



Universitat de Girona

RESPONSE OF THE BENTHIC MACROINVERTEBRATE COMMUNITY TO A POINT SOURCE IN LA TORDERA STREAM (CATALONIA, NE SPAIN)

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ISBN: 84-689-3755-X

Dipòsit legal: Gi.1073-2005

<http://hdl.handle.net/10803/7901>

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La Tordera stream (Catalonia, NE Spain)**

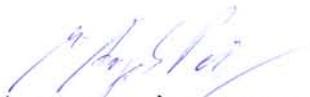
Jesús Ortiz Durà
2005

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La Tordera stream (Catalonia, NE Spain)**

Universitat de Girona
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Memòria presentada per
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per a optar al grau de Doctor en Biologia

Tesi realitzada sota la direcció de
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Dr. Manel Poch Espallargas



Dra. M. Àngels Puig



Dr. Manel Poch



Jesús Ortiz

Blanes, abril de 2005

For my family

橋は流れて河は流れず

Hashi wa nagarete, kawa wa nagarezu

As the Japanese saying goes, “robust structures, such as bridges (or dissertations), are often perishable while flowing systems, such as streams, tend to remain.”

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1. General introduction

From their outset, humans have considerably modified their environment, frequently with severe consequences in detrimental of themselves. The European Environment Agency estimated that more than 50% of flowing freshwaters in the European Union underwent significant eutrophication by the end of the 20th century (Crouzet et al. 2000). The same applies for other rich countries like the United States (USEPA 2002) or Japan (Water Environment Department 2001) and lower, but increasing, for developing countries (Kivaisi 2001, Kambole 2003). Since the Industrial Revolution, watercourses have been usually understood as channels for transport of wastes down to the sea, the immense waste disposal where everything is supposed to be diluted. Discharge of nutrients and organic matter into freshwater ecosystems can lead to eutrophication, having adverse effects on their ecology and limiting the suitability of water for human use (Paul and Meyer 2001). In the European Union, point sources, mainly through urban sewage, represent the second cause of water impairment after agriculture (Nixon et al. 2003). However, point sources are of major relevance given that the coverage of agricultural land is threefold the built-up area in Europe (EEA 2003). In addition, urbanization is increasing at unsustainable rates predominantly at expense of agricultural land.

Increasing the number of wastewater facilities and their operational efficiency led to a significant amelioration of freshwater quality in European countries during the last two decades (Nixon *et al.* 2003, EEA 2003). However, demand for freshwater is rapidly increasing (Council of the European Communities 2000) and the capacity of wastewater treatment plants (WWTPs) to reduce nutrient inputs into aquatic ecosystems is constrained by technological and economic limitations and the continuous growth of population and related

activities. Moreover, the climate conditions of the Mediterranean region, characterized by soft, wet winters and hot, dry summers, make it one of the areas most appropriate for human settlement and concomitant consequences on natural ecosystems. Mediterranean flowing ecosystems are particularly susceptible to human impact because water resources are in short supply (Gasith and Resh 1999). In addition, the strong seasonal variability in rainfall mostly occurs through storms that often translates in flooding events and may have severe consequences in stream ecosystems (Lake 2000).

The actual Framework Directive of the European Community highlighted the need of considering biologic quality to provide information for the efficient and effective design of future monitoring programs (Council of the European Communities 2000). The first documented reference regarding the effects of organic pollution on aquatic organisms was probably that of Aristotle (Margalef 1983), whereas the use of living organisms for biomonitoring was much earlier. However, it was not until the beginning of the 20th century that the concept of biological indicators of environmental conditions was developed for aquatic ecosystems. In their interesting research, Kolkwitz and Marsson (1909) introduced the Saprobien system in an attempt to provide a framework for the use of aquatic organisms as indicators of organic pollution. They provided a large list of organisms typically inhabiting in each trophic condition (oligosaprobic, α - and β -saprobic, and polysaprobic) including rotifers and macroinvertebrates that was constantly lengthen and revised (Cairns and Pratt 1993). The idea of using stream-dwelling organisms as indicators of water quality was extended to other organisms, from bacteria to fish (Margalef 1955, Hynes 1978). However, of all organisms that have been considered for bioassessment, algae, macroinvertebrates, and fish offer the greatest feasibility, and macroinvertebrates are, by far, the most frequently employed (Rosenberg and Resh 1993). The use of benthic

macroinvertebrates for biomonitoring hold remarkable advantages relative to other organisms, but certain difficulties must be considered (Table 1.1). Some of these difficulties can, however, be overcome by considering a multimetric approach. In this sense, Karr and Dudley (1981) underlined the need of attaining biotic integrity in bioassessments to provide a broader understanding of the processes controlling altered streams. They provided the now widely accepted definition of biotic integrity as “the ability to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region.” Following their multimetric approach, further research focused on the examination of the effects of human impact on a wide range of structural and functional characteristics of macroinvertebrate communities. Based on previous research, Kerans and Karr (1994) proposed the hypothetical response that will have several metrics of macroinvertebrate communities to human impact. The continuous testing and modification of these hypotheses (e.g., Barbour *et al.* 1996, Fore *et al.* 1996, Roy *et al.* 2003) allowed the establishment of several general patterns concerning the use of integrated metrics for biomonitoring (Table 1.2). The increase of nutrients and organic matter enhance respiration (Steinman and Lamberti 1996, Miltner and Rankin 1998) and lead to low DO concentrations, especially at night (Mulholland *et al.* 2001), that can be restrictive for some macroinvertebrates (McCormick *et al.* 2004). Point source discharges not only are known to increase nutrient concentrations into stream ecosystems but can also raise water availability. The resulting increase of discharge may convert a temporary stream into a permanent one (Gasith and Resh 1999) and have consequences for the macroinvertebrate community (Dieterich and Anderson 2000).

Table 1.1 Advantages and difficulties to consider in using benthic macroinvertebrates for biomonitoring (Modified from Rosenberg and Resh 1993 and Resh *et al.* 1996).

Advantages	Difficulties
Being ubiquitous, they can be affected by many disturbances in many aquatic systems and habitats	Their distribution and abundance can be influenced by factors other than water quality
Their high diversity offers a spectrum of responses to environmental stresses	The required taxonomic resolution for bioassessments is not clear ¹
The limited mobility of many taxa allows effective spatial analysis	Drift behavior of some macroinvertebrates may lead to ambiguous results
Their relatively long life cycles allow continuous monitoring enabling long-term analysis	Temporal variability may complicate interpretations and comparisons
Qualitative sampling requires simple and inexpensive equipment	Quantitative sampling requires large number of samples and effort
Taxonomy of many groups is well known and identification keys are available	Certain groups are taxonomically difficult
Many methods of data analysis have been developed	The multiplicity of methods of analysis may indicate that most of them are not satisfactory
Species-specific responses to particular stressors have been established	The importance of rare taxa is still in quarrel ²
Biochemical and physiological measures of the response of certain taxa to perturbations are being developed	They are not sensitive to some perturbations, such as human pathogens and trace amounts of some pollutants
They are well suited to experimental studies of perturbation	

¹ Wright *et al.* 1995, Marchant *et al.* 1995, Thompson and Townsend 2000, Bailey *et al.* 2001, Schmidt-Kloiber and Nijboer 2004

² Cao *et al.* 1998, Marchant 1999, Cao 1999, Cao *et al.* 2001, Marchant 2002, Nijboer and Schmidt-Kloiber 2004

Table 1.2 Hypothesized response of benthic macroinvertebrates to point sources.

Attribute	Expected response
Community structure	
Total macroinvertebrate density	Variable ²
Total macroinvertebrate biomass	Variable
Taxa richness	Decline ^{1, 2, 3}
Diversity	Decline ²
Evenness	Decline
Dominance	Increase ^{1, 2, 3}
Community composition	
Ephemeroptera	Decline ^{1, 2, 3}
Plecoptera	Decline ^{1, 2, 3}
Trichoptera	Decline ^{1, 2, 3}
Chironomidae	Increase ^{1, 3}
Functional organization	
Shredders	Decline ¹
Scrapers	Decline ¹
Gatherers	Increase ¹
Filterers	Increase ¹
Predators	Decline ¹
Microhabitat distribution	
Dependence to microhabitat conditions	Decline
Elemental stoichiometry	
C:N and C:P ratios	Decline
Consumer-resource elemental imbalances	Decline

Modified from ¹Kerans and Karr 1994, ²Barbour *et al.* 1996, and ³Fore *et al.* 1996)

A number of studies have demonstrated the consistent variation of certain structural metrics with increasing organic pollution (Wiederholm 1984, Suren 2000). However, the non-monotonic response of other metrics (e.g., total density) to human impact (Odum *et al.* 1979) may lead to conflicting conclusions. The functional organization of macroinvertebrates is known to reflect faithfully

ecosystem processes (Vannote *et al.* 1980, Minshall *et al.* 1983, Merritt and Cummins 1996a). However, clear patterns for the functional approach are not established yet given its dependence to other environmental conditions not strictly related to human disturbance such as riparian vegetation, seasonality, and hydrology (Wallace and Webster 1996, Hart and Finelli 1999). Several studies showed that human disturbance might also have detectable effects on macroinvertebrate biochemistry, physiology, morphology, behavior, life history, and bioaccumulation (Johnson *et al.* 1993). However, little attention has been paid to potential effects of point sources on macroinvertebrate microdistribution and elemental stoichiometry.

The general objective of this work is to assess how the macroinvertebrate community of a Mediterranean stream responds to a point source input from a multimetric approach. Moreover, unpredictable flooding events occurring during the sampling period allowed us to examine the effects of nutrient enrichment and its interaction with natural disturbance. The responses of macroinvertebrates to such disturbances and its temporal variability is here analyzed from several perspectives, including community structure and composition, functional organization, microhabitat distribution, and elemental stoichiometry. This work aim to contribute for a better understanding of the mechanisms that operate in stream ecosystems exposed to human and natural disturbance in an attempt to provide useful information for biomonitoring strategies and the development of future management plants for stream restoration.

2. Study site

The study was conducted in La Tordera stream in Catalonia (NE Spain; Figure 2.1). We selected one reach upstream and one reach downstream of the input of the wastewater treatment plant (WWTP) of Sta. Maria de Palautordera to examine the effects of this point source and related outflows on several descriptors of the benthic macroinvertebrate community.

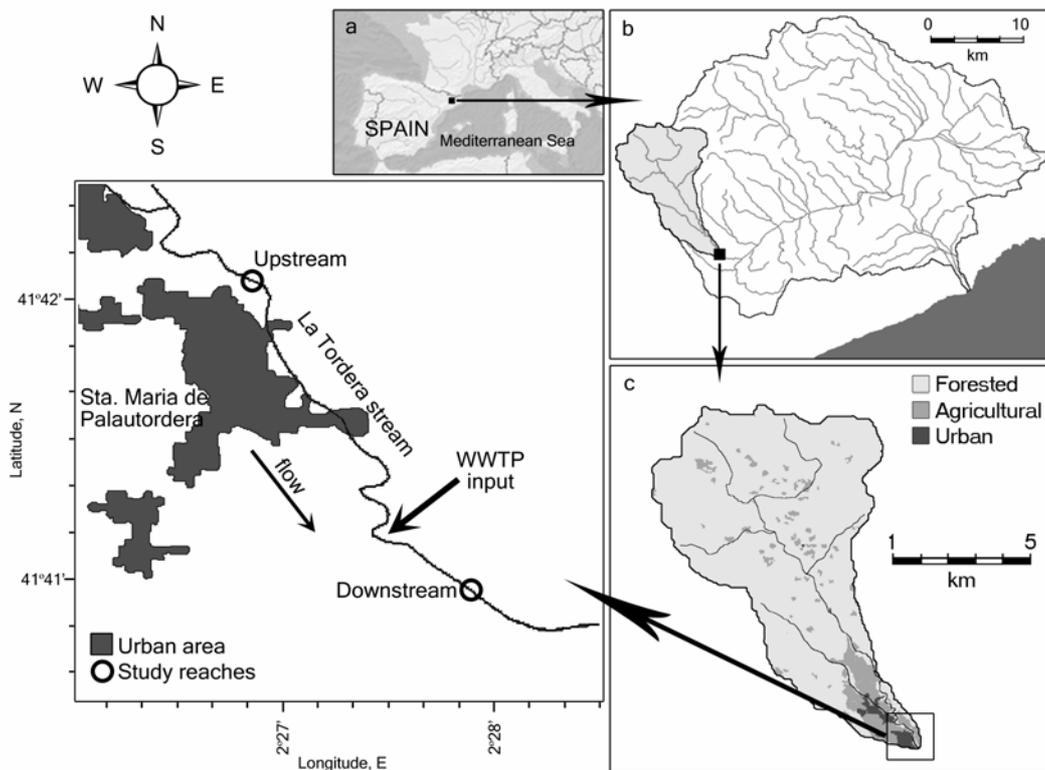


Figure 2.1 Location of La Tordera stream in Catalonia, NE Spain (a), La Tordera catchment and the subcatchment affecting the sampling site, highlighted in grey (b), land uses of the subcatchment affecting the study reaches (c), and location of the study reaches in relation to the wastewater treatment plant (WWTP) input (d). Data from the Catalan Cartographic institute (ICC).

