell, 1979; Higgins i Thie,l 1988; Giere, 1993). Tot i que la transferència directa de coneixement entre els dos ambients pot ser difícil donada les diferencies d'hàbitat (ex. la naturalesa unitària dels mars i la falta de marees a les aigües continentals), hauria de ser possible a nivell de processos generals (tant al laboratori com al camp), així com una font d'inspiració per a la recerca futura.

Durant molts anys, els estudis sobre la meiofauna han estat relegats a ambients hiporreics i d'aigües subterrànies. Per aquest motiu, molts limnòlegs estan més familiaritzats amb termes com psammic, hiporreic i stygobiòtic que amb meiofauna i meiobentònic. Al llarg dels noranta, alguns autors van començar a moure aquest concepte cap als sediments. Alguns treballs efectuats a nord-Amèrica (portats a terme per Palmer, Borchardt, Bott, Morin, Boulton, entre d'altres) i Europa (ex. Schmid-Araya, Robertson, Traunspurger, Bretschko) i la inclusió d'un capítol sencer dedicat a la meiofauna en el llibre "Methods in stream ecology" (editat per Hauer i Lamberti) van incentivar de nou, els estudis en meiofauna d'aigua dolça. Tot i això, només va ser un petit impuls per al següent pas: la publicació d'un volum especial sobre meiofauna en ambients lòtics a la revista Freshwater Biology al 2000; i un manual "Freshwater

Meiofauna: Biology and Ecology", editat per Simon Rundle, Anne Robertson i Jenny Schmid-Araya al 2002. En Richard Warwick, un reconegut científic especialitzat en meiofauna marina va escriure al pròleg del llibre: "(…) Això (aquest llibre) representa la nova era de la meiobentologia d'aigua dolça com a disciplina (…)". Però, ha esdevingut així?

Darrers 10 anys de recerca en meiofauna d'aigua dolça

Des de la publicació d'aquest especial en meiofauna lòtica l'any 2000, prop de 120 articles han estudiat la meiofauna d'aigua dolça. Aquests articles s'han sel·leccionat entre els que s'han dut a terme en aigües continentals (excloent interaccions amb aigües salobres i estuaris), on la meiofauna, per si mateixa, era l'objectiu d'estudi (excloent així inventaris en estudis sobre dietes de peixos). D'aquests estudis, 53 articles van ser realitzats en habitats lòtics, 32 en ambients lentics i un es va fer comparant els dos sistemes. Els altres eren: experiments realitzats al laboratori (15 articles), estudis taxonòmics (7 articles), aproximacions teòriques (7 articles) i estudis sobre sistemes hiporreics o coves (9 articles). De tots aquests treballs, el 85% van ser publicats en revistes amb factor d'impacte i només el 15% en revistes sense.

Com a mesura de l'impacte d'aquest volum especial de la meiofauna lòtica (Taula I.3), l'anàlisi del nombre de vegades que s'ha citat cada article (verificat per darrera vegada el 14/3/2011), indica que el grup de microcrustacis sembla ser el més citat així com l' article relacionat amb les interaccions tròfiques de la meiofauna. En segon terme, trobem els articles relacionats amb el rol de la meiofauna dins els processos funcionals dels ecosistemes i els patrons a petita escala que poden afectar les comunitats. Aquests dos temes estan estretament relacionats amb el desenvolupament d'aquesta tesi i probablement el seu coneixement actual segueix sent encara deficient. L'últim article d'aquest volum especial (Robertson et al., 2000) va resumir el fins llavors estat actual del coneixement sobre la meiofauna lòtica incidint el els punts febles que calia reforçar en futurs estudis. Una de les conclusions finals feia referència a la manca d'experiments manipulatius i d'estudis dirigits a esbrinar la influència dels processos a gran escala sobre les comunitats de la meiofauna.

Processos funcionals en ecosistemes lòtics

Els ecosistemes lòtics són en gran mesura controlats per la geomorfologia, la hidrologia, la temperatura de l'aigua i la influència del bosc de ribera (ex. Margalef, 1960; Minshall et al., 1983; Elwood et al., 1983 entre d'altres). La hidrologia i la influència del bosc de ribera han sigut àmpliament reconeguts com a factors claus dintre de la organització d'associacions biòtiques dintre de les aigües corrents (Cummins et al., 1984), així com de les més afectades per les activitats humanes. Des dels anys 50 del segle passat, una visió holística de l'ecosistema fluvial (sobretot influenciat pels treballs seminals d' Odum i Margalef) van impulsar una sèrie d'estudis integratius que intentaren generalitzar els processos que tenien lloc dins els rius. Des del començament, l'ecologia funcional va començar a reemplaçar els estudis taxonòmics per criteris funcionals que descriguessin els processos. Per exemple, entre d'altres científics brillants en aquest camp, Cummins (1974) es va centrar en la conversió eficient de la matèria orgànica a CO_2, relegant a un segon terme el rol dels productors primaris que van tenir la seva època daurada predominant en la majoria d'estudis fluvials portats a terme la primera meitat del segle XX. Passat aquest primer entusiasme, els ecòlegs fluvials van acordar que ambdues fonts de carboni, la provinent de l'ecosistema terrestre i la provinent dels productors primaris del riu, eren prou importants i que sovint els processos es complementaven l'un a l'altre (Minshall et al., 1985). A més, l'any 1996 Merritt i Cummins van desenvolupar una classificació funcional dels organismes basada en els mecanismes que empraven a l'hora d'adquirir el menjar (Functional Feeding Groups, FFG). Aquests grups funcionals, tenien l'avantatge de poder ser igualment representatius en qualsevol sistema, i aplicables en teories integratives, com el River Continuum Concept (Vannote et al., 1980).

A la literatura, el processos fluvials descrits inclouen la descomposició, la dinàmica de nutrients, el metabolisme fluvial (relacionant biomassa d'autòtrofs amb la respiració per part dels heteròtrofs), producció secundaria, etc...tots ells relacionats i probablement part d'un sol procés global. La inclusió de la meiofauna en aquests processos ha sigut minsa. Per exemple, la comunitat de meiofauna no ha estat específicament classificada en grups funcionals com els FFG, probablement per que en alguns casos (sobretot en la meiofauna temporal) algunes estructures corporals no estan totalment desenvolupades i adquirir una resolució taxonòmica a nivell d'espècie és gairebé impossible. Tot i això, alguns grups com els nematodes si que han desenvolupat la seva classificació pròpia basada en la morfologia bucal (Moens et al., 2006). Un altre grup considerat "complicat", com els protozous, han simplificat la seva dificultat taxonòmica creant grups basats en les bades ciliars de la part oral (ex. Foissner i Berger 1996; Franco et al, 1999).

Recentment, alguns estudis han inclòs la meiofauna dins dels càlculs de producció secundària demostrant que tot i que la seva biomassa pot ser relativament discreta, els seus cicles vitals curts i la seva capacitat de reproducció (d'entre els més alts dels metazous) fan que calgui tenir-los en consideració (ex. Stead et al., 2005, Lemke i Benke 2009).

Influència del bosc de ribera i entrades de matèria organica per la comunitat de meiofauna

Després de l'article seminal de Pomeroy sobre el "microbial loop" marí, alguns limnòlegs com en Cummins (1974) van reconèixer el paper crucial de bacteris i fongs com a descomponedors i com a recurs alimentari per als insectes. Amb tot, ell només va reconèixer el paper de les diatomees i els protozous com a únics posseïdors de suficient biomassa i activitat metabòlica en comparació a bacteris i fongs, deixant de banda la meiofauna. L'any 1994, Meyer va adaptar el reconegut "microbial loop" marí als sistemes lòtics (Fig. I.4) incloent totes les possibles entrades de carboni orgànic per als consumidors, incloent la microfauna, meiofauna i macroinvertebrats. Tot i això, totes les possibles interaccions dins els ambients lòtics no han sigut del tot descrites. Per exemple, tot i que la bioturbació i la coprofàgia han estat àmpliament estudiades en ambients marins, s'han ignorat en molts estudis de sistemes lòtics (Hakenkamp i Morin, 2000).

La distribució de la meiofauna en els sistemes fluvials esta estretament relacionada amb la distribució de la matèria orgànica (Swan i Palmer, 2000) i per tant, aquest vincle pot tenir una importància cabdal. La meiofauna pot alimentar-se directament de la matèria orgànica (ex. alguns membres de la meiofauna temporal amb els apèndixs bucals prou esclerotitzats), però generalment s'alimenta del biofilm associat a la matèria orgànica en descomposició. Els membres de la meiofauna són en gran mesura bacterívors (Meyer, 1994; Borchardt i Bott, 1995), i poden exercir una pressió significant en els bacteris i fongs que colonitzen la matèria orgànica (Perlmutter i Meyer, 1991). Però les acumulacions de matèria orgànica on la meiofauna s'alimenta i viu, també poden funcionar com a refugis durant les pertorbacions hidrològiques (ex. mantenint la humitat durant les sequeres o creant petites preses durant les avingudes), i també poden funcionar com a mecanisme transportador per recolonitzar trams riu avall (Robertson i Milner, 2001).

Els rius mediterranis

En tots els continents, algunes regions costaneres en altituds mitjanes, la majoria estenent-se entre 30° i 40° al nord i al sud de l'equador, són afectades per circulacions atmosfèriques simètriques que provoquen un clima caracteritzat per hiverns suaus i humits i estius secs i calorosos (Aschmann, 1973). La influència moderadora dels oceans manté les temperatures hivernals suaus, amb una temperatura mensual mínima d'entre 8° i 12°C, i gelades poc freqüents excepte a altes elevacions o terra endins; la màxima mensual a l'estiu sol variar entre 18° i 30°C (Dell et al., 1989).