At the sampling site (41°41' N, 2°27' E, 200 m a. s. l.), La Tordera is a 3rd-order stream draining a catchment of 80 km² dominated by a sclerophyllous forest of several species of *Quercus* (Figure 2.1). Small patches of irrigated crops are present in the lower part of the catchment, surrounding the urban area. The geology of the catchment is mainly siliceous, dominated by slates and phyllites. The climate is Mediterranean, with mean air temperatures ranging from 5 °C (January) to 23 °C (August). Mean annual precipitation is 575 mm, which mostly occurs in spring and fall (Figure 2.2). Stream discharge is highly variable within and among years. In dry years, most sections of the stream became isolated pools in the summer, but below the WWTP input the effluent outflow was sufficient to maintain continuous flow for hundreds of m of stream channel. Spates are associated with rainstorms and usually occur in spring and autumn. Mean annual discharge of the stream during the hydrologic year 2001-2002 (0.20 m³/s) was three times lower than the mean discharge for the period comprised between 1923

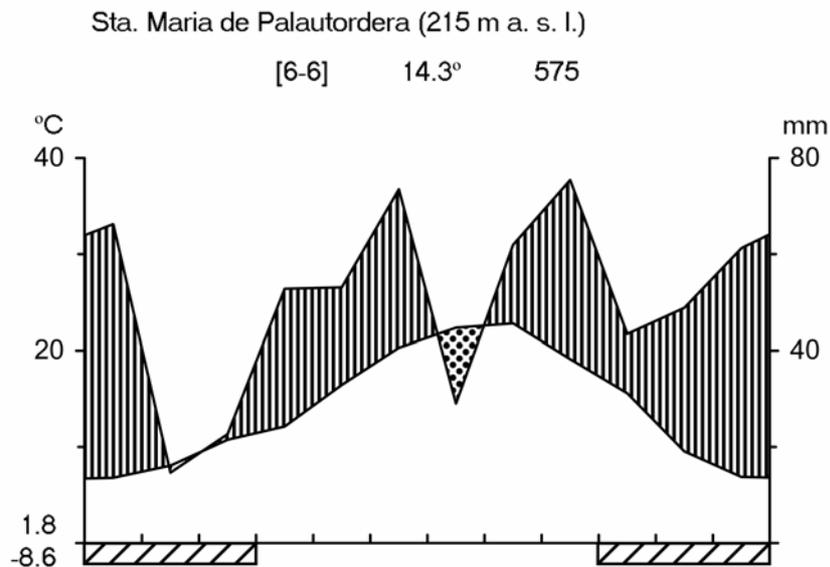


Figure 2.2 Ombrothermic diagram of Sta. Maria de Palautordera. Data from the Catalan Meteorological Service (SMC).

and 2002 ($0.77 \text{ m}^3/\text{s}$; Figure 2.3a). The hydrologic year 2001-2002 was preceded by four years of similar mean annual discharge ($0.27 \text{ m}^3/\text{s}$). However, during the sampling period discharge peaked in spring while during the previous four years the maximum occurred in winter. The upstream reach was dry from July to early October 2001. The two reaches were affected by spates in the middle of November of 2001, and early March, April, May and June of 2002 (Figure 2.3b). The most significant spate during the sampling period was in May, when stream discharge was over $7 \text{ m}^3/\text{s}$.

The population of the catchment in 2001 was 8564 inhabitants, 93% of which were concentrated in the lower part of the catchment, in the villages of Sta. Maria de Palautordera and St. Esteve de Palautordera. The WWTP treats a wastewater of 5808 inhabitant-equivalents¹ that is mostly from urban origin. The mean outflow of the WWTP effluent to La Tordera stream is $1300 \text{ m}^3/\text{day}$, while the discharge is highly variable both daily and seasonally (Figure 2.4). The WWTP perform a biological treatment with activated sludge, but lack the technology to actively remove nitrogen or phosphorus. In 2000, the mean efficiency was 80% for nitrogen and 58% for phosphorus, but nutrient concentrations were still much higher than in the receiving stream (Figure 2.5). Diffuse sources from small-scale agriculture and small tributaries also affect La Tordera stream, but they are located near the WWTP effluent input and were considered together as a point source for the downstream reach. The point source considerably increased nutrient concentrations in La Tordera stream and persisted several hundred m below. Any tributary joints the stream between the WWTP input and the downstream reach and there were not diffuse sources under dry conditions (Merseburger *et al.*, in press).

¹ One inhabitant-equivalent is the biodegradable organic matter load equivalent to a BOD_5 of $60 \text{ g O}_2/\text{day}$.

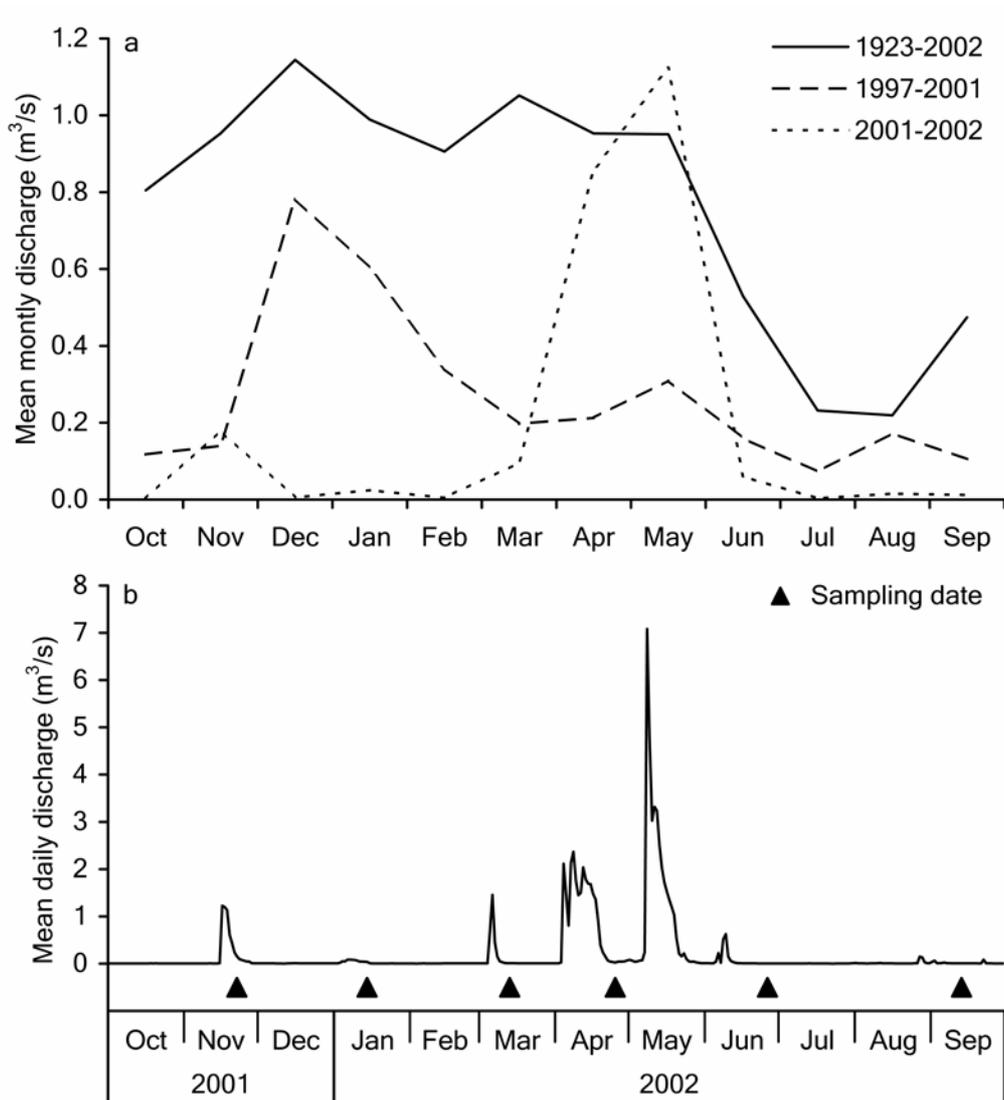


Figure 2.3 Mean monthly discharges (m³/s) of La Tordera stream over the periods 1923-2002, 1997-2001, and 2001-2002 (a), and mean daily discharge during the sampling period (2001-2002; b) continuously measured two km below the downstream reach. The arrowheads indicate sampling dates. Data from a gauging station maintained by the Catalan Water Agency (ACA).

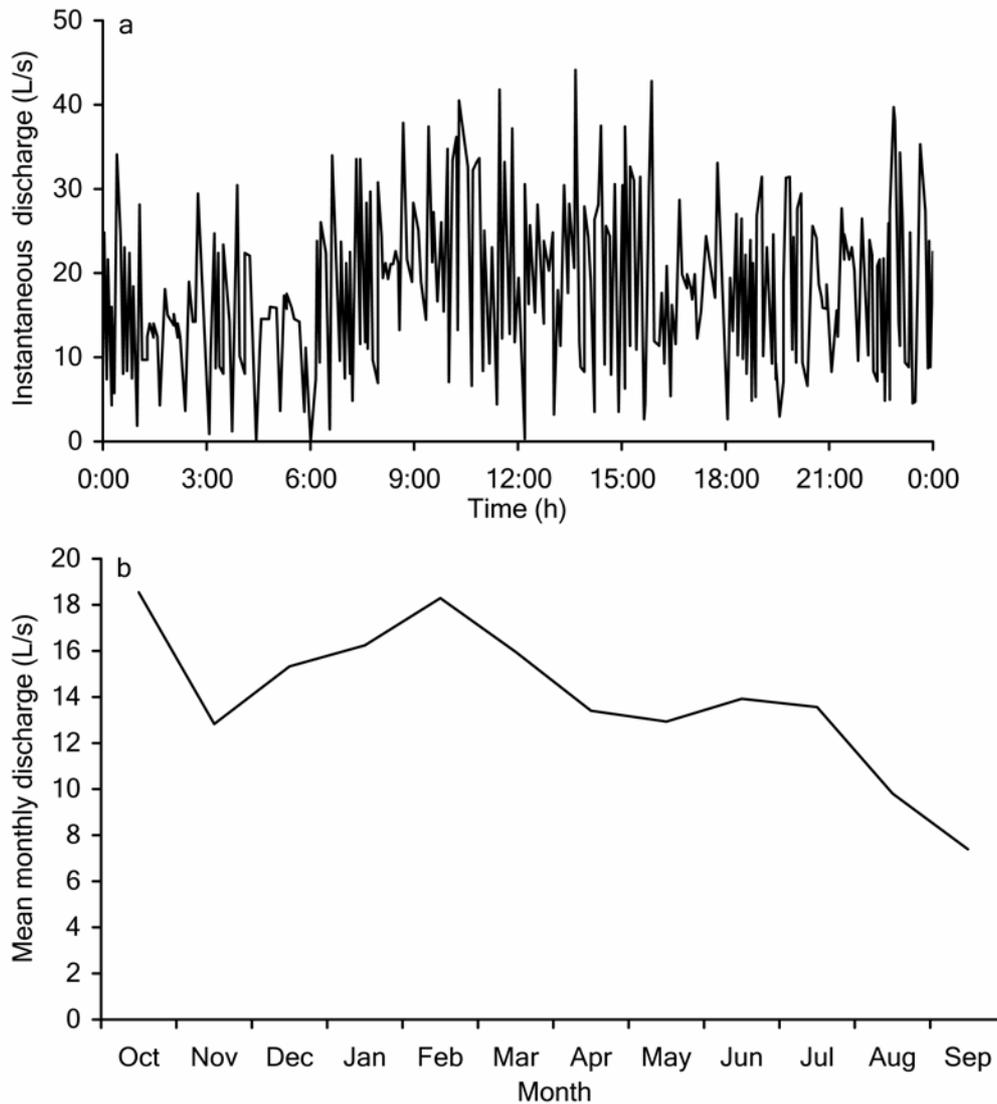


Figure 2.4 Instantaneous discharge (L/s) of the WWTP effluent over a typical 24-h cycle corresponding to March 2, 2001 (a) and mean monthly discharge (L/s) of the WWTP effluent over the hydrologic year 2000-2001 (b). Data provided by *Aigües de Catalunya*.

Response of benthic macroinvertebrates to a point source

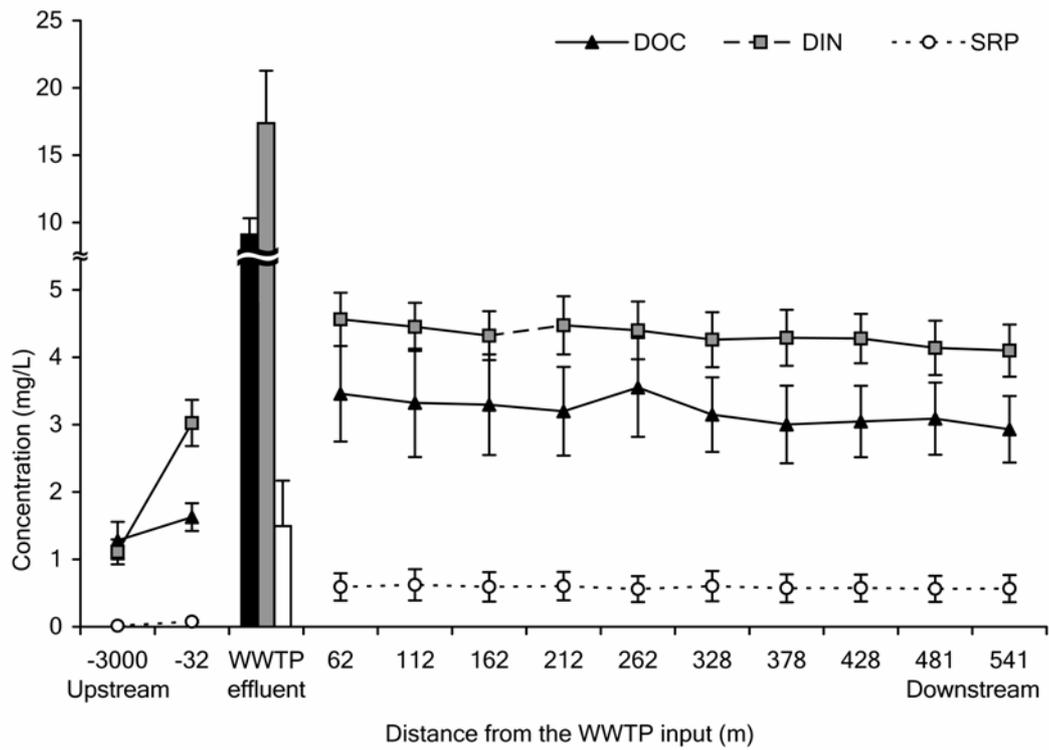


Figure 2.5 Mean concentrations (\pm SE) of dissolved organic carbon (DOC), dissolved inorganic nitrogen (DIN), and soluble reactive phosphorus (SRP) in two sampling points above the WWTP input, the WWTP effluent, and longitudinal variability over 541 m below the point source from August 2001 to September 2002. Upstream sites and WWTP effluent: $n = 8$, downstream sites: $n = 12$. The upstream reach was dry in four sampling dates. Unpublished data from G. Merseburger.