El règim de cabals d'una riera mediterrània típica, sol seguir el patró de les precipitacions ocorregudes i per tant, està marcat per un important component estacional i de variabilitat anual (Davies et al., 1994; Resh et al., 1990, Sabater et al., 1995) (ex. Capítol 2, Fig. 2.1). En les rieres mediterrànies, l'època d'avingudes sol començar a la tardor i es manté durant l'hivern fins a la primavera següent. Els sistemes mediterranis mostren una gran variabilitat hidrològica inter- i intraanual (Acuña et al., 2005). Aquesta variabilitat es reflecteix en la freqüència d'avingudes i sequeres. Les rieres mediterrànies estan moldejades, tant a nivell físic, químic com biològic, per aquests esdeveniments (Gasith i Resh, 1999; Lake, 2003). Anys secs i humits es van alternant doncs, i la llera del riu es pot assecar total o parcialment depenent dels anys (Sabater et al., 2001; Butturini et al., 2003). Aquests habitats, per tant, tenen la capacitat d'expandir-se i contraure's (Stanley et al., 1997).

Els efectes de les pertorbacions hidrològiques han estat constantment estudiades a les comunitats de macroinvertebrats (ex. Gasith i Resh, 1999; Bonada et al., 2007). En quant a les sequeres, els efectes directes inclouen a part de la pèrdua d'aigua, la pèrdua de l'hàbitat per organismes aquàtics i la pèrdua de connectivitat del curs fluvial. Els efectes indirectes inclouen, el deteriorament de la qualitat de l'aigua, l'alteració dels recursos alimentaris i els canvis en la robustesa i estructura d'interaccions interespecífiques (Lake, 2003). Quan el riu es comença a assecar, l'oxigen i els nutrients són consumits ràpidament i per tant, s'enceta una alta competència entre els organismes (Acuña et al., 2005; Beche i Resh, 2007). Pel contrari, les avingudes augmenten l'entrada d'oxigen dissolt i lixiviats de la conca, els quals augmenten el contingut de nutrients al riu (Bernal et al., 2003; Vázquez et al., 2007). Les avingudes també canvien dràsticament altres paràmetres relacionats amb el cabal (ex. velocitat de l'aigua , concentració de nutrients, conductivitat), afectant també la distribució del substrat bentònic, i la disponibilitat de la matèria orgànica dissolta (MOD) i particulada (MOP) (Bernal et al., 2002; Acuña et al., 2004). Els increments en la velocitat de l'aigua afecta severament l'estructura de les comunitats microbianes del bentos (Sabater et al., 2006), i indirectament afecta moltes altres condicions d'hàbitat necessàries pel desenvolupament de la comunitat microbiana (Stevenson, 1996; Artigas et al., 2009). Aquest biofilms microbians, són en gran part una font d'alimentació per la meiofauna. Per tant, els canvis dins aquest compartiment microbià, tindran efectes sobre la comunitat de la meiofauna.

Llocs de mostreig

Fuirosos

La riera de Fuirosos és una riera típicament mediterrània de tercer ordre que transcorre dins d'una conca granítica d'uns 18 Km^2 , altament forestada i situada a

Catalunya, a 150 km de Barcelona (Sala i Franch, 1980). Gran part del curs fluvial de la riera esta inclosa dintre d'un parc natural, el Parc del Montnegre-Corredor, en el que els impactes antròpics, com l'agricultura són molt baixos (sent petites plantacions forestals i maneig de petites parcel·les per tal d'augmentar la biodiversitat; Fig. I.5) i no hi ha cap activitat industrial.

Les característiques hídriques de la riera de Fuirosos estan marcadament determinades pel clima mediterrani. Normalment, el cabal oscil·la entre els 5 i els 20 L s^{-1} però pot augmentar fins a 100 cops durant les avingudes de la tardor i la primavera, mentre que a l'estiu es pot reduir fins a gairebé zero (Figura I.6). Les entrades principals de matèria orgànica provenen del bosc de ribera [dominat per *Platanus acerifolia* (Aiton-Willd.), *Populus nigra* (L.) and *Alnus glutinosa* (Gaertn.)] i esdevenen entre l'estiu (a causa de l'abscissió precoç deguda a l'estrès hídric dels arbres) i la tardor. La incidència lumínica és generalment baixa excepte a l'hivern i als inicis de la primavera quan els arbres encara no tenen fulla (Acuña et al., 2004).

Els primers estudis fets a Fuirosos daten de principis dels 80 i van consistir en descripcions geomorfològiques i de processos de lixiviació de la conca (Sala i Franch, 1980; Sala, 1983). La dinàmica freàtica i la seva relació amb el bosc de ribera va ser un dels primers objectius estudiats a Fuirosos com a part d'un projecte Europeu anomenat "Nitrogen Control by Landscape Structures" (NICOLAS) a finals dels 90 (Sabater et al., 2003). La rellevància de la vegetació en el consum de nitrats del freàtic, la baixa desnitrificació trobada a l'humus durant la sequera i la intrusió d'aigua de la riera al compartiment del bosc de ribera durant la recuperació de la sequera van ser els processos descrits més rellevants del projecte (ex. Sabater et al., 2001; Butturini et al., 2002; Bernal et al., 2002). Més endavant, els vincles entre la hidrologia i la biogeoquímica han sigut intensament estudiats. La calibració del model INCA (Integrated Nitrogen in CAtchments) en sistemes mediterranis va produir també una gran nombre de dades (Bernal et al., 2004). Recentment, els esforços s'han dirigit en estudiar els efectes de l'estacionalitat i la variabilitat interanual en el Carboni i Nitrogen Orgànic Dissolt (DOC i DON) així com la dinàmica de nutrients (Vázquez et al., 2007; von Schiller et al., 2008).

L'ecologia funcional de les comunitats ha estat estudiada des de diverses perspectives. El rol del biofilm i la seva interacció amb el compartiment animal, incloent protozous (Domènech et al., 2006; aquesta tesi), meiofauna (aquesta tesi) i macroinvertebrats (Acuña et al., 2005), ha estat explorat mitjançant aproximacions tant descriptives com manipulatives. Els vincles entre les variacions ambientals i el metabolisme de la riera, la dinàmica de la matèria orgànica, la dinàmica d'algues, meiofauna, macroinvertebrats i peixos, estequiometria, relacions tròfiques, traces isotòpiques, etc han estat àmpliament estudiats (ex. Acuña et al., 2005; Artigas et al., 2009; Mas-Martí et al., 2010). S'ha portat a terme diversos experiments *in situ* a Fuirosos per tal de testar algun d'aquests factors esmentats. Entre ells, les adicions de nutrients (Romaní et al., 2004; Sabater et al., 2011), colonització de sorres, pedres i fullaraca (Artigas, 2008; aquesta tesi), han aportat coneixement sobre el funcionament de la riera de Fuirosos.

La majoria d'experiments i mostrejos d'aquesta tesi doctoral s'han portat a terme en un tram de riu anomenat S3*, de 50 m de llargada (latitud 41° 42'N, longitud 2° 34'), uns 3-4 m d'amplada i 10-50 cm de profunditat de l'aigua (Figura 1.7). Els altres dos trams mostrejats (**Capítol 1**): el tram S2, 4000 m riu amunt de l'S3, i el tram S1 que es troba en un tributari de segon ordre més amunt dels altres dos trams. Les àrees de conca drenades pel riu a cada tram eren 1, 4.2 and 12.3 km^2 (S1, S2 and S3, respectivament; Institut Cartogràfic de Catalunya, http://www.icc.es, verificat 14/3/2011).

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*S1, S2 i S3 correspon als trams mostrejats al **Capítol 1**. Els experiments del **Capítol 2** es van efectuar al tram S3. En el **Capítol 3**, es van mostrejar els trams "Impacted" i S3 (en el text referit tan sols com a "Control"). La nomenclatura correspon a un disseny BACI (Abans-Després-Contol-Impactat, en anglès Before-After-Control-Impacted) inicial amb el que es va intentar minimitzar l'heterogeneïtat espaial emprant 3 trams control per comparar l'impacte de l'addició de nutrients (tram impactat). Tot i que els trams eren molt similars, la comunitat de la meiofauna era molt diferent (tal i com es veu al **Capítol 1**) com per utilitzar-los com a controls.

Tots tres trams tenen una morfologia similar, tot i que amb alguna petita diferència a nivell del llit del riu. La morfologia del llit de la riera alterna sovint zones de ràpids i zones lentes. El substrat groller (roques, pedres i còdols) se sol situar en zones de corrent mentre que el substrat fi (sorres i graves entre fines i mitjanes) tendeix a acumular-se a les zones lentes. La fullaraca i les branques també solen quedar-se acumulades al llit del riu, en bancs sorrencs o zones on l'aigua es mou lentament.

El riu Llobregat

El curs del riu Llobregat es caracteritza per una geologia calcària (Fig. I.6) i un règim hídric típicament mediterrani que causa freqüents avingudes a la primavera i tardor i un cabal mínim a l'estiu. El canal principal del riu pateix nombroses interrupcions i modificacions ja sigui per pantans, azuts, derivacions del canal, etc, que augmenta la inestabilitat del règim hídric sobretot en períodes de poc cabal. A més, el riu rep nombrosos efluents provinents de plantes depuradores amb aigües residuals urbanes o industrials en els seus trams mitjans i sobretot finals, empitjorant severament la qualitat de la seva aigua (veure López-Doval et al., 2010 i referències incloses).