3. Methods

Experimental setting

We selected two 100-m long run-riffle reaches with similar substrata type and canopy cover located upstream and downstream of the wastewater treatment plant (WWTP) effluent input. The upstream reach was located three km above the WWTP effluent input and served as a reference reach. The downstream reach was located 500 m below the WWTP effluent input. In each reach, we defined six equidistant transects where we measured hydraulic parameters and collected chemical and biological samples. We collected samples on six dates over the hydrologic year 2001-2002: November of 2001 and January, March, April, June, and September of 2002.

Physical and chemical parameters

We visually estimated substrata particle sizes along with each of the six transects of each reach and sampling date according to a simplified Wentworth scale (Allan 1995). In each reach and sampling date, we measured velocity using a Neurtek Instruments[®] Miniair 2 flow meter, depths and width at each of the six predefined transects. Discharge was calculated according to the velocity-area method described in Gordon *et al.* (1992). Water temperature and dissolved oxygen concentration (DO) were measured in each reach over 24 h cycles on each sampling date with a WTW[®] Oxi 340-A oxygen meter. We measured specific conductance using a WTW[®] LF 340 conductivity meter. We measured photosynthetically active radiation (PAR) in each reach over 24 h cycles on each sampling date using a spherical quantum sensor (Li-193SA, Lincoln, NB, USA) located within 20 cm of stream water level on the middle of the reach at one representative location.

Water samples for nutrient analysis (18 replicates per reach and sampling date), dissolved organic carbon (DOC), and major ions (six replicates per reach and sampling date) were taken in each transect, filtered on site through preashed Whatman[®] GF/F glass fiber filters and stored on ice. Ammonium (NH₄⁺-N) concentration was analyzed on a Bran-Luebbe[®] Technicon Autoanalyzer II. Nitrite (NO₂⁻-N), nitrate (NO₃⁻-N), and soluble reactive phosphorus (SRP) concentrations were analyzed on a Bran-Luebbe[®] TRAACS 2000 Autoanalyzer. NO₂⁻-N and NO₃⁻-N were analyzed using the cadmium-copper reduction method, and SRP was done using the molybdenum blue colorimetric method. Dissolved inorganic nitrogen (DIN) was calculated by summing the concentrations of NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N. DOC concentration was analyzed using high-temperature catalytic oxidation (Shimadzu[®] TOC 5000 analyzer). Chloride (Cl⁻) and sulfide (SO₄²⁻) concentrations were analyzed using the capillary electrophoresis technique with a Waters[®] CIA-Quanta 5000. Cations (Na⁺, K⁺, Ca²⁺, and Mg²⁺) were analyzed using the ICP-OES technique with a Thermo Optek[®] Polyscan 61 E.

Periphyton

We measured periphyton chlorophyll *a* and biomass by randomly collecting four stones from each transect. We broke each collected stone and selected flat pieces from the upper surface of approximately four cm². Samples were frozen until analysis. To determine periphyton chlorophyll *a* we selected three replicates per transect (18 replicates per reach and sampling date). Pigment extraction was performed with 90% acetone (4 °C for 24 h) and processed according to Steinman and Lamberti (1996). Absorbance was measured with a Shimadzu double-beam spectrophotometer (UV-2100). To determine periphyton biomass, we used one replicate of each transect (six replicates per reach and sampling date). Samples were dried at 60 °C until constant weight, weighed, ashed

at 450 °C for 4-5 h, and reweighed to obtain ash free dry mass (AFDM). We measured the area of each rock piece using the computer program Scion Image (for Windows release Beta 4.0.2, Scion Corp., Frederick, Maryland) through high-resolution digital photographs of the stone pieces to determine pigment concentration and AFDM per unit area.

Benthic macroinvertebrates

One modified Surber sample (625 cm², 250 µm mesh size) was taken in each transect (six replicates per reach and sampling date). Samples were preserved in the field with 4% formaldehyde solution or frozen. In the laboratory, heavier inorganic substrates were removed by elutriation. All large invertebrates (> 5 mm) were hand-picked from the samples and then preserved in 70% ethanol. If necessary, the smallest invertebrates (250 µm-5 mm) were subsampled on an area basis (Moulton II et al. 2000). Invertebrates were counted by handpicking with the aid of a dissecting microscope at 15x magnification, identified to the lowest practical taxonomic level, enumerated, dried (60 °C until constant weight) and weighed to obtain dry mass. Taxa were assigned to their relative contribution to each functional feeding group according to Moog (2002).

Benthic organic matter and primary producers

We quantified standing stocks of benthic organic matter (BOM) and large primary producers from the macroinvertebrate samples. After macroinvertebrates were removed, remaining organic matter was sorted into BOM, filamentous algae, mosses, and vascular plants. BOM was separated into coarse particulate organic matter (CPOM; > 1mm) and fine particulate organic matter (FPOM; 250 µm-1 mm) using nested sieves. Each fraction was dried at 60 °C for one week, weighed, ashed at 450 °C for 4-5 h, and reweighed to obtain AFDM. Suspended particulate

organic matter (SPOM) concentrations were obtained by filtering known volumes of stream water. Water samples were obtained through a peristaltic pump from the nearest four cm to the streambed and filtered with ashed and weighed Whatman[®] GF/F glass fiber filters (pore size = 0.7 μm). Filters were dried at 60 °C until constant weight, weighed, ashed at 450 °C for 4-5 h, and reweighed to obtain AFDM.

Microhabitat variables

Prior to sample collection, we located a frame with the same sampling area as the modified Surber sampler (625 cm²). We measured mean, maximum and minimum water velocity, at near bed depth in three transects within the frame with a Neurtek Instruments[®] Miniair 2 flow meter. We measured maximum and minimum water depth within the frame. We visually estimated substrata particle sizes, along with each macroinvertebrate sample according to a simplified Wentworth scale (Allan 1995). We also visually estimated coverage of primary producers. We estimated chlorophyll *a* concentrations of each sample by multiplying the visually estimated coverage of periphyton by the mean chlorophyll *a* concentration of each reach and sampling date. We quantified standing stocks of BOM and large primary producers from the macroinvertebrate samples as described above. Substrate roughness (k_v), Froude number (Fr), Reynolds number (Re), and roughness shear velocity (V_*) were calculated from measured microhabitat variables according to Doisy and Rabeni (2001).

Elemental analysis

We collected samples of macroinvertebrates, CPOM, FPOM, SPOM, periphyton, filamentous algae, and mosses in each reach and sampling date when present. Samples of SPOM, periphyton, filamentous algae, and mosses were dried

at 60 °C until constant weight and homogenized. Samples of macroinvertebrates, CPOM, and FPOM were immediately frozen. When samples were thawed, macroinvertebrates were identified, dried and homogenized. Remaining BOM was sorted into CPOM and FPOM using nested sieves as described above, dried, and homogenized. All samples were weighed on a microbalance to the nearest μg . For C and N analysis, samples were weighed in tin capsules and analyzed with a Carlo Erba NA 2100 CHN analyzer (Carlo Erba Instruments, Milan, Italy). For P analysis, samples were oxidized with potassium persulphate in a microwave and were analyzed using the malachite green colorimetric technique (Fernández *et al.* 1985). When sample biomass for elemental analysis was limited, we combined macroinvertebrate taxa from different sampling dates. Mollusks were analyzed including the shell, because the shell is an intrinsic part of mollusks and it was virtually impossible to separate the shell from living tissue of certain taxa and small individuals.

Data analysis

Structure and composition of the macroinvertebrate community

We calculated several macroinvertebrate community descriptors for each reach and sampling date, including density (individuals/m²), biomass (g DW/m²), taxa richness (S), rarefaction coefficient (ES₁₀₀), as number of taxa expected for 100 individuals (Heck *et al.* 1975, Walsh *et al.* 2001), Shannon-Wiener diversity (H'; Lloyd *et al.* 1968), Pielou's evenness (J'; Clarke and Warwick 1994), and Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness (Lenat 1983, Barbour *et al.* 1999). Rarefaction coefficient, Shannon diversity, and Pielou's evenness were calculated from averaged macroinvertebrate densities of each reach and sampling date using the DIVERSE analysis routine of the computer package PRIMER (for Windows, version 5.2.2, Plymouth Marine Labs, Plymouth, UK).

We correlated the temporal variability of the considered community descriptors to the effect of the relative contribution of the point source and environmental conditions using two-tailed Spearman's rank correlation (R_s). The relative contribution of the point source was determined in terms of discharge and concentrations of DIN and SRP and was calculated as the difference between the value at the downstream reach and that at the upstream reach standardized for the value at the upstream reach in each sampling date. The considered environmental conditions were mean water temperature and peak discharge during the previous spate to each sampling date. The response of each community descriptor was measured as the difference between the value at the upstream reach and that at the downstream reach in each sampling date. We determined the weight of Pielou's evenness and taxa richness on Shannon diversity by using two-tailed Pearson's correlation (r). These analyses were performed using the statistical package SPSS (for Windows, version 11.0.1, SPSS Inc., Chicago, Illinois).

Similarities in taxa composition between the upstream reach and the downstream reach among sampling dates were measured using the Jaccard's index of similarity (Townsend et al. 1987). We compared individual macroinvertebrate densities and biomasses between the two reaches for each sampling date using the Bray-Curtis similarity (Bailey et al. 1998) included in the SIMPER analysis routine of the computer package PRIMER (for Windows, version 5.2.2, Plymouth Marine Labs, Plymouth, UK).

Graphical comparisons of community composition were made using abundance/biomass comparison (ABC) plots, whereby the cumulative percentage of total abundance and biomass accounted by each taxa is plotted on the y axis against the rank order of taxa on the x axis in logarithmic scale (Clarke and Warwick 1994). The shape and arrangement of the curves reflects community responses to disturbance. Elevated abundance curves represent high dominance by

few taxa, while a steeper slope indicates a more evenly structured community. In undisturbed communities, the curve for biomass lays above the curve for abundance for its entire length. In disturbed communities, the two curves are closely coincident and may cross each other. As disturbance becomes more severe, the abundance curve moves above the biomass curve.

We used ordination analysis to explore taxa and sample distribution within reaches and sampling dates. Initial analysis using detrended correspondence analysis (DCA) showed that the macroinvertebrate data set had a gradient length shorter than two standard deviation units (SD). Hence, we used linear models such as principal component analysis (PCA) for further analysis as recommended by ter Braak and Šmilauer (1998). Macroinvertebrate densities were $\log_{10}(x + 1)$ transformed to stabilize variances and normalize the data sets, and rare taxa were not excluded from the analysis (Cao *et al.* 1998, Cao 1999, Cao *et al.* 2001, Nijboer and Schmidt-Kloiber 2004). These analyses were conducted by the computer program CANOCO (for Windows, version 4.5, Plant research International, Wageningen, The Netherlands). The response of the PCA scores of axis I to the relative contribution of the point source and environmental conditions was measured as the difference between reach means of the first axis scores for each sampling date.

Functional organization of the benthic community

We assessed the effect of the relative contribution of the point source on the temporal variability of the difference in primary producers and organic matter between the two reaches using two-tailed Spearman's rank correlation (R_s). We compared macroinvertebrate densities and biomasses of each functional feeding group between the two reaches for each sampling date using the Bray-Curtis similarity (Bailey *et al.* 1998) included in the SIMPER analysis routine of the

computer package PRIMER (for Windows, version 5.2.2, Plymouth Marine Labs, Plymouth, UK). We used regression analysis to examine the relationship between density and biomass of each FFG and their presumed food resources. In each case, we tested linear, power, logarithmic and exponential regression procedures as being the most common relationships in nature. We reported the results from the procedure that best explained the relationship in each case. All analysis were done by using the statistical package SPSS (for Windows, version 11.0.1, SPSS Inc., Chicago, Illinois).

Microhabitat distribution of benthic macroinvertebrates

Correlations between density, biomass, and taxa richness of macroinvertebrates with microhabitat variables were determined using two-tailed Spearman's rank correlation (R_s) because data for many of the microhabitat variables could not be normalized (Doisy and Rabeni 2001).

We used ordination analysis to explore relationships between taxa distribution and microhabitat variables. Initial analysis using detrended correspondence analysis (DCA) revealed that the macroinvertebrate data sets had gradient length shorter than 2 SD. We used linear models for further analysis as recommended by ter Braak and Šmilauer (1998) when gradient lengths are short (< 3 SD). We obtained correlations of macroinvertebrate densities and biomasses with microhabitat variables for each reach through redundancy analysis (RDA). We used forward stepwise selection of the environmental variables that best determined the distribution of taxa densities or biomasses in each reach. Variables were tested through Monte Carlo permutation tests (9999 permutations) and only significant variables ($p < 0.05$) were included in the analyses. Macroinvertebrate densities and biomasses were $\log_{10}(x + 1)$ transformed to stabilize variances and normalize the data sets and all taxa were included (Cao *et al.* 1998, Cao 1999, Cao

et al. 2001). We considered separately the different live stages (larvae or nymph, pupae, and adult) of the recorded macroinvertebrates because they are susceptible to show different microhabitat requirements. Coverage of each substrata particle size was arcsin-square root transformed and all other microhabitat variables were $\log_{10}(x + 1)$ transformed. These analyses were conducted by the computer program CANOCO (for Windows, version 4.5, Plant research International, Wageningen, The Netherlands).

Elemental stoichiometry of macroinvertebrates

We compared %C, %N, and %P and C:N, C:P, and N:P ratios in taxa from the two reaches with paired *T*-tests to examine differences in macroinvertebrate elemental contents and elemental ratios between the two reaches. We compared elemental contents and elemental ratios between the two reaches and among taxonomic groups or functional feeding groups using two-way analysis of variance (ANOVA) procedures. Pairwise comparisons among group means were made using Tukey's studentized range test (HSD). Data were either arcsin-square root or $\log_{10}(x + 1)$ transformed prior to analysis. All analysis were done by using the statistical package SPSS (for Windows, version 11.0.1, SPSS Inc., Chicago, Illinois).

4. Effects of the point source on composition and structure of the macroinvertebrate community

Introduction

Human activity has long been known to have dramatic effects on stream invertebrates (Hynes 1978, Wiederholm 1984, Rosenberg and Resh 1993, Suren 2000). Nutrient enrichment decrease macroinvertebrate richness (Paul and Meyer 2001) by elimination of sensitive taxa, mostly represented by the insect orders Ephemeroptera, Plecoptera, and Trichoptera (Lenat 1983). Simultaneously, taxa considered resistant to pollution and adapted to unstable habitats such as Chironomidae and Oligochaeta are enhanced (Hynes 1978). This response to nutrient enrichment is well established worldwide, in arctic (Milner and Oswood 2000, Benstead *et al.* 2005), tropical (Ometo *et al.* 2000, Thorne *et al.* 2000), desert (Dor *et al.* 1976, Voelz *et al.* 2005), temperate (Roy *et al.* 2003), and Mediterranean streams (Prat *et al.* 1984, Prenda and Gallardo-Mayenco 1996). The effects of point source inputs on macroinvertebrate densities are not consistent. Several studies found a decrease in total density while others noticed an increase. Paul and Meyer (2001) attributed such incongruence to the separate effects of toxic compounds and nutrient enrichment, respectively. However, high nutrient concentrations can also result in a decreased density (Hynes 1978). Wallace and Webster (1996) argued that when resource availability is high, invertebrates invest less energy in searching for food resulting in an increase of biomass. Although not difficult to test, few studies have examined changes in macroinvertebrate biomass due to nutrient enrichment and common patterns remain unclear. Biotic indices based on benthic macroinvertebrates are widely used for bioassessments, but temporal variation of the benthic community is rarely considered (however, see Sandin and Johnson 2000, Morais *et al.* 2004).

Moreover, little attention has been paid to flow disturbance in polluted streams, even though it is known to play a central role in determining the structure of stream communities (Lake 2000).

We sampled the benthic macroinvertebrate community on six sampling dates over the hydrologic year 2001-2002 in one reach upstream and one reach downstream of a point source in La Tordera stream (Catalonia, NE Spain). Our objectives were to assess the effect of the point source on the structure and community composition of the benthic macroinvertebrate community in La Tordera stream and to examine the temporal variability of the considered community descriptors over a hydrologic year. We hypothesized that density and biomass of macroinvertebrates would increase below the point source according to previous studies conducted in stream ecosystems under moderate nutrient enrichment. We also expected a decrease of taxa richness and an increase of dominance by tolerant taxa below the point source that would translate into a lower diversity and community structure.

Results

Physical and chemical parameters

Discharge was higher in spring and autumn in the two reaches (Table 4.1). During the sampling period, discharge was on average 1.4 times higher at the downstream reach than at the upstream reach. Water temperatures were slightly lower in winter and higher in summer at the downstream reach than at the upstream reach. The downstream reach also had a higher diel temperature range, especially during the summer. Daily light was similar in the two reaches. Mean DO concentration at the downstream reach was consistently lower (1.1 mg/L lower on average) than at the upstream reach (Table 4.2). Specific conductance was relatively low in the two reaches, but it was almost two times higher at the

Table 4.1 Mean values of physical variables measured in the upstream reach (up) and the downstream reach (dw) over the six sampling dates in La Tordera stream. Q = discharge, W = width, D = depth, V = water velocity, T = water temperature, PAR = daily photosynthetically active radiation. n.a. = not available.

variables	Nov-01		Jan-02		Mar-02		Apr-02		Jun-02		Sep-02	
	up	dw	up	dw	up	dw	up	dw	up	dw	up	dw
Q (L/s)	158.9	331.5	50.1	106.5	254.7	280.6	414.9	508.4	64.5	83.1	105.6	185.7
W (m)	7.72	5.10	7.38	4.92	7.97	6.05	8.82	6.68	6.75	6.33	6.78	6.58
D (m)	0.15	0.15	0.09	0.14	0.16	0.17	0.16	0.20	0.09	0.11	0.11	0.13
V (m/s)	0.15	0.27	0.08	0.18	0.21	0.29	0.30	0.40	0.13	0.14	0.16	0.23
T ¹ (°C)	10.8	10.1	9.1	8.8	10.2	10.8	12.3	13.7	17.9	20.5	17.1	17.8
PAR ² (mol·m ⁻² ·d ⁻¹)	(10.0-11.8)	(9.1-11.5)	(8.0-10.6)	(7.5-10.1)	(8.9-12.0)	(8.2-13.2)	(10.4-15.2)	(11.4-16.6)	(16.4-20.0)	(18.3-22.8)	(16.3-18.0)	(16.0-20.0)
	n.a.	8.30	16.30	11.50	24.80	31.00	45.83	33.73	n.a.	37.50	12.06	n.a.

¹ Minimum and maximum values in parenthesis of temperature correspond to values registered during 24 h records performed for each reach and sampling date.

² Values of daily light correspond to values registered over 24 h cycles performed for each reach and sampling date.

Table 4.2 Mean values of chemical variables measured in the upstream reach (up) and the downstream reach (dw) over the six sampling dates in La Tordera stream . DO = dissolved oxygen, cond. = specific conductance, DOC = dissolved organic carbon, DIN = dissolved inorganic nitrogen, SRP = soluble reactive phosphorus. n.a. = not available.

variables	Nov-01		Jan-02		Mar-02		Apr-02		Jun-02		Sep-02	
	up	dw										
DO ¹ (mg/L)	10.30	9.70	11.10	8.60	10.60	10.10	10.60	9.70	8.60	7.70	8.60	7.50
cond. (µS/cm)	205.2	272.2	165.6	318.1	124.2	175.1	92.0	173.8	163.9	327.7	165.4	307.3
DOC (mg/L)	2.39	2.71	0.83	2.38	2.08	2.07	0.86	1.73	0.68	1.01	0.55	1.56
NH ₄ ⁺ -N (mg N/L)	0.01	0.42	0.05	2.14	0.06	0.50	0.01	0.48	0.07	0.15	0.02	0.20
NO ₂ ⁻ -N (mg N/L)	0.00	0.06	0.01	0.09	n.a.	0.02	0.03	0.04	0.02	0.07	n.a.	0.06
NO ₃ ⁻ -N (mg N/L)	2.20	2.73	1.51	3.30	0.80	2.16	0.90	1.72	0.74	4.11	1.18	1.87
DIN (mg N/L)	2.21	3.21	1.57	5.53	0.86	2.68	0.93	2.24	0.83	4.33	1.20	2.13
SRP (mg P/L)	0.02	0.06	0.01	0.06	0.01	0.05	0.01	0.23	0.01	0.54	0.01	0.63
DIN:SRP (molar)	326	116	496	200	212	114	148	22	310	18	295	8
Na ⁺ (meq/L)	0.57	0.89	0.56	1.40	0.38	0.75	0.36	0.56	0.53	1.08	0.51	0.99
K ⁺ (meq/L)	0.04	0.08	0.03	0.11	0.02	0.05	0.01	0.03	0.02	0.07	0.02	0.07
Ca ²⁺ (meq/L)	0.74	1.05	0.62	0.99	0.47	0.68	0.48	0.71	0.72	1.34	0.70	1.02
Mg ²⁺ (meq/L)	0.42	0.48	0.35	0.42	0.28	0.33	0.27	0.33	0.38	0.51	0.38	0.43
Cl ⁻ (meq/L)	0.34	0.78	0.34	0.99	0.26	0.48	0.26	0.36	0.42	0.62	0.36	0.64
SO ₄ ²⁻ (meq/L)	0.38	0.64	0.44	0.65	0.29	0.38	0.30	0.33	0.42	0.57	0.78	0.78

¹ Minimum and maximum values of DO concentration correspond to values registered during 24 h records performed for each reach and sampling date.

downstream reach than at the upstream reach. Nutrient concentrations were higher at the downstream reach on all dates, but the effect differed among nutrients (Table 4.2). On average, NO_3^- -N concentration increased twofold below the point source while NH_4^+ -N and SRP concentrations increased 20-fold. The effect on DOC concentration was less noticeable. Major ions concentrations were also higher at the downstream reach than at the upstream reach, especially K^+ , Na^+ , and Cl^- . Substrate composition of the streambed was similar in the two reaches and was dominated by boulders, cobbles, and pebbles (Figure 4.1).

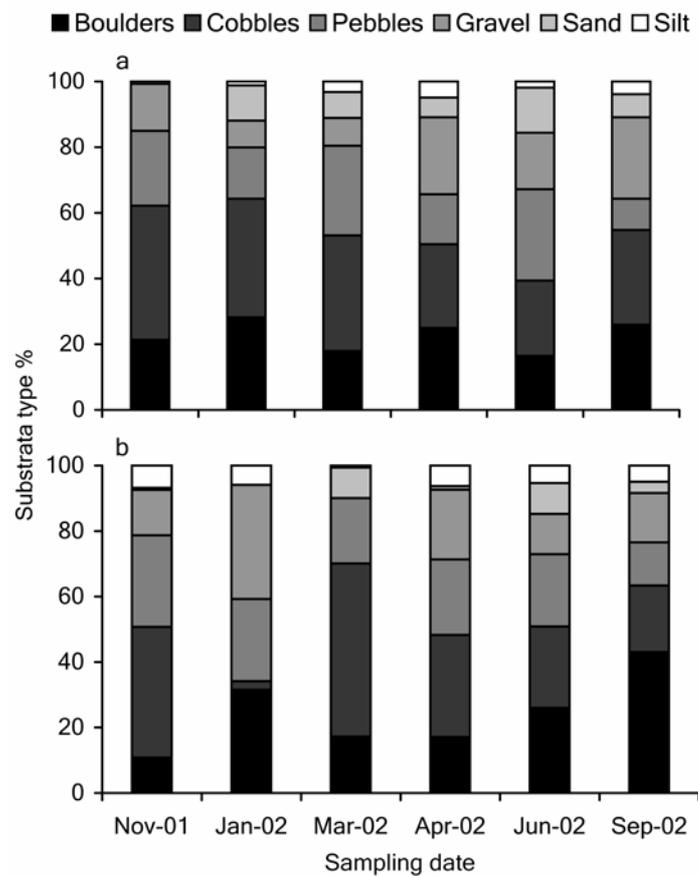


Figure 4.1 Streambed substrate composition at the upstream reach (a) and at the downstream reach (b) in La Tordera stream over the six sampling dates.

Density and biomass of benthic macroinvertebrates

We collected over 150,000 benthic macroinvertebrates distributed in 90 taxonomic groups in the two reaches over the six sampling dates. The most abundant taxon from November 2001 to March 2002 at the upstream reach was the chironomid subfamily Orthocladiinae (Appendix 4.1). Water mites sharply increased in April 2002 and *Baetis rhodani* was the dominant taxon in the samples of June and September 2002. At the downstream reach, the most common taxa were chironomids (Orthocladiinae and tribe Tanytarsini), tubificids, nematodes and nematomorphs, but like in the upstream reach, *B. rhodani* became dominant in June and September 2002. Several taxa were only found in the upstream reach including the mayfly *Epeorus torrentium*, most stoneflies such as *Capnioneura mitis*, *Siphonoperla torrentium* or *Isoperla grammatica*, and many caddisflies such as *Rhyacophila dorsalis*, *Mystacides azurea* or *Sericostoma personatum* (Appendix 4.1). However, after the spate of April 2002, some taxa that were previously restricted to the upstream reach, such as *Leuctra geniculata* or *Ecdyonurus angelieri*, appeared at the downstream reach. Other invertebrates such as the clam *Pisidium casertanum* and the leech *Glossiphonia* sp. were found only in the downstream reach. Jaccard's index of similarity in community composition revealed higher resemblances between the two reaches in summer samples (0.62 of similarity) than in the rest of the samples (from 0.44 to 0.51 of similarity; Table 4.3).

Table 4.3 Bray-Curtis index of similarity in individual density (B-C d), Bray-Curtis index of similarity in individual biomass (B-C b), and Jaccard's index of similarity in community composition (Jacc) between the upstream reach and the downstream reach.

	Nov-01	Jan-02	Mar-02	Apr-02	Jun-02	Sep-02
B-C d	26.86	23.08	15.47	22.77	64.25	23.53
B-C b	6.55	11.06	11.52	27.42	43.30	17.56
Jacc	0.48	0.48	0.51	0.44	0.62	0.62

At the upstream reach, Orthoclaadiinae, *B. rhodani*, and Hydracarina represented, on average and in this order, the higher contribution to total macroinvertebrate density. At the downstream reach, the most abundant taxa were Tanytarsini and Orthoclaadiinae. Total macroinvertebrate density was consistently higher at the downstream reach than at the upstream reach over the sampling period (Figure 4.2a). However, the difference in total macroinvertebrate density between the two reaches was lower in April and June 2002. Bray-Curtis index of similarity in individual macroinvertebrate densities was more than two times higher in June 2002 than in the rest of the samplings (Table 4.3).