El mostreig dut a terme al **Capítol 4** es va realitzar a Pont de Vilomara (U.T.M. 406502, 4604544), un punt del tram mitjà del riu, de gener a maig del 2002. Les propietats físico-químiques de l'aigua durant l'època de mostreig ja han estat àmpliament descrites a treballs com Sabater et al. (2003). La taula I.4. resum algunes característiques més generals per comparar aquest riu amb la riera de Fuirosos.

Objectius i desenvolupament de la tesi

L'objectiu principal d'aquesta tesi doctoral és caracteritzar la comunitat de la meiofauna en sistemes mediterranis i integrar-la com a baula dins dels processos funcionals d'ambients lòtics. Tot i que el coneixement sobre la meiofauna d'aigua dolça és encara escàs, aquesta tesi no ha pretès tenir un caire descriptiu de la comunitat des d'un punt de vista taxonòmic sinó emprar classificacions funcionals que permetin integrar la meiofauna en processos i contextos més amplis.

La tesi esta articulada en 5 capítols i en cadascun s'ha mostrejat diferents substrats en funció de les interaccions entre organismes i les característiques dels processos a petita escala. En aquests experiments, s'han adreçat diferents qüestions depenent de l'hàbitat o de les característiques del lloc. Essencialment, aquesta tesi s'ha centrat en els processos lòtics més influents: la hidrologia i les entrades de nutrients (ja siguin orgànics com inorgànics).

- En el **Capítol 1**, la comunitat de la meiofauna s'estudia durant un període de dos anys durant els quals s'observa que la seva estructura reflexa una gran variabilitat intra- i interanual coincident amb els períodes alternants de pertorbacions i estabilitat hidrològica. Donat que el cabal és un factor abiòtic ben reconegut com a determinant del patró de distribució dels invertebrats en els rius i el seu paper en la distribució de la meiofauna també ha estat extensament estudiat (veure Swan i Palmer, 2000 i referències incloses), l'estudi eminentment s'adreça a mesurar les respostes adaptatives dels organismes a avingudes i sequeres dins un context mediterrani. Aquestes adaptacions van ser mesurades en termes de trets avantatjosos (tant estructurals com de comportament) que poguessin conferir resistència o resiliència als organismes.

- L'entrada de matèria orgànica i la seva disponibilitat per als consumidors és un dels processos més importants en rius de capçalera. En el **Capítol 2**, es va analitzar el patró de colonització de fullaraca experimentalment dipositada al riu dintre de xarxes de plàstic, incloent tots els organismes involucrats en el procés per tal de poder seguir la traçabilitat del carboni.

- L'eutrofització dels sistemes lòtics ha sigut des de sempre, la part negativa de les activitats humanes. Per aquest motiu, es va parar especial atenció als efectes de la disponibilitat de nutrients per a la meiofauna ja que la seva petita mida i el seu cicle de vida curt ens poden donar informació interessant sobre la seva adaptabilitat a aquests tipus de pertorbacions. En sistemes mediterranis l'escassetat d'aigua pot arribar a potenciar els efectes negatius de l'eutrofització i per tant aquesta interacció també es va analitzar. En el **Capítol 3** s'estudia la resposta de la comunitat de meiofauna a una addició experimental de nutrients en una riera de condicions quasi prístines enfocant-nos en les diferències en la composició de la comunitat i en la producció secundària dels microcrustacis. En aquest sistema, les pertorbacions hidrològiques es produïren de forma natural i inevitable com a "soroll de fons". Com a contrapunt, en el **Capítol 4** es va estudiar la comunitat de nematodes en un sistema inevitablement eutròfic i allà es va estudiar l'estabilitat del substrat (flocs de cianobacteris adherits al fons vs. flotants). En el **Capítol 5** es va analitzar, en un experiment de laboratori, la ràpida resposta de nematodes a l'eutrofització tenint en compte diferents estratègies vitals i adaptacions a l'elevada disponibilitat de nutrients.

Al llarg d'aquesta tesi doctoral,

1. s'han estudiat dos nivells d'organització diferents:

   Escala de comunitat: meiofauna (**Capítols 1**, **2** i **3**) i nematodes (**Capítol 4**).

   Escala de població: ciclòpids i ostracodes (**Capítol 3**, producció secundària), i nematodes (**Capítol 5**).
2. i també s'ha realitzat diferents aproximacions funcionals:

   Resiliència(**Capítol 1**)

   Trets específics (**Capítols 1** i **5**)

   Producció secundària (**Capítols 3** i **5**)

   Colonització (**Capítols 2, 4** i **5**)

   Traça del carboni (**Capítol 2**)

   Taxes de consum-excreció (**Capítol 5**)

Capítol 1

Trets específics i resiliencia de la meiofauna en front d’avingudes i sequera en una riera mediterrània

Ainhoa Gaudes, Joan Artigas, and Isabel Muñoz

Marine and Freshwater Research, 2010, 61, 1336–1347

En els sistemes mediterranis, les avingudes i les sequeres són processos bàsicament estacionals i predictibles, arribant a ocórrer entre dos i tres cops l’any. Sota aquestes condicions, el multivoltinisme i els cicles vitals curts haurien de ser afavorits, especialment per organismes amb una capacitat migratòria limitada. La comunitat de la meiofauna és doncs un clar candidat a posseir aquestes adaptacions en front a les pertorbacions hidrològiques. Amb tot, la meiofauna ha sigut ignorada en la majoria d’estudis lòtics. L’estudi va examinar la variabilitat temporal de la densitat i biomassa de la meiofauna durant un període de 2 anys en tres trams d’una riera mediterrània d’ordre baix. Es va investigar la relació entre els trets biològics dels organismes i les característiques hídriques i ambientals. La resiliencia de la meiofauna en front de les avingudes es va quantificar mitjançant regressions. Les petites diferències en el drenatge de la conca van comportar diferents respostes a la comunitat. L’abundància i la resiliència van ser majors en el tram d’aigües amunt en comparació amb els dos d’aigües avall. Per fer front a les pertorbacions hidrològiques, trets com ser petit, tenir una estructura del cos vermiforme i tenir estructures locomotores actives confereixen una major resistència i resiliència. Els trams d’ordre baix són sovint un refugi per a la meiofauna funcionalment importants que poden eventualment, recolonitzar trams d’aigües avall.

Capítol 2

Contribució de les comunitats microbianes i d'invertebrats a la colonització de fullaraca en una riera mediterrània

Ainhoa Gaudes, Joan Artigas, Anna Mª Romaní, Sergi Sabater and Isabel Muñoz

Journal of the North American Benthological Society, 2009, 28(1):34–43

Els aports de fullaraca i la seva retenció al riu juga un paper molt important en el funcionament dels ecosistemes dels rius forestats. Es va analitzar la colonització de fulles per part dels microbis (bacteris, fongs i protozous) i la fauna de Fuirosos, una riera mediterrània intermitent. Paquets de fullaraca de pollancre (*Populus nigra*) i plàtan (*Platanus acerifolia*) es van dipositar a la riera durant 4 mesos. Es va calcular les densitats i biomasses de bacteris, fongs, protozous, meiofauna, i macroinvertebrats per determinar la seva dinàmica i potencials interaccions durant el procés de la colonització. La colonització va estar estretament relacionada amb la variabilitat hidrològica (definida bàsicament pel cabal i la temperatura de l'aigua). La primera setmana de colonització es va caracteritzar per l'estabilitat hidrològica i temperatures càlides, i la transició del carboni del compartiment microbià al dels invertebrats va ser ràpid. Agregats densos de matèria orgànica particulada fina (FPOM) van anar quedant retinguts pels paquets de fullaraca permetent una ràpida colonització per part de la microfauna i col·lectors-recol·lectors de la meiofauna (ostracodes i copèpodes). Després de dues setmanes, una avinguda va augmentar el cabal de la riera fins a 20 cops. Aquest alt cabal i les temperatures més baixes de l'aigua van fer que els agregats de FPOM i la seva fauna associada fossin rentats de la superfície de la fullaraca modificant així la seqüència de colonització. Els fongs van tenir llavors els màxims de biomassa, amb valors similars als obtinguts a l'inici de l'experiment. Prop de 70 dies després d'aquesta avinguda els fongs van decréixer fins el gairebé 40% del carboni total dels paquets de fullaraca mentre que els invertebrats van esdevenir més abundants aconseguint el 60% del carboni. L'ocurrencia natural d'avingudes en rieres mediterrànies podria esdevenir doncs un procés clau dintre de la colonització i el processat de la matèria orgànica.