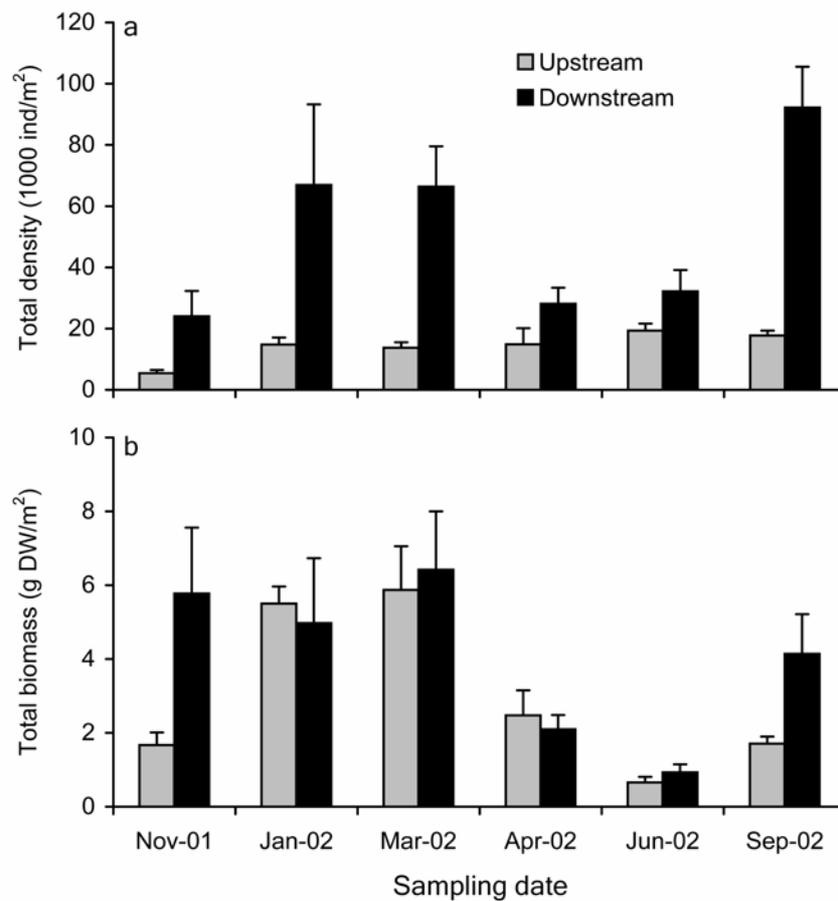


Figure 4.2 Mean \pm SE values of total macroinvertebrate density (a) and biomass (b) in the two reaches of La Tordera stream over the sampling period.

Total macroinvertebrate biomass was dominated by *Eiseniella tetraedra*, *Ancylus fluviatilis*, and *B. rhodani* at the upstream reach, and by *Erpobdella* sp., *Physella acuta*, Tanytarsini, Orthoclaadiinae, and Simuliidae, at the downstream reach. The temporal variability of total macroinvertebrate biomass was very similar in the two reaches over the sampling period (Figure 4.2b). Total biomass was only different between the two reaches in November 2001 and September 2002, when it was higher at the downstream reach. On most dates, the downstream reach presented a higher ratio of total density to total biomass indicating predominance of smaller body size of benthic macroinvertebrates relative to the upstream reach. Bray-Curtis index of similarity in taxa biomass between the two study reaches were quite low on all dates but increased in April 2002 and especially in June 2002 (Table 4.3).

The response of total density and total biomass of macroinvertebrates to the point source was negatively correlated with the peak discharge of the previous spate ($R_s = -0.812$, $p = 0.050$ for the two descriptors; Table 4.4). The difference in total macroinvertebrate biomass between the upstream and the downstream

Table 4.4 Two-tailed Spearman rank correlations between the difference of each community descriptor and centroids by date and reach of the sample PCA scores of axis I between the two reaches and the relative contribution of the WWTP effluent to the downstream reach in terms of discharge (rel. Q), dissolved inorganic nitrogen (rel. DIN), and soluble reactive phosphorus (rel. SRP), and environmental conditions as mean water temperature (T) and peak discharge of the previous spate event (Q max). $n = 6$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	rel. Q	rel. DIN	rel. SRP	T	Q max
Δ Total density	0.086	-0.371	-0.257	-0.257	-0.812*
Δ Total biomass	0.314	-0.829*	-0.657	-0.429	-0.812*
Δ Taxa richness	0.463	0.062	-0.741	-0.926**	-0.329
Δ EPT richness	0.986***	-0.232	-0.203	-0.551	-0.618
Δ Shannon diversity	-0.257	0.314	-0.086	-0.257	-0.203
Δ Pielou's evenness	-0.200	0.486	0.029	-0.143	-0.145
Δ PCA scores (axis I)	0.371	-0.086	-0.714	-0.886*	-0.551

reaches was also negatively correlated with the relative contribution of DIN of the point source ($R_s = -0.829$, $p = 0.042$).

Diversity of benthic macroinvertebrates

Taxa richness was between eight and 18 units higher at the upstream reach than at the downstream reach (Figure 4.3a). At the downstream reach, 24 taxa present at the upstream reach were absent (Appendix 4.1). Only four taxa that were present at the downstream reach were not found at the upstream reach. In the two reaches, taxa richness was higher in winter and decreased in spring. The rarefaction coefficient was higher at the upstream reach than at the downstream reach on all sampling dates, but in June 2002 when it was similar in the two reaches (Figure 4.3b). Shannon diversity was much higher at the upstream reach than at the downstream reach on three of the six sampling dates (Figure 4.3c). Values were very similar between the two reaches in November 2001, April and June 2002. Similarly, the shapes of the Pielou's evenness curves of the two reaches through the sampling period followed the same pattern as Shannon diversity (Figure 4.3d). At the upstream reach, Shannon diversity was strongly correlated with Pielou's evenness ($r = 0.992$, $p < 0.0005$), whereas the weight of taxa richness was not significant ($r = 0.384$, $p > 0.05$). In contrast, at the downstream reach the weight of the two components on Shannon diversity, evenness and taxa richness, were similar ($r = 0.998$, $p < 0.0005$ and $r = 0.832$, $p = 0.040$, respectively). EPT (Ephemeroptera, Plecoptera, and Trichoptera) richness was between six and ten units higher at the upstream than at the downstream reach (Figure 4.4). The percentage of EPT taxa responsible for differences in taxa richness between the two reaches ranged from 46% in March 2002 to 100% in September 2002.

The response of taxa richness was negatively correlated with mean water

temperature ($R_S = -0.926$, $p = 0.008$), whereas the response of EPT richness was higher with decreasing stream dilution of the point source ($R_S = 0.986$, $p < 0.0005$; Table 4.4). On the other hand, our results did not show any significant relationship between the response of Shannon diversity or Pielou's evenness with the relative contribution of the point source, water temperature or peak discharge of the previous spate event.

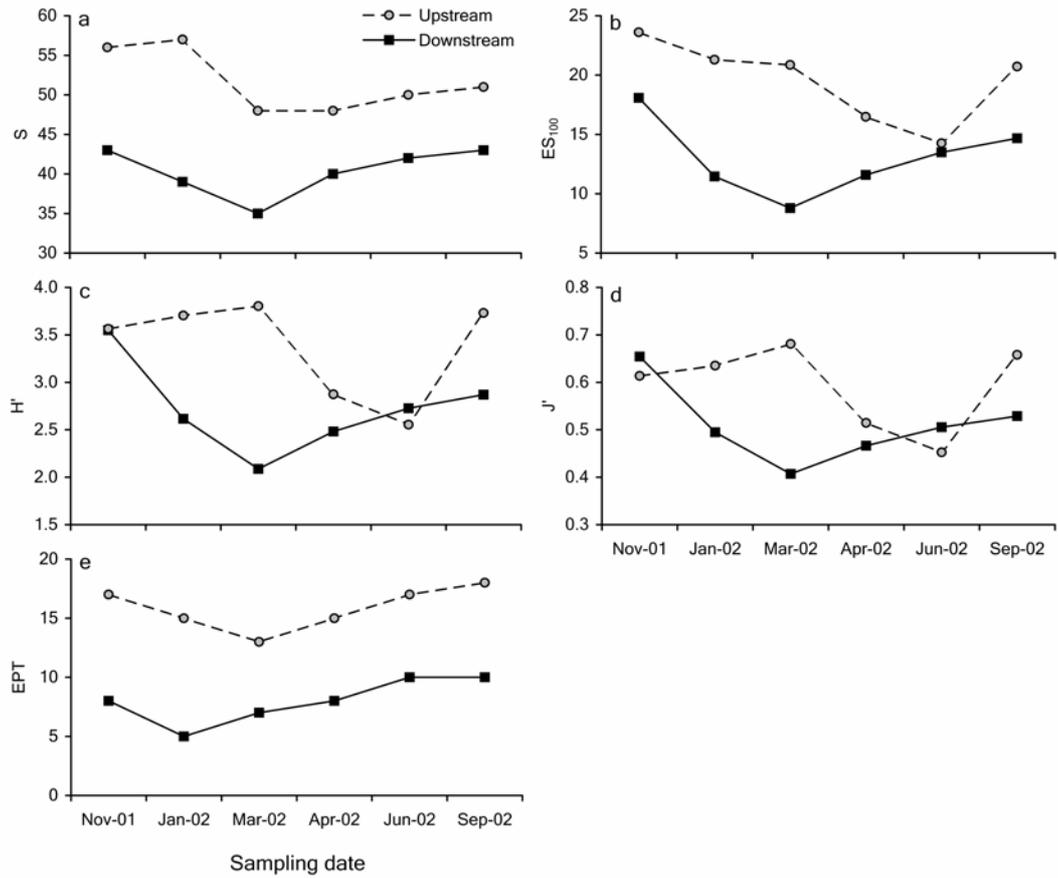


Figure 4.3 Taxa richness (S), rarefaction coefficient (ES_{100}), Shannon diversity (H'), Pielou's evenness (J'), and EPT richness (EPT) in the two reaches of La Tordera stream over the sampling period.

Structure of the benthic communities

In the abundance/biomass comparison (ABC) plots for the upstream reach, the *k*-dominance curve of macroinvertebrate biomass lied above the curve for abundance for its entire length in November 2001, January and March 2002 (Figure 4.4). After the spate of April 2002, the *k*-dominance curve of macroinvertebrate biomass was placed below the curve for abundance. The lower elevation of the *k*-dominance curve of macroinvertebrate abundance in March and September 2002 relative to the other sampling dates revealed that the macroinvertebrate community was more evenly structured. At the downstream reach, the *k*-dominance curve of macroinvertebrate biomass lied below the curve for abundance on all sampling dates but in November 2001 (Figure 4.5). In addition, the *k*-dominance curve of macroinvertebrate abundance and the distance between taxa indicate that the dominance was lower in November 2002.

Ordination analyses

The PCA performed for 72 samples and 104 taxa explained 40.5% of the variance in the first two axes (axis I = 26.8%; axis II = 13.7%). Axis I was best explained by a positive relationship with *Erpobdela* sp., *Helobdella stagnalis*, Tubificidae, Nematoda, Nematomorpha, Tanytarsini, and *Pisidium casertanum*, and by a negative relationship with *Serratella ignita*, *Ecdyonurus angelieri* and *Leuctra geniculata* (Figure 4.6a). Axis II was positively related to *Baetis fuscatus*, Tanyptodinae, *Hydropsyche instabilis*, and *B. rhodani*, and negatively related to Naididae, Cladocera, *Potamopyrgus antipodarum*, *Agabus* sp., *Capnioneura mitis*, and *Isoperla grammatica*.

Distribution of sites along axis I of the PCA clearly separated the samples from the two study reaches whereas axis II discriminates cases according to the temporal pattern (Figure 4.6b). Samples of the upstream reach plotted to the left

Response of benthic macroinvertebrates to a point source

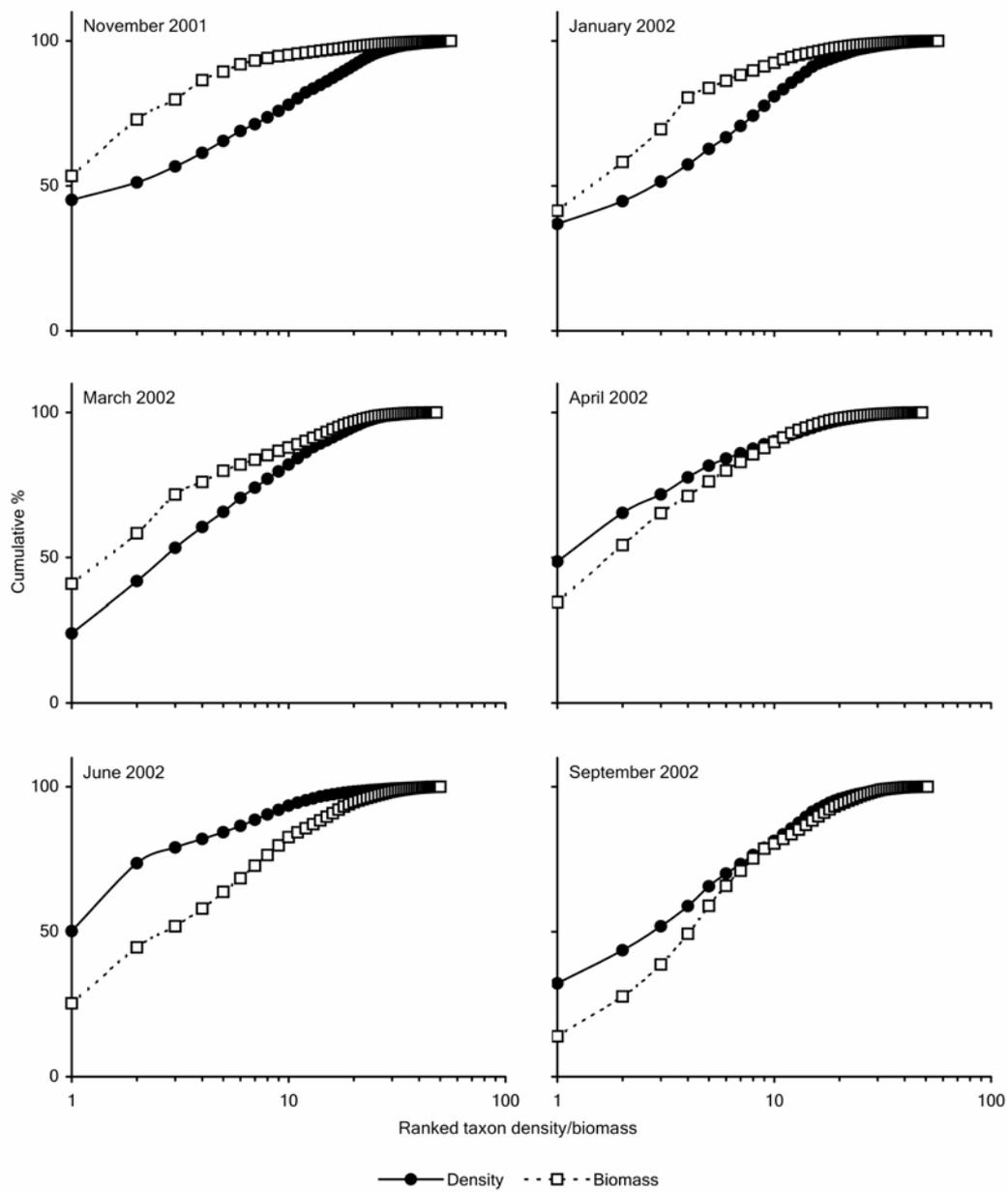


Figure 4.4 Abundance/biomass comparison (ABC) plots of benthic macroinvertebrates within the upstream reach over the six sampling dates.

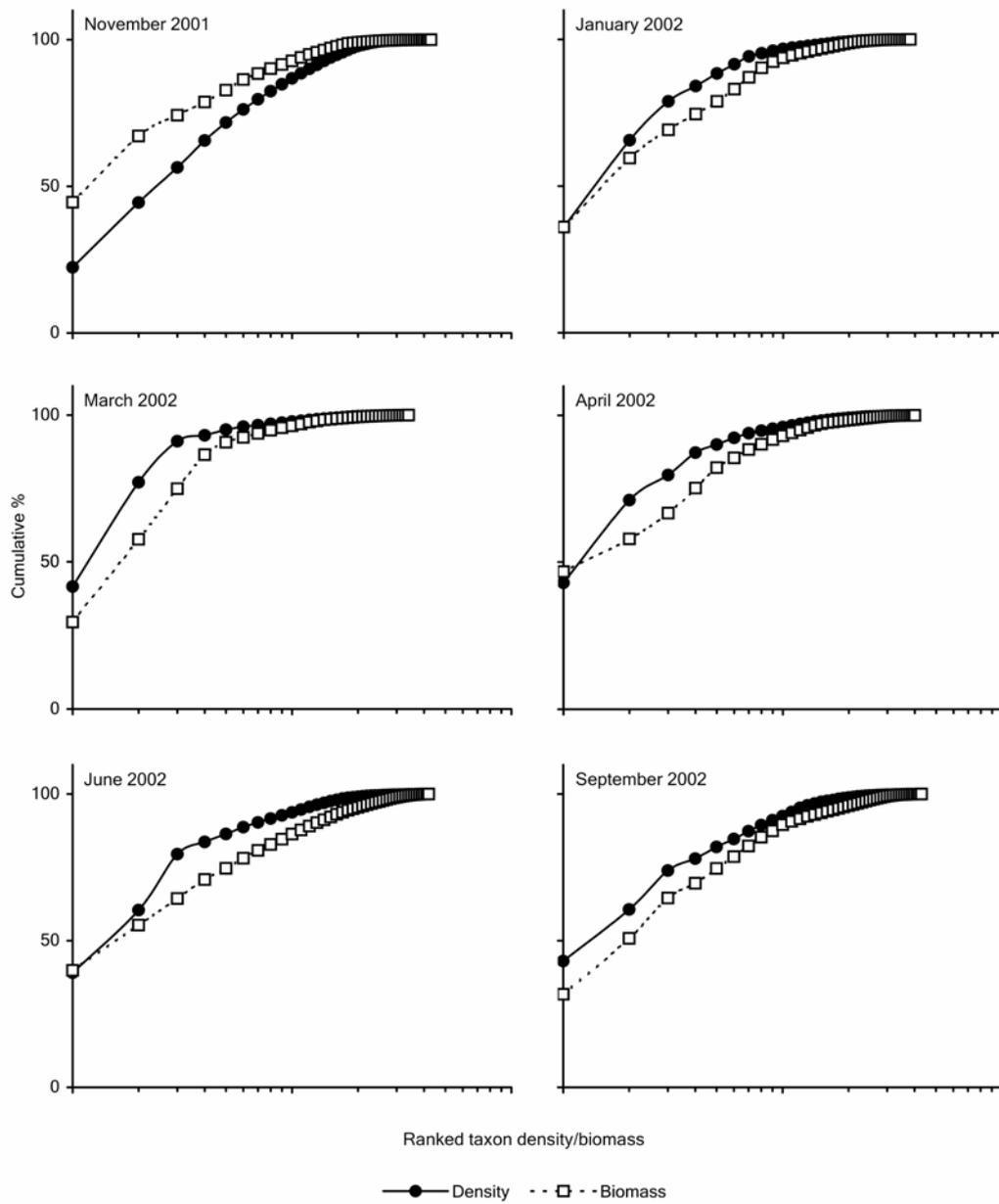


Figure 4.5 Abundance/biomass comparison (ABC) plots of benthic macroinvertebrates within the downstream reach over the six sampling dates.

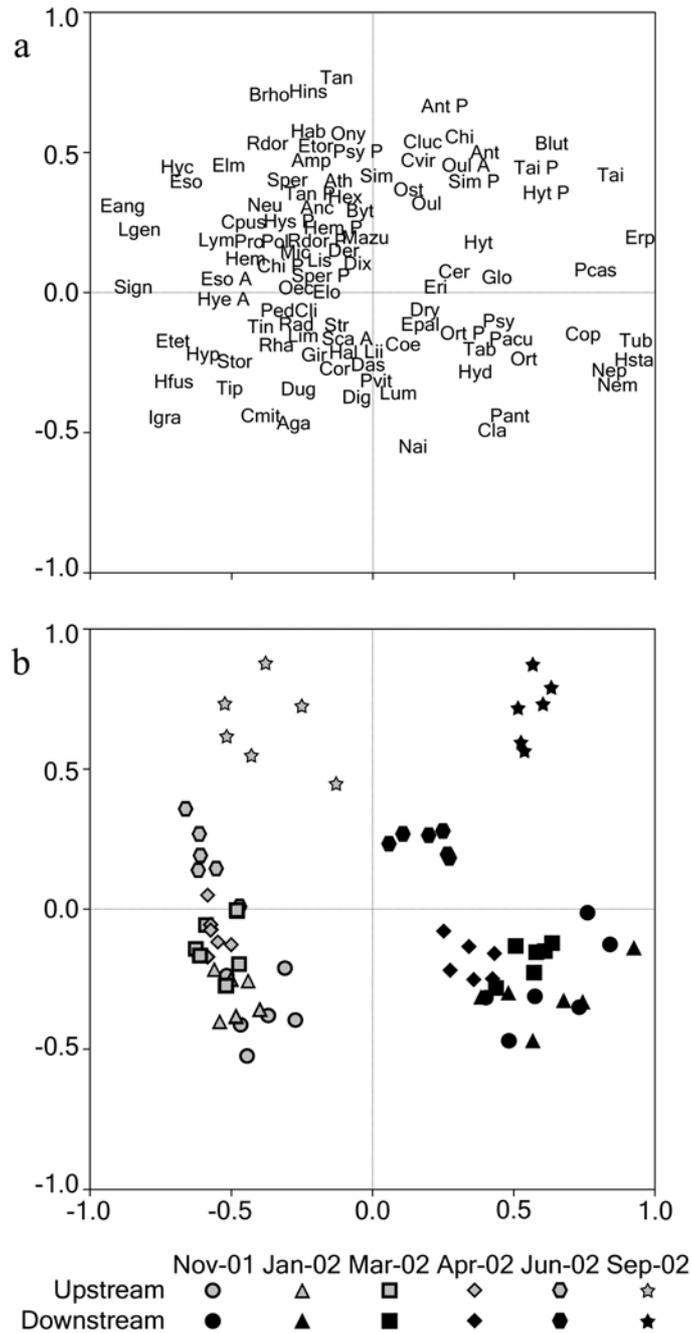


Figure 4.6 Principal component analysis (PCA) ordination of taxa composition (a) and macroinvertebrate samples (b) of the two reaches of La Tordera stream over the sampling period. See Appendix 4.1 for taxon codes.

while those of the downstream reach plotted on the right. Winter samples remained at the bottom of the diagram and summer samples were located at the top of the diagram.

No significant correlations were found between the relative contribution of discharge, DIN or SRP by the point source input and the PCA site scores of axis I, whereas mean water temperature explained a significant part of the variability of this response ($R_S = -0.886$, $p = 0.019$; Table 4.4).

Discussion

Effects of the point source on the benthic macroinvertebrate community

At La Tordera stream, degradation of water quality was moderate compared to studies performed in nearby streams (Martí *et al.* 2004). However, the effects of the point source were sufficient to lead to observable consequences on the macroinvertebrate community. The conditions favored taxa with small body sizes, short life spans, and high reproduction rates. As a result, total density of macroinvertebrates was much higher at the downstream reach than at the upstream reach. Several studies maintained that human impact decreased total density of macroinvertebrates (Garie and McIntosh 1986, Kerans and Karr 1994, Prenda and Gallardo-Mayenco 1996). In contrast, other studies found that total density of macroinvertebrates was not affected by urbanization (Jones and Clark 1987, Roy *et al.* 2003), or even increase under certain nutrient enrichment (Wiederholm 1984, Miltner and Rankin 1998, Morais *et al.* 2004). Such contradictory results may be explained by the subsidy-stress hypothesis proposed by Odum *et al.* (1979). According to this assumption, certain community descriptors follow a response curve to usable inputs rather than a linear trend. Thus, density of macroinvertebrates will increase under moderate nutrient enrichment and decrease under high nutrient inputs. Therefore, linear trends might

result from studies performed on narrow ranges of organic pollution or influenced by other factors such as toxic compounds.

Total biomass of macroinvertebrates did not differ between the two reaches. This result contradicts our initial hypothesis that total biomass would also be enhanced with nutrient enrichment. An increase in food availability may allow consumers to invest less energy in searching for food, thus increasing their feeding efficiency (Wallace and Webster 1996). Similarly, a study in a plains stream of north central Colorado observed an increase in biomass of macroinvertebrates below point and diffuse sources (Shieh *et al.* 2003). Our results indicate that macroinvertebrate biomass may have been limited by factors other than nutrient concentrations.

The changes induced by the point source into the stream resulted in a decrease in taxa richness. The decline in EPT richness was responsible for a high proportion of the decrease in taxa richness at the downstream reach. Although diversity indices were suggested as a useful metric to monitor water quality in the middle of the 20th century (Pinder and Farr 1987, Lenat and Resh 2001), they were rarely tested. A similar study in a southwestern Portuguese stream found that Shannon diversity and evenness were rather stable and were always lower in a reach affected by a wastewater treatment plant (WWTP) input relative to a non-impaired reach (Morais *et al.* 2004). In our study, Shannon diversity and evenness were highly variable and the differences between the two reaches were not consistent over the six sampling dates. Our results support the criticisms made by Lenat (1983) that diversity indices are not convenient for bioassessments because can be highly dependent on factors other than organic pollution.

The point source input clearly affected taxa composition of the benthic community of La Tordera stream. At the downstream reach, some taxa were eliminated while other were subsidized. Stoneflies, mayflies, and caddisflies

were the most sensitive groups to the point source, in agreement with previous research (Lenat 1983, Roy *et al.* 2003). On the contrary, chironomids, oligochaetes, and nematodes were favored by nutrient enrichment as other studies have reported (Hynes 1978, Prenda and Gallardo-Mayenco 1996). The ambient conditions at the downstream reach allowed the colonization of few taxa but were, at the same time, restrictive for a number of taxa. Indeed, taxa that appeared only at the downstream reach (e.g., *Pisidium casertanum* and *Glossiphonia* sp.) were rather tolerant to pollution while the taxa eliminated (e.g., *Epeorus torrentium*, *Isoperla grammatica*, and *Sericostoma personatum*) were considered sensitive to pollution (Tachet *et al.* 2000).

Temporal variability

Our results show that community descriptors commonly used for bioassessment might present a high temporal variability not directly related to organic pollution. Therefore, results may lead to misguided conclusions. At the downstream reach, total density of macroinvertebrates was higher for most, but not all dates. Our results suggest that flooding can decrease macroinvertebrate density. This is consistent with the findings of others (e.g., Grimm and Fisher 1989, Boulton *et al.* 1992, Townsend *et al.* 1998). Although detailed studies are required, our research suggests that the community of the upstream reach was more resistant and resilient to spates, in terms of total density, than the community of the downstream reach. Such different response to natural disturbance led to similar total density of macroinvertebrates in the two reaches after the spates of April and May 2002. In this sense, the relatively low densities at the upstream reach may allow a faster recovery of the benthic community. The higher relative density of mayflies and stoneflies relative to dipterans and mollusks in the upstream reach may also have supplied higher stability (Scarsbrook 2002). We

also detected higher similarities in terms of individual biomass after spates but, in contrast, total biomass of macroinvertebrates did not differ between the two reaches for any sampling date. Total biomass of macroinvertebrates is not commonly used in bioassessments because of the effort to obtain good estimates (Bernardini *et al.* 2000) and the lack of clear patterns in response to organic pollution. Flow disturbance occurring during the sampling period may play an important role in biomass constraints as reported by Grimm and Fisher (1989). However, the observed independence of macroinvertebrate biomass to nutrient concentrations was not simply a consequence of flow disturbance or macroinvertebrate biomass would have been higher at the downstream reach in January and March 2002. On the other hand, even though the biomass of the two reaches appeared to be equally affected by spates, at the downstream reach total macroinvertebrate biomass increased faster than at the upstream reach. However, the macroinvertebrate biomass of the two reaches seemed to be limited by the same asymptote. The higher recovery of macroinvertebrate biomass below the point source may result from an increase in productivity of nutrient enriched ecosystems as seen in previous studies (Shieh *et al.* 2003).

Differences in taxa richness and EPT richness between the two reaches were relatively invariable. Consistent with the findings of Gasith and Resh (1999), at the upstream reach, taxa richness was higher in winter than in summer, and was not affected by the spates of April and May 2002. At the downstream reach, taxa richness was also relatively high in winter and decreased in March 2002, but increased again in the summer. Similarities in taxa composition between the two reaches were higher in the summer and the difference of taxa richness between the reaches was significantly correlated with water temperature. These results contradict the findings of Gasith and Resh (1999) that the effect of pollution will be greater in the summer because stream dilution is lower in the Mediterranean

climate. Such incongruence probably occurred because spates that occurred in the spring of 2002 increased stream dilution improving water quality (Prenda and Gallardo-Mayenco 1996), and because flooding may enhance drift from upstream reaches (Gasith and Resh 1999). EPT richness was consistently higher at the upstream reach than at the downstream reach and the variability was relatively low in the two reaches. The difference in EPT richness between the two reaches seemed to be directly related to the effect of the point source on the stream. The high statistical power of taxa richness and EPT richness (Sandin and Johnson 2000) along with the ease of calculation makes these two indicators good candidates for water quality assessments (Roy *et al.* 2003).