Capítol 3

Resposta de la meiofauna a una addició de nutrients en una riera mediterrània

Ainhoa Gaudes, Jordi Ocaña, and Isabel Muñoz

(sota revisió a Limnology and Oceanography)

Es van examinar els efectes d'una addició moderada de nutrients, durant un període de dos anys, per tal de determinar la resposta de la comunitat de meiofauna d'hàbitats sorrencs d'una riera mediterrània, en front d'aquesta entrada de nutrients. El patró de la comunitat de meiofauna, tant d'abundàncies com de composició, mostra un elevat grau de variabilitat intra- i interanual. Aquest patró alterna períodes d'estabilitat hidrològica amb pertorbacions hidrològiques com avingudes i sequeres, característiques dels sistemes mediterranis. Es va emprar un diseny BACI (abans-després-control-impactat, en anglès Before-After-Control-Impacted) per tal de determinar l'efecte de l'addició comparant un tram control aigües amunt i un d'aigües avall on s'havia fet l'addició. L'anàlisi de les dades es va fer mitjançant mètodes no-paramètrics de permutació de dades (PERMANOVA) i va mostrar que la fertilització va tenir efectes positius. Les diferències a nivell de densitat i biomassa van ser significatives pels grups de meiofauna més abundants com són microcrustacis, oligoquets i quironòmids. Els microcrustacis van ser el grup més abundant de la meiofauna. També vam examinar diferències a nivell de la producció secundària en ambdós trams. Els ostracodes i els ciclòpids van incrementar la seva producció secundària en el tram impactat com a conseqüència de l'addició de nutrients. En aquesta riera, el compartiment de la meiofauna podria veure's afectat directament per l'addició de nutrients o indirectament per l'enriquiment del detritus en el que la meiofauna viu i s'alimenta.

Capítol 4

La comunitat de nematodes de flocs de cianobacteris en el riu Llobregat

Ainhoa Gaudes, Sergi Sabater, Elisabet Vilalta, and Isabel Muñoz

Nematology, 2006, Vol. 8(6), 909-919

L'objectiu de l'estudi va ser entendre el rol de la comunitat de nematodes en un biofilm dominat per cianobacteris del riu Llobregat (NE Spain). Aquest biofilm estava dominat per espècies oscil·latorials i diatomees. Fraccions significatives d'aquests flocs de cianobacteris es poden desprendre del substrat i surar lliurement dispersant-se riu avall i adquirint propietats estructurals i fisiològiques diferents. Ambdós biofilms de cianobacteris, tant l'adherit com el flotant, es van comparar amb una altra comunitat algal que també es trobava al tram durant l'estudi. Es va trobar una baixa diversitat de nematodes, amb la comunitat dominada per *Chromadorita leuckarti* (De Man), *Diplogaster rivalis* (Leydig), *Plectus parvus* Bastian, *Neotobrilus diversipapillatus* (Daday), *Monhystera* spp., *Dorylaimus* sp. and *Mononchus* sp. La densitat i biomassa de nematodes va ser significativament més alta en el floc flotant (valors màxims de 752 ind/cm^2 i 171,3 µgC/cm^2). Es van observar diferents estratègies tròfiques i sexuals durant l'estudi. El floc flotant va demostrar posseir més abundància de juvenils i una major proporció de femelles prenyades. També es va observar una correlació positiva entre la densitat de cianobacteris i l'abundància de juvenils i adults de l'espècie més abundant de nematode indicant la seva potencialitat com a font d'alimentació.

Capítol 5

Efectes *Bottom-up* a les poblacions de nematodes bacterívors: una aproximació emprant microcosms

Ainhoa Gaudes, Isabel Muñoz, and Tom Moens

(In prep.)

L’objectiu d’aquest estudi va ser testar experimentalment l’efecte de diferents concentracions de nutrients en el desenvolupament de les poblacions de dos nematodes bacterívors de vida lliure a l’aigua dolça. *Bursilla monhystera* i *Plectus aquatilis*. El disseny experimental tenia com a objectiu complementar les dades obtingudes en experiments previs realitzats *in situ* a la riera de Fuirosos, situada al Parc del Montnegre-Corredor. Els nutrients afegits en ambdós sistemes, la riera i els microcosms, van ser fosfat, nitrat i amoni. Addicionalment, en l’experiment de laboratori, es va afegir glucosa com a font de carboni orgànic dissolt biodegradable (BDOC) creant així les condicions basal (baix carboni) i 20 cops incrementada la condició basal (elevat carboni). A cada condició de carboni, es van crear diferents condicions de nitrogen i fòsfor (basal i x3, x10 de N o P sols o una combinació dels dos). Els microcosms es van omplir d’una capa fina de sediments del riu prèviament autoclavada i aigua milliQ amb la corresponent sol·lució de nutrients. Es van inocular amb bacteris aïllats prèviament del riu i tres dies més tard es van afegir els nematodes.

L’experiment va durar 2 mesos. En cada mostreig, es van retirar quatre rèpliques de cada tractament i es va analitzar la concentració de nutrients dissolts a l’aigua, la densitat de bacteris i la densitat i biomassa de nematodes. En el primer mostreig es va obtenir un increment de la població de 2 a 6 cops més alt, demostrant l’elevada capacitat d’ adaptació i colonització de *B. monhystera*. Malgrat això, si els nutrients no eren prou abundants, les densitats tendiren a disminuir, probablement per que no invertien en reproducció i les poblacions eren mantingudes per individus de mida petita i/o mitjana. Per altra banda, sota les condicions més enriquides*, B. monhystera* va tendir a crèixer en nombre durant tot l’experiment, tot i que tampoc es va observar un augment significatiu d’individus reproductors. *Plectus aquatilis* només va augmentar significativament sota les condicions més enriquides, tot i que el seu increment va ser menor que el dels individus *Bursilla*. Els nostres resultats semblen indicar doncs, que l’addició puntual de nutrients poden afectar les poblacions de nematodes a curt plaç però que més enllà, les seves dinàmiques venen més aviat determinades per l’obtenció de nutrients a través de la remineralització de detritus a través de la seva activitat o de la seva ingesta

Discussió general

L'objectiu general d'aquesta tesi ha sigut el de caracteritzar i integrar la meiofauna dintre de l'actual coneixement dels processos funcionals de sistemes lòtics. En els capítols anteriors s'ha intentat enfocar diversos aspectes dels processos lòtics tenint en compte la seva múltiple interacció amb el context en que es troben, el clima mediterrani. És més, la resposta general de la meiofauna trobada al llarg d'aquesta tesi ha estat sempre molt vinculada als factors climàtics. Això implica la natural variabilitat intra- i interanual exhibida pels sistemes mediterranis (Gasith i Resh, 1999; Acuña et al., 2005). El règim anual de temperatures i precipitacions en el clima mediterrani directament determina la hidrologia fluvial i les seqüències d'avingudes i sequeres. Indirectament, aquests efectes també influencien processos lòtics com la deposició i descomposició de la matèria orgànica (Acuña et al., 2004; Artigas et al., 2009), la dinàmica de la matèria orgànica dissolta (Vàzquez et al., 2007), el consum i reciclatge de nutrients (von Schiller et al., 2010; Sabater et al., 2011), el transport de sediment i l'estabilitat de l'hàbitat. Alguns d'aquests processos i la seva relació amb la comunitat de meiofauna han sigut estudiats al llarg d'aquesta tesi emprant experiments de camp i de laboratori i els seus resultats es resumeixen a la figura D.1. i es discutiran a continuació.

Efectes de la hidrologia en la comunitat de meiofauna

Alguns meiobentòlegs han definit el cabal i el substrat com els processos més importants que actuen a petita escala, per a determinar la distribució de la meiofauna (Swan i Palmer 2000; Silver et al., 2002). Aquests dos factors poden interactuar de forma directa amb la meiofauna. Per exemple Palmer (1992) va determinar que velocitats de l'aigua superiors a 12 cm/s incrementava el desenganxament de larves de quironòmids i les dispersava riu avall. Però sovint ambdós factors, cabal i substrat, poden actuar conjuntament, perquè al mateix temps que els organismes són enduts pel corrent, el substrat arrossegat per l'augment de cabal pot actuar com a medi de transport per a altres organismes del seu interior, fins a trams d'aigües avall. Al llarg d'aquesta tesi aquest efecte s'ha pogut observar en sediments sorrencs (**Capítol 1**), en el detritus (**Capítol 2**) i en flocs de cianobacteris (**Capítol 4**), i la seva implicació per a la dispersió dels organismes es discutirà més endavant.

Les avingudes prou fortes com per a modificar el llit del riu poden tenir conseqüències dramàtiques pels organismes, donat que amb la seva força, ho arrassa tot. Tot i això, els organismes de la meiofauna han desenvolupat respostes adaptatives per fer front a aquestes pertorbacions. Per tant, tenir una mida de cos petit, vermiforme i amb aparells locomotors actius semblen ser trets avantatjosos als trams de Fuirosos. Les avingudes poden aturar temporalment el metabolisme, especialment la producció primària (GPP), però solen ser esdeveniments efímers i la recuperació sol ser ràpida (Uehlinger, 2006). En el **Capítol 1**, la resposta resilient de la comunitat ha demostrat actuar d'una manera similar, com a resposta a aquests pulsos curts en els trams de capçalera. Pel contrari, en trams més avall els esdeveniments plujosos van provocar augments majors del cabal (com a conseqüència d'una major area de conca drenada), i les comunitats de meiofauna van mostrar una recuperació més lenta. A Fuirosos, la història hidrològica prèvia també va influir la resposta dels invertebrats a les avingudes (Boulton i Lake, 1992), disminuint la capacitat de recuperació de la comunitat sobretot en trams d'aigües avall. Pel contrari, aquest efecte no es va observar al tram de més amunt (S1). Els trams de capçalera de Fuirosos, alguns d'ells tributaris o trams de primer-ordre, han evidenciat la complexitat de les propietats de l'hàbitat i de l'heterogeneïtat. En aquests trams, molts d'ells intermitents i en els que l'aigua ocasionalment flueix, la matèria orgànica arrossegada per l'aigua durant les avingudes tendeix a agregar-se i a acumular-se interrompent el pas de l'aigua, creant petites preses de pals i fullaraca entre les pedres i els troncs dels arbres de ribera caiguts sobre el riu (Gooderham et al. 2007). Aquestes preses poden proveir una certa estabilitat als organismes, directament oferint-los refugi amb aliment o bé, desviant l'aigua i creant "zones mortes" amb poc corrent i on les comunitats poden desenvolupar-se. Amb tot, la idea de que aquestes zones puguin esdevenir una font per a la recolonització dels organismes aigües avall i mantenir així la biodiversitat, sovint topa amb l'argument de l'hiporreic com a refugi. Per exemple, Olsen i Townsend (2005) van aportar poques evidències d'emprar l'hiporreic com a refugi durant una avinguda en un riu sorrenc de quart- ordre. Van observar que l'avinguda va afectar de manera similar les densitats d'invertebrats al bentos (0-10 cm) i a l'hiporreic (10-50 cm). De manera similar, Boulton et al. (2004) tampoc van trobar una transferència significativa d'hidràcars de zones epibèntiques a zones de "downwelling" o una disminució d'espècies hiporreiques després d'una avinguda simulada in situ. Això suggereix que ambdues respostes haurien de ser possibles en funció de la brusquedat de l'esdeveniment. Les avingudes als sistemes mediterranis generalment ocorren de forma abrupta i, mentre que la seva severitat és del tot impredictible, els seus temps de retorn sovint són menors. Per altra banda, les sequeres solen donar-se de forma progressiva (permetent un cert grau d'adaptació per part de les comunitats d'organismes) i algunes respostes ecològiques poden mostrar una resposta pautada: canvis graduals que s'alternen amb transicions ràpides arribant a un llindar determinat, ex. quan l'hàbitat desapareix o es fragmenta (Boulton et al., 2003).

Les sequeres en el clima mediterrani són predictibles i periòdiques, i són tipificades com a sequeres estacionals (Lake, 2003). Tot i això, la intensitat de la sequera pot variar d'un any a un altre degut a la variabilitat interanual. Aquestes pertorbacions són resultat d'una declinació de les precipitacions, aixaragallament, humitat del sòl, nivells freàtics i cabal del riu (Dahm et al., 2003). La disminució del cabal inclou la disminució de la capacitat de transport del sistema i un increment del recobriment del llit del riu per part dels detritus. Acuña et al. (2005) van descriure el procés de sequera de la riera de Fuirosos i la fragmentació del corrent del riu en petits tolls d'aigua aïllats. Durant aquest procés, l'heterogeneïtat espaial de l'hàbitat va augmentar i les característiques físico-químiques de l'aigua van canviar de forma brusca convertint-se en un punt crític per l'ecosistema (ex. Boulton i Lake, 1990; Stanley et al., 1997). En aquesta situació, l'hiporreic de Fuirosos podria haver funcionat com a via d'escapament durant el complet assecat dels tolls, especialment pels organismes que posseïen trets avantatjosos com el cos vermiforme. Amb tot, donades les característiques geològiques de la conca de Fuirosos i la gran complexitat del seu hiporreic, aquest procés es mereix una atenció especial en investigacions futures en aquesta riera.

Variabilitat intraanual de la comunitat de meiofauna

En el **Capítol 1** i el **Capítol 3**, la comunitat de meiofauna de Fuirosos va patir un increment de densitat i abundància durant el procés de dessecament del riu, a la primavera (anys 2003 i 2005) o a l'estiu (any 2004). A la primavera, aquests increments coincideixen amb els pulsos autotròfics que de forma natural ocorren a Fuirosos (Artigas et al. 2009, von Schiller 2008). La producció primària de les algues del bentos incrementa quan la disponibilitat de la llum i l'estabilitat del cabal convergeixen cap un òptim. Tot i que aquesta resposta es pot observar fàcilment a les algues epilítiques, el substrat sorrenc i les comunitats que hi habiten també podrien estar directa o indirectament afectades per aquestes condicions ambientals. Els beneficis per a la meiofauna podrien venir directament per un augment de la font d'aliment, augmentant densitats de diatomees i flagel·lats autotròfics, o bé indirectament, degut a l'enriquiment del detritus amb exudats provinents de les algues epilítiques, que al seu torn, podrien afavorir l'increment de les poblacions de bacteris i fongs. Amb tot, aquest procés sovint coincideix també amb una disminució de cabal i una contracció de l'ecosistema (Stanley et al., 1997). Aquesta contracció implica una concentració de nutrients i més endavant, a causa de l'aturada del corrent, una interrupció del transport de la matèria orgànica (Acuña et al., 2005). Durant la formació i aïllament dels tolls, algunes espècies oportunistes de la meiofauna (com alguns quironòmids, oligoquets i nematodes) podrien sortir beneficiats. En molts estudis de macroinvertebrats s'ha remarcat la pèrdua de biodiversitat durant aquest procés. Però tal i com s'ha vist al **Capítol 5**, inclús comparant espècies de nematodes oportunistes, petits atributs o diferències de comportament poden donar a lloc a un patró de successió a petita escala que donaria lloc a un constant reemplaçament d'espècies de microorganismes i de la meiofauna. No obstant, aquests processos romandran ocults als investigadors d'ambients lòtics que només fixin la vista per sobre els 250 µm.

En el **Capítol 4**, la comunitat de nematodes que habitaven els flocs de cianobacteris també van mostrar una successió d'espècies directament relacionada amb els canvis en la composició algal. L'evolució d'aquests flocs al seu torn, estava estretament lligada a l'increment de temperatura entrada la primavera, i a la disminució de cabal.

Variabilitat interanual de la comunitat de meiofauna

En el **Capítol 1** i el **Capítol 3**, la variabilitat interanual va ser un determinant important en la dinàmica de les comunitats, ja que es varen succeir dos anys amb diferencies significatives en quant a precipitació. Acuña et al. (2004) va trobar diferències significatives en el total de matèria orgànica dipositada al llit del riu entre anys secs i humits. Per tant, aquestes diferències també van provocar diferències significatives en les propietats físico-químiques i en els valors de respiració de l'ecosistema. La producció secundària anual estimada pels ostracodes i ciclòpids del **Capítol 3** també van ser majors al tram control el segon any d'estudi, indicant que aquest increment va ser en gran part causat per l'estabilitat del cabal i les precipitacions més baixes que van caracteritzar aquest any (**Capítol 1**). Tot i això, aquest increment de la producció secundària va ser potenciada per l'addició de nutrients tal i com es va observar al tram impactat (**Capítol 3**).

Les prediccions pel canvi climàtic per a la conca Mediterrània (IPCC, 2007) auguren un increment de les temperatures i una precipitació menor, especialment a l'estiu. Els canvis en el patró de precipitacions podria alterar la durada, freqüència i magnitud del cabal i els pulsos de riuades. Sota aquestes condicions, l'alternança entre anys secs i humits es pot expandir, afectant la resposta de la meiofauna i altres comunitats bentòniques davant aquests esdeveniments extrems. Estudis de llarga durada sobre la variabilitat de cabal portats a terme en rius intermitents van revelar que casos extrems d'avingudes o períodes de sequera podrien tenir efectes de llarga durada per l'estructura de la comunitat (Sponseller et al., 2010). Per tant, les prediccions més pessimistes sobre el canvi climàtic i els seus efectes a les regions mediterrànies són que les comunitats aquàtiques podrien simplificar-se en gran mesura. Amb tot, tot i que això podria afectar de ple a la meiofauna temporal, la meiofauna permanent podria exhibir una resposta més ràpida per tal d'adaptar-se a aquestes condicions extremes (mitjançant cicles de vida més curts, un millor accés a l'hiporreic, etc). Per tant, aquestes adaptacions podrien minimitzar els impactes de la hidrologia sobre aquests organismes. Tot i així, els efectes del canvi climàtic sobre la disponibilitat de l'aliment també estarien moldejant indirectament aquestes comunitats, considerant que els processos de descomposició disminuiran com a resposta de la modificació del regim d'inundació del riu (Langhans i Tockner, 2006).

Patrons de la colonització de la matèria orgànica i dispersió dels organismes

La matèria orgànica es probablement el major determinant de la distribució de la meiofauna, així com de l'abundància i composició d'espècies, ja que proporciona als organismes aliment, estructura, hàbitat i material per construir-se estoigs i altres (veure Silver et al., 2002 i referències incloses). Tot i això, un cop més, el cabal és de nou un determinant clau en la deposició i distribució de la matèria orgànica en el llit del riu. Els resultats obtinguts al **Capítol 2** recolzen aquest efecte, ja que la hidrologia i la temperatura van influir molt en la colonització de la fullaraca en descomposició. El patró successional observat durant l'experiment va permetre una visió interna de la colonització i la posterior descomposició de les fulles. La traça del carboni i la seva transferència entre compartiments va demostrar ser una eina útil en la descripció del procés. Es més, al llarg de l'experiment es va poder observar una transferència directa entre la meiofauna temporal i els macroinvertebrats, així com una transferència global entre els bacteris i fongs cap al compartiment de la fauna.