The variation of Shannon diversity revealed a higher dependence on the evenness component than on taxa richness in the two reaches of La Tordera stream. The decrease of taxa richness and the increase of dominance by few taxa at the downstream reach resulted in a lower Shannon diversity than at the upstream reach on three dates. Morais *et al.* (2004) found that Shannon diversity and evenness increased at the impaired reach after flood disturbance. Our results show that these metrics not only increased at the downstream reach but also decreased at the upstream reach. In addition, the response was more noticeable, probably because the flood event was more severe, indicating that flood disturbance can homogenize the communities of impaired and pristine sites. Previous studies in a nearby river found that Shannon diversity was not related to pollution but with flow disturbance (Puig *et al.* 1987). A study in a chalk stream in England (Pinder and Farr 1987) attributed an increase of Shannon diversity below a WWTP input to a subsidy effect on oligotrophic waters. Although nutrient concentrations in our stream were comparable to that of the streams studied by Pinder and Farr (1987), we did not detect such a relationship. The observed independence of the Shannon diversity response to the relative

contribution of the point source suggests that it was driven by factors other than the point source. In this sense, flow related disturbance and population dynamics of certain taxa had important consequences on the structure of the benthic community in this stream. From July to October 2001, the two reaches were affected by a drought when the upstream reach completely dried. In November 2001, the increase of flow increased habitat suitability (Boulton 2003) and translated to an unusually high evenness at the downstream reach. In April 2002, water mites suddenly increased at the upstream reach and decreased evenness. Previous studies reported relevant alterations of the community structure due to population dynamics of water mites (Di Sabatino *et al.* 2000). The spate of May 2002 considerably decreased the abundance of all macroinvertebrate taxa in the two reaches. This disturbance had harmful consequences on the structure of the macroinvertebrate communities, as has been seen before in nearby streams (Argerich *et al.* 2004). In June 2002, the communities of the two reaches were dominated by a large number of young *Baetis rhodani* nymphs. This translated to an important decay of evenness at the upstream reach, where the proportion of this mayfly was much higher. The sudden dominance by *B. rhodani* may have simply been to population dynamics (Humpesch 1979), or determined by the spate that occurred one month prior (Boulton *et al.* 1992). Such erratic fluctuations in evenness led many authors to question the reliability of diversity indices in bioassessment (Lenat 1983), and locate richness measures among the best indicators to detect water degradation (Sandin and Johnson 2000, Ofenböck *et al.* 2004).

The two reaches were clearly separated by community composition over the sampling period. We expected to find higher differences in taxa composition between the two reaches during the summer. The summer drought that characterizes Mediterranean-type streams led to low stream dilution accentuating

the effect of organic pollution. Contrarily, the differences between means of the sample scores of the first axis in the PCA were lower in summer, when water temperature was higher but also after major spates occurred. This supports our hypothesis that flooding may modulate the effect of the point source. The second axis of the PCA split the samples of the two reaches according to the same temporal variability indicating that the point source did not modified the seasonal patterns of the macroinvertebrate community.

Few studies considered potential effects of flooding in streams affected by point sources. Although our study was not designed to assess the effects of flow disturbance, we detected higher similarities in total and individual densities and biomasses, taxa richness, EPT richness, and community composition after the spates of April and May 2002. In addition, the response of total density and total biomass were negatively related to the magnitude of the preceding spate. These results suggest that intermediate frequencies and intensities of flooding (Townsend *et al.* 1997) might act as a reset mechanism (Lake 2000). Flow disturbance is probably the most important organizing factor for stream ecosystems (Resh *et al.* 1988) that could contribute in restoring stream benthos to a certain degree. Our findings lend support to the opinion of Collier and Quinn (2003) that regular sampling is required to obtain valuable assessments. Population dynamics, hydraulic regime, and natural disturbance may result in marked changes in the macroinvertebrate community and lead to false conclusions about the effects of human impact.

Conclusions

The WWTP effluent of Sta. Maria de Palautordera and related outflows substantially increased nutrient concentrations, organic matter, and discharge into La Tordera stream. As a result, sensitive taxa, mainly mayflies, stoneflies, and

caddisflies, declined while tolerant taxa, mainly midges, blackflies, leeches, and snails increased. This translated in an increase of total macroinvertebrate density but did not altered total biomass. Calculation of different community metrics over regular samplings in combination with detailed analysis of hydraulic regimes, natural disturbances, and population dynamics provide valuable information for stream bioassessments. There is now a substantial body of research on the effects of different human impacts on stream ecosystems but the multivariate nature of macroinvertebrate communities makes difficult to establish clear patterns for density, biomass, and diversity indices. In contrast, the differences in taxa richness and EPT richness were consistent over the sampling period lending support to previous research that found these two indices as the most promising for biomonitoring. Shannon diversity, evenness, and ABC plots supplied valuable information about the structure of macroinvertebrate communities but were highly dependent to factors other than nutrient enrichment.

The damaging effects of flooding and drying on macroinvertebrate communities have been well documented. However, our results revealed that flow disturbance could substantially contribute to amelioration of water quality in human altered streams. The similarity between the two reaches, reference and altered, was higher after flooding. This happened because flooding scoured out the two reaches but also allowed certain taxa get to the downstream reach. We suggest that further research should focus on the study of the use of drying and flooding manipulations for future management plants understood as reset mechanisms for stream ecosystems.

Summary

Below the point source, discharge and specific conductance were higher than at the upstream reach, dissolved oxygen decreased 1 mg/L, $\text{NH}_4^+\text{-N}$ and SRP concentrations were 20 times higher, and periphyton chlorophyll *a* was five times higher. Total macroinvertebrate density was higher at the downstream reach than at the upstream reach. The two reaches did not differ in macroinvertebrate total biomass suggesting that macroinvertebrates may be constrained by factors other than nutrient concentrations. On average, taxa richness at the upstream reach was 20% higher than at the downstream reach. Several taxa, especially mayflies, stoneflies and caddisflies, were present only at the upstream reach. Shannon diversity was similar between the two reaches on 50% of the dates. Ordination analysis clearly separated the samples of the two reaches in the first axis and corroborated the effect of the point source on the benthic community. The two reaches followed a similar temporal pattern with respect to the distribution of taxa along the second axis. Higher similarities between the two reaches in taxa composition, densities and biomass after the spates of April and May 2002, suggest that flooding events may act as a reset mechanism for benthic communities and play an important role in stream restoration.

Appendix 4.1 Taxa list, taxon code, and mean densities of benthic macroinvertebrates in individuals/m² (\pm ES) recorded in the upstream reach and the downstream reach over the six sampling dates in La Tordera stream ($n = 6$). A = adult, P = pupae.

Taxa nomenclature	code	Nov-02		Jan-02		Mar-02		Apr-02		Jun-02		Sep-02	
		Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
Arthropoda													
Insecta													
Ephemeroptera													
Heptageniidae													
<i>Ecdyonurus angeliari</i>	Eang	56 (18)	0 (0)	267 (71)	0 (0)	351 (38)	0 (0)	72 (18)	0 (0)	568 (173)	80 (18)	400 (104)	20 (11)
<i>Epeorus torrentium</i>	Etor	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	317 (153)	0 (0)
Baetidae													
<i>Baetis fuscatus</i>	Bfus	3 (4)	24 (21)	6 (3)	0 (0)	0 (0)	4 (3)	7 (7)	39 (28)	130 (65)	873 (110)	1232 (539)	2457 (1236)
<i>Baetis lutheri</i>	Blut	34 (17)	84 (43)	25 (18)	193 (148)	0 (0)	40 (19)	3 (4)	34 (22)	7 (6)	524 (163)	51 (40)	16239 (2902)
<i>Baetis rhodani</i>	Brho	59 (26)	4 (3)	588 (128)	49 (16)	168 (38)	26 (10)	875 (171)	777 (138)	9716 (1998)	12569 (2403)	5711 (1444)	3660 (453)
Ephemerelellidae													
<i>Serratella ignita</i>	Sign	27 (11)	8 (8)	341 (87)	0 (0)	1582 (409)	9 (9)	368 (82)	27 (12)	405 (67)	64 (7)	35 (12)	20 (20)
Caenidae													
<i>Caenis lactinosa</i>	Cluc	117 (37)	370 (243)	72 (31)	162 (97)	152 (59)	40 (14)	31 (17)	13 (6)	0 (0)	16 (10)	1469 (652)	12241 (2158)
<i>Caenis pusilla</i>	Cpus	0 (0)	0 (0)	0 (0)	0 (0)	6 (5)	0 (0)	11 (5)	0 (0)	0 (0)	0 (0)	27 (15)	0 (0)
Leptophlebiidae													
<i>Habroleptoides</i> sp.	Hab	0 (0)	0 (0)	0 (0)	0 (0)	38 (29)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	427 (141)	9 (9)
<i>Habrophlebia fusca</i>	Hfus	221 (67)	5 (3)	1005 (237)	8 (8)	421 (191)	9 (9)	165 (62)	5 (3)	203 (41)	21 (7)	0 (0)	0 (0)
Plecoptera													
Nemouridae													
<i>Amphinemura</i> sp.	Amp	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	16 (10)	0 (0)
<i>Nemoura</i> sp.	Neu	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	24 (5)	0 (0)	8 (8)	0 (0)
<i>Protonemura</i> sp.	Pro	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	59 (19)	0 (0)	0 (0)	0 (0)
Leuctridae													
<i>Leuctra geniculata</i>	Lgen	5 (3)	0 (0)	19 (12)	0 (0)	660 (94)	18 (18)	595 (115)	0 (0)	283 (44)	8 (5)	131 (38)	0 (0)
Capniidae													
<i>Capnioneura mitis</i>	Cmit	128 (47)	0 (0)	480 (60)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Appendix 4.1 Continued

Chloroperlidae												
<i>Siphonoperla torrentium</i>	Stor	11 (8)	0 (0)	16 (13)	0 (0)	16 (7)	0 (0)	21 (8)	0 (0)	5 (3)	0 (0)	0 (0)
Perlodidae												
<i>Isoperla grammatica</i>	Igra	112 (15)	0 (0)	861 (181)	0 (0)	283 (79)	0 (0)	125 (40)	0 (0)	0 (0)	3 (3)	0 (0)
Odonata												
Calopterygidae												
<i>Calopteryx virgo</i>	Cvir	0 (0)	3 (3)	0 (0)	0 (0)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	31 (12)
Gomphidae												
<i>Onychogomphus</i> sp.	Ony	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	9 (9)
Cordulegasteridae												
<i>Cordulegaster</i> sp.	Cor	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Coleoptera												
Halipidae												
<i>Halipus</i> sp.	Hal	0 (0)	0 (0)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dytiscidae												
<i>Hydroporus</i> sp.	Hyp	0 (0)	0 (0)	37 (15)	0 (0)	58 (15)	9 (9)	29 (15)	0 (0)	16 (6)	3 (3)	0 (0)
<i>Scarodytes</i> sp. A	Sea A	0 (0)	0 (0)	5 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Deronectes</i> sp.	Der	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (5)	5 (5)	0 (0)
<i>Agabus</i> sp.	Aga	19 (6)	0 (0)	27 (8)	3 (3)	0 (0)	0 (0)	0 (0)	3 (3)	0 (0)	0 (0)	0 (0)
Hidrophilidae												
<i>Coelostoma</i> sp. A	Coe A	3 (3)	9 (9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hidraeniidae												
<i>Hydraena</i> sp. A	Hye A	3 (3)	0 (0)	8 (5)	0 (0)	16 (7)	0 (0)	5 (3)	0 (0)	8 (8)	3 (3)	5 (5)
Dryopidae												
<i>Dryops</i> sp.	Dry	0 (0)	0 (0)	0 (0)	9 (9)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Elmidae												
<i>Elmis</i> sp.	Elm	0 (0)	0 (0)	8 (5)	0 (0)	20 (10)	0 (0)	13 (6)	0 (0)	19 (10)	0 (0)	104 (56)
<i>Esolus</i> sp.	Eso	99 (53)	5 (5)	72 (33)	0 (0)	75 (35)	9 (9)	35 (13)	0 (0)	120 (75)	5 (5)	341 (76)
<i>Esolus</i> sp. A	Eso A	19 (10)	0 (0)	24 (21)	0 (0)	23 (17)	0 (0)	0 (0)	0 (0)	8 (5)	0 (0)	11 (5)
<i>Limnius</i> sp.	Lis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)	0 (0)	0 (0)
<i>Oulimnius</i> sp.	Oul	0 (0)	50 (28)	0 (0)	17 (11)	3 (3)	0 (0)	3 (3)	0 (0)	0 (0)	0 (0)	32 (8)