El cabal generalment emmascara altres processos a petita escala, i els seus efectes generalment tenen conseqüències importants en els organismes de la meiofauna. Alguns organismes de la meiofauna temporal poden posseir estructures del cos que els permetin augmentar la seva resistència a l'increment de cabal (ex. ganxos, estructures adhesives, etc) però normalment aquestes estructures no s'acaben de desenvolupar completament fins que no tenen una mida de macroinvertebrat. Per tant, en general la meiofauna, com d'altres organismes de cicle vital curt, fan front a aquestes pertorbacions hidrològiques augmentant la resiliència i beneficiant-se del poder del cabal com agent dispersador. Silver et al. (2002) van definir la deriva passiva com un dels processos més importants per a la redistribució de la meiofauna. En el **Capítol 1**, es va observar una menor resposta resilient en el tram S2 ja que el pendent de la densitat de la comunitat era menor. Però al menys en un dels casos, observacions in situ van revelar que immediatament després de la riuada, enlloc de trobar un tram escurat i sense sorres, n'estava ben ple de sorres amb organismes provinents de trams riu amunt. En aquest cas, aquesta elevada densitat a l'inici va influenciar el pendent de la resiliència de la comunitat.

En el **Capítol 2** el patró natural de la colonització de la fullaraca va ser probablement accelerat per l'arribada d'agregats de FPOM densament habitats i provinents de trams riu amunt i arrossegat per la riuada. En conseqüència, la flora associada al detritus i els microcrustacis (principalment ostracodes i ciclòpids) van ser retinguts pels nostres paquets de fullaraca, per les petites preses de matèria orgànica i als marges sorrencs de la riera.

En el **Capítol 4**, l'augment de nematodes probablement va accelerar el procés de separació dels flocs de cianobacteris del substrat, gràcies a un augment de la bioturbació. Palmer et al. (1992) va demostrar que la meiofauna aparentment tenia un cert control en la seva entrada i sortida del corrent quan aquest era baix. A més, la capacitat dels nematodes a moure's activament cap a zones amb abundant aliment (ex. detectant CO_2 bacterià, O'Halloran et al., 2006) o de detectar compostos volàtils com la geosmina (Höeckelmann et al., 2004), podria estar indicant que aquestes comunitats van romandre en aquests flocs flotants per que els oferien unes condicions millors que els cianobacteris enganxats al substrat. Una major temperatura en els flocs flotants a la superfície, podria haver estat un factor important com a accelerador de les taxes de reproducció. Però el fet curiós va ser observar que en aquells flocs flotants, les femelles gràvides de *Diplogaster rivalis* dominaven sobre les altres. Aquestes femelles tenien un comportament vivípar ja que portaven els embrions en desenvolupament dins d'ous que duien dintre dels seus cossos enlloc d'expulsar-los a l'exterior, i això podria esdevenir un tret avantatjós a l'hora de recolonitzar un nou substrat. Donat que aquest procés de separació del substrat no és un procés aïllat, podria ser que els nematodes haguessin adaptat el seu mode de dispersió a aquest mecanisme.

Efectes de l'addició de nutrients

Els resultats obtinguts al **Capítol 3** van indicar que les comunitats del meiobentos en rieres forestades de clima mediterrani podien ser sensibles a una entrada moderada de nutrients. L'addició de nutrients va incrementar la densitat i biomassa de la comunitat de la meiofauna així com va incrementar la producció secundària anual i la relació P/B d'alguns microcrustacis. Tot i que al camp, una addició augmentant tres vegades la concentració basal va ser suficient per observar els canvis, sota condicions de laboratori, les poblacions de nematodes del **Capítol 5** van necessitar un enriquiment més alt, tot i que es va observar un clar control bottom-up per par dels bacteris. Això pot ser degut a que les espècies escollides eren marcadament oportunistes i en el camp, la resposta general pot estar emmascarant les múltiples reaccions i interaccions entre organismes amb estratègies diferents. Al mateix temps, la reducció de cabal que es va donar l'estiu del 2004 podria haver afavorit que en alguns tolls els organismes estiguessin sotmesos a concentracions de nutrients més elevades.

En aquest sentit, el rol de la hidrologia va ser un factor important mentre la fertilització es va a dur a terme. Les diferències observades durant els dos anys hidrològics en el **Capítol 1**, podrien estar emmascarant l'efecte de l'addició de nutrients. En el tram control, una major estabilitat hidrològica durant l'any en que es va fertilitzar l'altre tram, podria haver afavorit un major desenvolupament de la comunitat. Així i tot, els canvis en el tram fertilitzat van ser més evidents, indicant que la combinació d'ambdós factors, cabal i entrada de nutrients, poden tenir un gran impacte en les comunitats. Això pot tenir efectes més enllà, ja que suggereix que els efectes dels nutrients poden ser altament potenciats sota condicions d'escassetat d'aigua.

En el riu Llobregat, la hidrologia i els nutrients també van tenir una influència important en les comunitats tot i que els seus mecanismes van actuar a una escala diferent a la de la riera de Fuirosos. La conca del Llobregat ha estat altament afectada durant els últims segles (veure López-Doval et al., 2010 i referències incloses). A part de l'entrada massiva de nutrients i aigües residuals, la seva morfologia ha estat modificada i cenyida a una sèrie de pantans, preses i azuts. Això implica que en anys amb menor cabal (com a resultat d'una menor precipitació) els productors primaris poden proliferar degut a una combinació d'una alta disponibilitat de nutrients i una estabilitat hidrològica. En el **Capítol 4**, els nostres flocs de cianobacteris van dominar el compartiment dels productors primaris durant la primavera, en el període comprés entre les comunitats dominades per diatomees d'hivern i les comunitats dominades per les algues verdes típiques de l'estiu. Aquesta proliferació de cianobacteris pot tenir conseqüències per l'ús de l'aigua per part de les persones. Els flocs d'Oscillatoria produeixen un compost orgànic volàtil (VOC), inofensiu per l'esser humà, però molest ja que és massa car d'extraure en les plantes potabilitzadores i produeix una olor desagradable en l'aigua potable.

En aquests flocs de cianobacteris , les densitats de nematodes que es van trobar eren de 100 a 600 vegades superior al total de la comunitat de meiofauna de les sorres de Fuirosos. En ambdós substrats, hi havia una diferència considerable en el contingut de C:N. Mentre que al Llobregat, el biofilm de cianobacteris es caracteritzava per tenir una proporció de C:N baixa i un alt contingut de matèria orgànica (Vilalta 2004) que podria haver afavorit un major desenvolupament del nematodes, a Fuirosos, la gran quantitat de carboni orgànic provinent de les entrades del bosc de ribera (Acuña et al., 2004; Artigas et al., 2009) podria haver augmentat la relació C:N. En el **Capítol 5** això també es va poder observar degut al detritus que contenien les sorres de Fuirosos que es van afegir als microcosms. Per tant, un alt contingut de carboni refractari i una alta relació de C:N podria ser la responsable de les baixes densitats i biomasses trobades al camp (**Capítol 1** i **Capítol 3**), i en les condicions basals i de baixos nutrients de l'experiment del **Capítol 5,** suggerint així una possible estequiometria sub-optima pels individus.

El carboni, en les seves formes més refractàries han demostrat afectar la reproducció dels nematodes (Höss et al., 2001). En el **Capítol 5** les poblacions de nematodes sota condicions de baixos nutrients tendien a decréixer, probablement perque no podien invertir en reproducció i la població es mantenia bàsicament amb individus juvenils de mida petita o mitjana. Pel contrari, en les condicions en que s'augmentava per 10 el nitrogen i el fòsfor, els nematodes presentaven un creixement exponencial tot i que tampoc s'observava un increment significatiu de formes reproductives. En els microcosms es va observar un increment de compostos de nitrogen inorgànic dissolt (sobretot amoni) suggerint que l'activitat dels nematodes podria estar remineralitzant els compostos orgànics provinents del detritus o de la ingestió de bacteris.

Ambdues especies*, Bursilla monhystera* i *Plectus aquatilis*, han demostrat ser bons colonitzadors sota condicions enriquides. Amb tot, la resposta dels dos colonitzadors va ser diferent ja que el creixement de *B. monhystera* va ser molt més ràpid en totes les condicions experimentals i *P. aquatilis* només va incrementar sota els tractaments més enriquits. Aquesta resposta diferenciada entre dues espècies oportunistes també ens indica que petites diferències en atributs biològics i del comportament pot estar influint en el procés de successió després d'una pertorbació.

Al llarg d'aquesta tesi doctoral, els trets adaptatius de la meiofauna en front a pertorbacions hidrològiques o d'entrada de nutrients han demostrat ser nombroses i merèixer una investigació més exhaustiva en la recerca futura.

Conclusions

Capítol 1

1. La densitat i biomassa de la meiofauna trobada a Fuirosos va ser de l'ordre de la descrita a altres estudis, amb valors màxims durant la tardor i la primavera, quan s'arribava a un punt òptim entre cabal, temperatura i disponibilitat de matèria orgànica.

2. La meiofauna temporal va tenir més biomassa al llarg de tot l'estudi i majors densitats durant els períodes de disminució de cabal.

3. Els organismes amb trets morfològics vermiformes com oligoquets, quironòmids i nematodes van ser els més representatius de la meiofauna a tots tres trams. Els microcrustacis, amb apèndixs que els permeten una locomoció activa, també van ser abundants durant els períodes amb abundant retenció i processament de detritus al riu.

4. Les petites diferències a nivell de conca van afectar directament el cabal i l'estabilitat de l'hàbitat i al seu torn l'estructura de les comunitats de meiofauna de la riera de Fuirosos. És més, el tram situat més amunt (S1) va mostrar major densitat, biomassa i resposta resilient, i amb potencial com a font de recolonització després de riuades de trams riu avall.

Capítol 2

5. A Fuirosos, la successió biològica de la matèria orgànica en descomposició va estar fortament correlacionada amb el cabal i la temperatura de l'aigua.

6. Es va observar una transferència de carboni de la meiofauna temporal als macroinvertebrats, així com una transferència global dels compartiments de bacteris i fongs cap als compartiments de la fauna durant el transcurs del procés.

7. La biomassa fúngica va rondar entre el ~32 i el ~93% del total de carboni dels organismes (sense comptar el de la fullaraca) dels paquets de fullaraca durant l' experiment, mentre que la biomassa bacteriana va assolir entre el ~0.2% i el ~6%, la

biomassa de la meiofauna entre el ~0.03% i el ~8.4% i els macroinvertebrats entre el ~1.7% i el ~58% .

Capítol 3

8. Les comunitats meiobentòniques de Fuirosos han demostrat ser sensibles a una addició moderada de nutrients.

9. Els grups principals de la meiofauna temporal (quironòmids i oligoquets) i de la meiofauna permanent (microcrustacis i nematodes) van ser els que van respondre a aquesta addició.

10. La producció secundària dels ciclòpids (dominats pel gènere *Eucyclops*) i els ostràcodes (sobretot Candoninae i Cypridopsinae) van incrementar després de la fertilització al tram impactat.

11. La relació P/B d'ostracodes al tram control va disminuir després de la fertilització mentre que va incrementar al tram impactat.

12. Les diferències en la freqüència i durada de les pertorbacions hidrològiques entre els dos anys que va durar l'experiment, han estat un factor determinant per l'estructura de la meiofauna i la producció secundària de microcrustacis en els dos trams. És més, la variabilitat interanual pot potenciar o disminuir els efectes de l'addició de nutrients en la comunitat de meiofauna.

Capítol 4

13. La densitat de nematodes trobada als flocs de cianobacteris van ser similars als trobats a altres estudis de substrats similars. Amb tot, en el floc flotant, les densitats i biomasses van arribar a superar els valors trobats a qualsevol ambient lòtic.

14. Els flocs de cianobacteris van mostrar una baixa diversitat amb una gran densitat i biomassa d'espècies oportunistes com *Cromadorita leuckarti, Diplogaster rivalis* i *Neotobrilus diversipapillatus*.

15. El patró d'histèresi de la comunitat de nematodes al floc flotant i a l'adherit al substrat, confirmaria l'acoblament entre el cicle dels nematodes i el creixement i evolució dels cianobacteris.

16. Cianobacteris i nematodes es podrien beneficiar mútuament, ja que l'acció brostejadora i l'excreció de femtes i mucus podria estimular el creixement dels bacteris i cianobacteris. Els nematodes també poden potenciar la permeabilitat del biofilm a través de la bioturbació i afavorir així el desenganxament del substrat i la seva dispersió.

Capítol 5

17. Sota les condicions més enriquides, *Bursilla monhystera* (cp-1) va tendir a créixer de forma constant al llarg del temps tot i que no es va observar un increment significatiu de formes reproductores. Per altra banda, *Plectus aquatilis* (cp-2) només va augmentar sota les condicions més enriquides tot i que la seva eficiència va ser molt menor que la de les poblacions de *Bursilla*.

18. La correlació entre les abundàncies de bacteris i *Bursilla* van indicar un clar control bottom-up.

19. Aquest augment d'abundàncies així com una major presència de nematodes grans, va anar acompanyat d'un augment de nitrats i amonis dissolts a l'aigua. Això es podria deure en part, a l'alliberament de nutrients per part del detritus o bé a una excreció de N com a resultat d'un augment en l'activitat dels nematodes

20. Només els tractaments amb addició de fòsfor (sobretot els x10) van ser capaços de mantenir un increment constant en les poblacions de nematodes.

APPENDIX

APPENDIX

```r
# This function computes the annual secondary production using the size-frequency
# method using the bootstrap resampling method. The final output gives the annual
# production (yearprod; mg DM·m-2 year-1)and the Production to Biomass ratio (ratio P/B)
#
# Usage:
#   yearProd(prod.data, nintervals = 10, sampl.area = 0.067, cpi = 90)
#
# Arguments:
#   prod.data        A numeric vector with values of organisms biomasses(note: not lenghts!)
#   nintervals       Number of classes or intervals in which the 'prod.data' values are
#                    tabulated in frequency form. It defaults to 10
#   sampl.area       Sampling area. It is used to convert the preceding
#                    frequency counts into densities. It defaults to 0.067
#   cpi              CPI value "Cohort Production Interval"
#
# Value:
#   A vector of length 2 with the annual secondary production (yearprod) and the
#   Production to Biomass ratio (ratio P/B)
#
# Examples:
#   produc <- read.table(file="prodCICLOPB2.txt", header=T)
#   yearProd(produc[,1])  # Equivalent to  yearProd(produc[,1], cpi = 90)
#   yearProd(produc[,1], cpi=182.5)
#   yearProd(produc[,1], sampl.area = 0.1, cpi=(90+182.5)/2)

yearProd <- function(prod.data,
  nintervals = 10, sampl.area = 0.067, cpi = 90)
{
  min.prod.data <- min(prod.data)
  max.prod.data <- max(prod.data)
  histo <- hist(
    prod.data,
    breaks = seq(from=min.prod.data, to=max.prod.data, by = (max.prod.data - min.prod.data) /
nintervals),
    plot = FALSE
  )
  dens <- histo$counts / sampl.area
  loss <- dens
  loss[-nintervals] <- loss[-nintervals] - loss[-1]
  pCla <- histo$mids
  pCla[-nintervals] <- 0.5 * (pCla[-nintervals] + pCla[-1])
  biom <- loss * pCla
  sumBioms <- rep(TRUE, nintervals)
  sumBioms[1] <- biom[1] >= 0
  prod.year <- sum(biom[sumBioms]) * nintervals * 365 / cpi
  result <- c(prod.year, prod.year / sum(histo$mids * dens))
  names(result) <- c("year prod","ratio P/B")
  return(result)
```

```
}


# This function generates "nb" number of bootstrap replicates of the annual production value.
# It defaults 10000 iterations.
#
# Usage:
#   bootReplicates.prod(prod.data,
#     nintervals = 10, sampl.area = 0.067,
#     cpi = expression(rnorm(1,mean=mean.cpi, sd=sigma.cpi)),
#     min.cpi = 90,  max.cpi = 182.5,
#     mean.cpi = (min.cpi + max.cpi)/2,
#     sigma.cpi = (max.cpi - mean.cpi) / qnorm(0.975),
#     nb = 10000)
#
# Arguments:
#   prod.data        A numeric vector with the same meaning than in function
#                    'yearProd'
#   nintervals       Number of intervals in which the 'prod.data' values are
#                    tabulated in frequency form. It defaults to 10
#   sampl.area       Sampling area. It is used to convert the preceding
#                    frequency counts into densities. It defaults to 0.067
#   cpi              an object of class 'expression' defining how CPI values
#                    are generated when each bootstrap resample is produced.
#                    It defaults to random normal values generated from 'rnorm'
#                    with mean = (min.cpi + max.cpi)/2 and
#                    sd = (max.cpi - mean.cpi) / qnorm(0.975). Other
#                    expressions are admisible, including a constant value,
#                    e.g. cpi = expression(90) or simply cpi = 90
#   min.cpi          Hypothetical minimum value of CPI (species specific).
#                    It is ignored if mean.cpi and sigma.cpi are provided
#   max.cpi          Hypothetical maximum value of CPI (species specific).
#                    It is ignored if mean.cpi and sigma.cpi are provided
#   mean.cpi         Mean of the normal distribution used to generate CPI
#                    resampling values. It defaults to (min.cpi + max.cpi)/2
#   sigma.cpi        Standard deviation of the normal distribution used to
#                    generate CPI resampling values.
#                    It defaults to (max.cpi - mean.cpi) / qnorm(0.975)
#   nb               Number of bootstrap resamples
#
# Value:
#   A numeric matrix with 2 rows and nb columns, each column stands for a
#   bootstrap replicate of values produced from function 'yearProd'
#
# Examples:
#   produc <- read.table(file="prodCICLOPB2.txt", header=T)
#   boot.prods <- bootReplicates.prod(produc[,1])
#
#   Show first 10 bootstrap replicates:
#   boot.prods[,1:10]
```

```
#
#   Resample generating uniform CPI values:
#   boot.prods <- bootReplicates.prod(produc[,1], cpi = expression(runif(1,90,182.5)))
#
#   Resample with constant (90) CPI values:
#   boot.prods <- bootReplicates.prod(produc[,1], cpi = 90)
#
# Details:
#   Note that 'cpi' argument does not have the same meaning in
#   'bootReplicates.prod' than in 'yearProd'.
#   If argument 'cpi' receives a value other than its default, arguments
#   'min.cpi', 'max.cpi', 'mean.cpi' and 'sigma.cpi' are ignored.
#   Each resample is generated from a nonparametric bootstrap resample from
#   data 'prod.data' and a possibly random CPI value provided by argument 'cpi'

bootReplicates.prod <- function(prod.data,
  nintervals = 10, sampl.area = 0.067,
  cpi = expression(rnorm(1,mean=mean.cpi, sd=sigma.cpi)),
  min.cpi = 90,
  max.cpi = 182.5,
  mean.cpi = (min.cpi + max.cpi)/2,
  sigma.cpi = (max.cpi - mean.cpi) / qnorm(0.975),
  nb = 10000)
{
  # Generation of nb production bootstrap replicates with possibly random cpi
  replicate(nb,
    yearProd(
      sample(prod.data, replace=T),
      nintervals = nintervals, sampl.area = sampl.area,
      cpi = eval(cpi)
    )
  )
}


# Bootstrap percentile confidence interval (CI) for year production.
# Creates a simple CI for the bootstrap generated data.
#
# Usage:
#   bootPercCI.prod(prod.data,
#     nintervals = 10, sampl.area = 0.067,
#     cpi = expression(rnorm(1,mean=mean.cpi, sd=sigma.cpi)),
#     min.cpi = 90,  max.cpi = 182.5,
#     mean.cpi = (min.cpi + max.cpi)/2,
#     sigma.cpi = (max.cpi - mean.cpi) / qnorm(0.975),
#     nb = 10000,
#     boot.replicates,
#     alphas = c(0.025, 0.975)
#   )
#
```

```
# Arguments:
#   prod.data        A numeric vector with the same meaning than in function
#                    'yearProd'
#   nintervals       Number of intervals in which the 'prod.data' values are
#                    tabulated in frequency form. It defaults to 10
#   sampl.area       Sampling area. It is used to convert the preceding
#                    frequency counts into densities. It defaults to 0.067
#   cpi              an object of class 'expression' defining how CPI values
#                    are generated when each bootstrap resample is produced.
#                    It defaults to random normal values generated from 'rnorm'
#                    with mean = (min.cpi + max.cpi)/2 and
#                    sd = (max.cpi - mean.cpi) / qnorm(0.975). Other
#                    expressions are admisible, including a constant value,
#                    e.g. cpi = expression(90) or simply cpi = 90
#   min.cpi          Hypothetical minimum value of CPI (species specific).
#                    It is ignored if mean.cpi and sigma.cpi are provided
#   max.cpi          Hypothetical maximum value of CPI (species specific).
#                    It is ignored if mean.cpi and sigma.cpi are provided
#   mean.cpi         Mean of the normal distribution used to generate CPI
#                    resampling values. It defaults to (min.cpi + max.cpi)/2
#   sigma.cpi        Standard deviation of the normal distribution used to
#                    generate CPI resampling values.
#                    It defaults to (max.cpi - mean.cpi) / qnorm(0.975)
#   nb               Number of bootstrap resamples
#   boot.replicates  A matrix with resampled production values. Its must have
#                    the same structure than the output of 'bootReplicates.prod'
#   alphas           The probabilities in the tails of the bootstrap
#                    distribution. The confidence interval will have nominal
#                    1 - (alphas[1] + alphas[2]) confidence level
#
# Value:
#   A numeric matrix with 2 rows and nb columns, each column stands for a
#   bootstrap replicate of values produced from function 'yearProd'
#
# Examples:
#   produc <- read.table(file="prodCICLOPB2.txt", header=T)
#   boot.prods <- bootReplicates.prod(produc[,1])
#
#   Show first 10 bootstrap replicates:
#   boot.prods[,1:10]
#
#   bootPercCI.prod(boot.replicates = boot.prods)
#
#   Confidence interval resampling with uniform CPI values:
#   bootPercCI.prod(produc[,1], cpi = expression(runif(1,90,182.5)))
#
#   With constant (90) CPI values:
#   bootPercCI.prod(produc[,1], cpi = 90)
#
# Details:
```

```
#   If argument 'boot.replicates' is missing, the bootstrap replications are
#   generated by a call to bootReplicates.prod.
#   If argument 'boot.replicates' is provided, all arguments except 'alphas'
#   are ignored

bootPercCI.prod <- function(prod.data,
  nintervals = 10, sampl.area = 0.067,
  cpi = expression(rnorm(1,mean=mean.cpi, sd=sigma.cpi)),
  min.cpi = 90,
  max.cpi = 182.5,
  mean.cpi = (min.cpi + max.cpi)/2,
  sigma.cpi = (max.cpi - mean.cpi) / qnorm(0.975),
  nb = 10000,
  boot.replicates,
  alphas = c(0.025, 0.025)
)
{
  if (missing(boot.replicates)) {
    boot.replicates <- bootReplicates.prod(prod.data,
      nintervals, sampl.area,
      cpi = cpi, mean.cpi = mean.cpi, sigma.cpi = sigma.cpi, nb = nb
    )
  }
  # Bootstrap percentile confidence intervals. Only include values failing within the 95% CI.
  limits <- apply(boot.replicates, 1, quantile, prob = c(alphas[1], 1 - alphas[2]))
  limits
}


# Graphical and numerical comparative summary of the bootstrap values (only the 95%CI)
# associated to two data sets. By means of a box plot graphic compares two data sets.
#
# Usage:
#   compareProds(boot1, boot2, name1 = "First series", name2 = "Second series",
#     alphas = c(0.025, 0.025),
#     graphic = c("hist", "boxplot")
#   )
#
# Arguments:
#   boot1           A matrix with resampled production values. Its must have
#                   the same structure than the output of 'bootReplicates.prod'
#   boot2           A matrix with resampled production values. Its must have
#                   the same structure than the output of 'bootReplicates.prod'
#   name1           Character, name of the first dataset
#   name2           Character, name of the second dataset
#   alphas          The probabilities in the tails of the bootstrap
#                   distribution. The confidence interval will have nominal
#                   1 - (alphas[1] + alphas[2]) confidence level
#   graphic         Name of the graphic to be displayed comparing 'boot1' and
#                   'boot2'. The possible values are "hist" (default) to
```

```
#                  plot the combined histogram or "boxplot"
#
# Examples:
#   exemple1 <- read.table(file="prodCICLOPA1.txt", header=T)
#   exemple2 <- read.table(file="prodCICLOPA2.txt", header=T)
#
#   boot.prods1 <- bootReplicates.prod(exemple1[,1])
#   boot.prods2 <- bootReplicates.prod(exemple2[,1])
#
#   compareProds(boot.prods1, boot.prods2, "A1", "A2")
#   compareProds(boot.prods1, boot.prods2, "A1", "A2", graphic = "boxplot")
#
#
# Details:
#   For each set of bootstrap values, the percentile confidence interval and
#   the mean of all bootstrap values lying inside de confidence interval are
#   displayed. These numercial values are accompanied by a joint diagram
#   consisting on joint histograms or boxplots for each series of bootstrap
#   values (those lying inside the confidence intervals)

compareProds <- function(boot1, boot2,
 name1 = "First series", name2 = "Second series",
 alphas = c(0.025, 0.025),
 graphic = c("hist", "boxplot"))
{
 smmry1 <- summaryProd(boot1, name1, alphas)
 smmry2 <- summaryProd(boot2, name2, alphas)
 varnams <- rownames(boot1)
 old.par <- par(mfrow = c(2,1))
 switch (
  graphic[1],
   hist = {
    groupedHist(list(smmry1[[2]][[1]], smmry2[[2]][[1]]), tittle = paste(varnams[1],
name1,"vs",name2))
    groupedHist(list(smmry1[[2]][[2]], smmry2[[2]][[2]]), tittle = paste(varnams[2],
name1,"vs",name2))
   },
   boxplot = {
    boxplot(list(smmry1[[2]][[1]], smmry2[[2]][[1]]), names = paste(varnams[1], c(name1,
name2)))
    boxplot(list(smmry1[[2]][[2]], smmry2[[2]][[2]]), names = paste(varnams[2], c(name1,
name2)))
   }
 )
 par(mfrow = old.par)
}
```

```
#
***************************************************************************************
*
#  AUXILIARY FUNCTIONS, NOT DESIGNED FOR DIRECT USE
#
***************************************************************************************
*

centralPoints <- function(samplePoints, limits) {
  result <- vector("list", length = nrow(samplePoints))
  for (i in 1:nrow(samplePoints)) {
    result[[i]] <- samplePoints[i, (samplePoints[i,] > limits[1,i]) & (samplePoints[i,] < limits[2,i])]
  }
  names(result) <- rownames(samplePoints)
  result
}

groupedHist <- function(x, tittle, ...)
{
  junk = NULL
  grouping = NULL
  for(i in 1:length(x)) {
    junk = c(junk,x[[i]])
    grouping <- c(grouping, rep(i,length(x[[i]])))
  }
  grouping <- factor(grouping)
  n.gr <- length(table(grouping))
  xr <- range(junk)
  histL <- tapply(junk, grouping, hist, plot = FALSE, ...)
  maxC <- max(sapply(lapply(histL, "[[", "counts"), max))
  h.den <- c(10, 15, 20)
  h.ang <- c(45, 15, -30)
  plot(histL[[1]], xlim = xr, ylim= c(0, maxC), density = h.den[1], angle = h.ang[1], xlab = "x",
main = tittle)
  for(j in 2:n.gr)
      plot(histL[[j]], add = TRUE, density = h.den[j], angle = h.ang[j], main = tittle)
  invisible()
}


summaryProd <- function(boot.replicates, nam = "", alphas = c(0.025, 0.025))
{
  print(nam)
  limits <- bootPercCI.prod(boot.replicates = boot.replicates, alphas = alphas)
  print("Confidence intervals:")
  print(limits)
  central <- centralPoints(boot.replicates, limits)
  print("Mean value of central (inside confidence interval) bootstrap replicates:")
  for (i in 1:nrow(boot.replicates)) {
    cat(rownames(boot.replicates)[i], "  ", mean(central[[i]]), "\n")
```

```
  }
  return(list(limits, central))
}
```

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