Appendix 4.1 Continued

Tabanidae	Tab	0 (0)	8 (4)	3 (3)	11 (8)	0 (0)	3 (3)	0 (0)	3 (3)	0 (0)	0 (0)	0 (0)	3 (3)	0 (0)
Anthomyiidae	Ant	0 (0)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)	0 (0)	0 (0)	8 (4)	0 (0)	54 (21)
Anthomyiidae P	Ant P	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)	53 (20)
Crustacea														
Cladocera	Cla	72 (21)	564 (268)	584 (415)	685 (218)	986 (412)	1253 (195)	240 (73)	445 (74)	8 (4)	43 (28)	27 (15)	284 (97)	
Copepoda	Cop	64 (36)	661 (291)	269 (140)	3464 (2039)	300 (36)	1351 (194)	165 (58)	640 (327)	64 (23)	413 (90)	45 (15)	1289 (327)	
Ostracoda	Ost	53 (23)	830 (506)	504 (113)	427 (213)	490 (64)	249 (105)	120 (41)	51 (23)	152 (52)	192 (43)	165 (36)	1289 (289)	
Amphipoda														
Niphargidae	Mic	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (3)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Microniphargus</i> sp.														
Arachnida														
Acari	Hye	299 (73)	97 (56)	528 (105)	44 (24)	720 (167)	100 (40)	7235 (3582)	117 (19)	1051 (435)	195 (29)	776 (161)	578 (155)	
Hydracarina														
Mollusca														
Gastropoda														
Hydrobiidae														
<i>Potamopyrgus antipodarum</i>	Pant	56 (23)	199 (112)	85 (36)	167 (103)	8 (5)	109 (35)	0 (0)	5 (3)	0 (0)	27 (12)	0 (0)	14 (11)	
<i>Bythiospeum</i> sp.	Byt	19 (19)	0 (0)	8 (8)	5 (5)	8 (5)	0 (0)	11 (8)	0 (0)	0 (0)	0 (0)	355 (351)	74 (48)	
Ancyliidae														
<i>Ancylus fluvialilis</i>	Aflu	187 (105)	220 (98)	797 (292)	161 (109)	2460 (680)	148 (56)	952 (204)	176 (62)	69 (28)	149 (31)	581 (135)	1893 (892)	
Planorbidae														
<i>Gyraulus</i> sp.	Gyr	0 (0)	9 (9)	8 (5)	0 (0)	13 (5)	18 (18)	8 (5)	5 (5)	0 (0)	0 (0)	0 (0)	0 (0)	
Lymnaeidae														
<i>Lymnaea</i> sp.	Lym	40 (28)	0 (0)	72 (25)	0 (0)	244 (90)	98 (57)	227 (73)	40 (15)	69 (42)	53 (27)	67 (32)	187 (117)	
<i>Radix</i> sp.	Rad	5 (3)	5 (3)	3 (3)	0 (0)	3 (3)	0 (0)	19 (8)	0 (0)	0 (0)	0 (0)	5 (5)	0 (0)	
Physidae														
<i>Physella acuta</i>	Pacu	43 (11)	1476 (720)	277 (97)	1800 (987)	123 (31)	341 (160)	45 (20)	19 (10)	5 (5)	3 (3)	195 (138)	288 (138)	
Bivalvia														
Sphaeriidae														
<i>Psidium caesertanum</i>	Peas	0 (0)	327 (218)	0 (0)	87 (41)	0 (0)	89 (41)	0 (0)	8 (5)	0 (0)	8 (5)	0 (0)	169 (117)	

Appendix 4.1 Continued

Annelida													
Oligochaeta													
Naididae	Nai	328 (111)	2209 (1316)	1160 (393)	24061 (8869)	76 (11)	0 (0)	107 (36)	155 (40)	32 (12)	245 (53)	13 (8)	44 (25)
Tubificidae	Tub	3 (3)	2869 (736)	13 (10)	2097 (620)	0 (0)	676 (321)	19 (12)	2136 (1050)	8 (8)	755 (213)	0 (0)	269 (81)
Lumbriculidae	Lum	24 (15)	176 (74)	3 (3)	183 (90)	139 (53)	8 (8)	32 (8)	163 (113)	19 (13)	16 (7)	16 (8)	5 (5)
Lumbricidae													
<i>Eisenella tetraedra</i>	Eiet	128 (23)	0 (0)	168 (26)	0 (0)	125 (54)	0 (0)	91 (18)	8 (5)	5 (3)	3 (3)	16 (7)	21 (10)
Hirudinea													
Glossiphoniidae													
<i>Glossiphonia</i> sp.	Glo	0 (0)	13 (8)	0 (0)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (3)
<i>Helobdella stagnalis</i>	Hsta	5 (5)	488 (135)	0 (0)	270 (122)	0 (0)	188 (68)	0 (0)	67 (27)	0 (0)	19 (5)	0 (0)	31 (12)
Erpobdellidae													
<i>Erpobdella</i> sp.	Erp	0 (0)	211 (48)	0 (0)	157 (53)	3 (3)	181 (39)	0 (0)	64 (23)	5 (3)	293 (148)	5 (5)	340 (68)
Nematoda	Nem	67 (29)	1071 (268)	107 (24)	2888 (807)	146 (44)	9307 (2449)	107 (34)	2411 (337)	21 (8)	360 (93)	61 (25)	516 (104)
Nematomorpha	Nep	11 (5)	323 (138)	29 (12)	222 (75)	12 (9)	284 (86)	3 (3)	253 (77)	0 (0)	141 (35)	5 (5)	111 (57)
Cnidaria													
Hydrozoa													
Hydridae													
<i>Hydra</i> sp.	Hyd	0 (0)	3 (3)	3 (3)	32 (17)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Platyhelminthes													
Turbellaria													
Tricladida													
Planariidae													
<i>Plagocata vitia</i>	Pvit	3 (3)	3 (3)	8 (5)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dugesidae													
<i>Dugesia</i> sp.	Dug	256 (126)	0 (0)	43 (32)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Trematoda													
Digenea	Dig	16 (13)	5 (5)	35 (11)	41 (35)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

5. Effects of the point source on the functional organization of the benthic community

Introduction

The analysis of the trophic organization in benthic communities has been successfully used as surrogates of ecosystem functional attributes (Vannote *et al.* 1980, Hawkins and Sedell 1981, Minshall *et al.* 1983, Merritt *et al.* 2002). Point sources, among other human disturbances, may have lead to severe changes in the trophic basis for production in stream ecosystems (Merritt and Cummins 1996a, Paul and Meyer 2001). Thus, ecosystem analysis from a functional perspective (Wallace and Webster 1996) may provide a complementary understanding of the processes involved in the ecology of human-altered streams. Given the yielding offered by taxonomic guides and traits (Merritt and Cummins 1996a, Merritt and Cummins 1996b, Tachet *et al.* 2000, Moog 2002), the functional organization approach provides a conceptual framework that made easy functional feeding groups to have been widely used in bioassessments.

Based on previous research and reasoning, Kerans and Karr (1994) hypothesized that human impact, including organic enrichment, would enhance relative densities of gatherers and filterers, and decrease those of shredders, scrapers, and predators. Subsequent studies demonstrated that changes driven by human impact in macroinvertebrate functional organization are likely to be taken as a trend (Hachmöller *et al.* 1991, Shieh *et al.* 1999, Roy *et al.* 2003). However, the reliability of using the functional approach for biomonitoring purposes is not free of controversy and specifications are not uncommon in literature (Barbour *et al.* 1996, Fore *et al.* 1996, Delong and Brunsen 1998). Indeed, the effects of point sources on relative density of functional feeding groups might not be consistent everywhere, given the multivariate nature of human impacts and the habitat

complexity of stream ecosystems. The response of macroinvertebrate functional feeding groups to the conditions driven by point sources is often related to the availability of food resources and the sensitivity to low dissolved oxygen concentrations. Gatherers are generalist feeders that can tolerate a broad range of food materials (Cummins and Klug 1979) and usually having specific mechanisms to obtain oxygen under low oxygen concentrations given their association with low-current depositional zones. Point sources are known to increase suspended solid concentrations in stream ecosystems (Brunet *et al.* 2001, Roy *et al.* 2003), which is known to enhance filterers (Wotton 1987, Wallace and Webster 1996). However, filterers may also reach high densities in pristine ecosystems under certain habitat conditions (Wallace and Merritt 1980, Smock *et al.* 1985, Barbour *et al.* 1996). Most shredders are characteristic from headwater streams, where leaf litter represents the main resource basis for heterotrophic production (Vannote *et al.* 1980, Hawkins and Sedell 1981, Minshall *et al.* 1983, Naiman *et al.* 1987). Such ecosystems are often characterized by steep slopes that ensure sufficient water oxygenation while human impact is generally low. Therefore, dwelling organisms, including shredders, are more susceptible to be sensitive to pollution through low oxygen concentrations and changes on microbial colonization in coarse particulate organic matter (CPOM). Little is known about the effects of point sources on leaf litter (Paul and Meyer 2001) and observed changes are hardly attributed to the point source but to changes on riparian vegetation (Shieh *et al.* 1999). High levels of organic pollution may lead to extremely low oxygen concentrations and limit autotrophic production and their consumers (Ortiz *et al.*, in press, Hynes 1978, Masseret *et al.* 1998). However, several studies found that moderate nutrient enrichment can enhance periphyton under sufficient light exposure (Biggs 2000, Suren 2000) and hence, enhance scraper density in both field experiments (Elwood *et al.* 1981, Hart and Robinson

1990) and human altered streams (Welch *et al.* 1992, Delong and Brunsen 1998). Predators are expected to decrease in human altered streams even though the abundance of potential prey may increase, because most invertebrates included in this functional feeding group are highly sensitive to pollution (Moog 2002). The term “strict” predators (Kerans and Karr 1994), which excludes taxa considered resistant to pollution such as leeches and midges, has been used in search of clear patterns. However, the relevance of predators is often left in a second term because their response to nutrient enrichment may be variable (Barbour *et al.* 1996, Fore *et al.* 1996).

Relationships between consumers and their presumed resources are commonly used to examine specific mechanisms leading changes on functional organization (Hawkins and Sedell 1981). A number of studies examined relationships between consumer density and standing stocks of their presumed food resources (e.g., Delong and Brunsen 1998, Martinez *et al.* 1998, Shieh *et al.* 1999, Doisy and Rabeni 2001). However, the use of consumer-resource relationships in bioassessment merging reference and altered sites may lead to improper assessments because it assumes that the relationship between consumers and their food is not altered by the disturbance. In addition, most studies were restricted to macroinvertebrate density, while biomass may offer higher accuracy (Hawkins *et al.* 1982).

We sampled biomass compartments including benthic macroinvertebrates and their presumed food resources in one reach upstream and one reach downstream of a point source in La Tordera stream on six sampling dates over the hydrologic year 2001-2002. Our objectives were to examine the changes on the potential food resources for macroinvertebrates below the point source, and assess the effects of the point source on the functional organization of the

macroinvertebrate community considering potential alterations in consumer-resource relationships.

Results

Primary producers and organic matter standing stocks

Mean chlorophyll *a* concentration was, on average, five times higher at the downstream reach than at the upstream reach and differences between the two reaches were substantial in January and March 2002 (Table 5.1). The differences in chlorophyll *a* concentration between the two reaches was positively correlated to DIN concentration (linear regression, $r^2 = 0.495$, $p = 0.011$, $n = 12$), but was not correlated to the relative contribution of the point source to stream discharge ($R_S = -0.200$, $p = 0.704$, $n = 6$). On the other hand, the difference in chlorophyll *a* between the two reaches was correlated to mean water temperature ($R_S = 0.886$, $p = 0.019$, $n = 6$), indicating higher disparities in winter than in summer. The two reaches had the lowest values of chlorophyll *a* in June 2002, after the spate of May 2002. Periphyton standing stocks differed little between the two reaches over the sampling period (Table 5.1). Periphyton biomass was higher in March 2002 at the downstream reach, and in March and September 2002 at the upstream reach. Standing stocks of periphyton were lowest in November 2001 in the two reaches. Standing stocks of filamentous algae (mostly *Cladophora glomerata*) and mosses (mostly *Amblystegium riparium*) were substantially higher at the downstream reach than at the upstream reach. At the upstream reach, biomass of filamentous algae and mosses were very low over the sampling period. At the downstream reach, the higher biomasses occurred in March and January 2002 and the lowest in June 2002. The most common vascular plants found in the two reaches were *Apium nodiflorum*, *Ranunculus* sp., and *Callitriche* spp. The two reaches had similar standing stocks of vascular plants over the sampling period (Table 5.1).

Table 5.1 Mean \pm SE, values of chlorophyll a (mg chl a/m^2), primary producers and benthic organic matter standing stock (mg AFDM/ m^2), and SPOM (mg AFDM/L) measured in the two reaches over the six sampling dates in La Tordera stream .

	Nov-01	Jan-02	Mar-02	Apr-02	Jun-02	Sep-02
Upstream						
Chlorophyll a ¹	43.9	110.0	23.7	31.3	6.0	65.3
Periphyton ¹	7218.7	7927.7	34591.5	25949.1	14457.0	41699.1
Filamentous algae	0 \pm 0	74.6 \pm 33.1	424.5 \pm 41.4	9.3 \pm 8.6	4.8 \pm 3	428.3 \pm 218.7
Moss	78.8 \pm 53.1	22.4 \pm 6.5	44.6 \pm 17.3	176.6 \pm 95.6	10.1 \pm 7.7	532.9 \pm 383.1
Vascular plants	32.8 \pm 21	105.1 \pm 74.4	0 \pm 0	302.7 \pm 264.7	0 \pm 0	14.5 \pm 11.5
CPOM	6125.2 \pm 739.6	4406.5 \pm 1776.7	11538.2 \pm 2165.9	21134.7 \pm 14363.4	4756.5 \pm 2010.7	2289.2 \pm 3174.6
FPOM	2993.6 \pm 724.1	2567.8 \pm 798.4	3282.4 \pm 542	3335.2 \pm 1064.9	1347.8 \pm 512.2	502.6 \pm 177.7
SPOM	0.3 \pm 0.1	0.3 \pm 0	1.3 \pm 0.1	0.8 \pm 0	0.7 \pm 0	0.6 \pm 0.1
Downstream						
Chlorophyll a ¹	89.5	915.9	229.4	68.0	4.0	11.3
Biofilm ¹	13675.0	30830.2	69812.2	23369.6	29173.1	14781.9
Filamentous algae	315 \pm 178.5	2982.1 \pm 1748.9	4933.3 \pm 1314.2	593.4 \pm 162.9	9.9 \pm 2.4	687.3 \pm 276.8
Moss	3952.4 \pm 3013.9	5967.4 \pm 5247	10851.6 \pm 5584.1	3595.3 \pm 1658.3	219.7 \pm 169.3	1056.3 \pm 461.9
Vascular plants	28.8 \pm 28.8	96.5 \pm 85.2	369.6 \pm 253.9	0 \pm 0	0 \pm 0	29.1 \pm 14.3
CPOM	32581.8 \pm 20162.3	11358.5 \pm 3349.2	15677.5 \pm 3284.2	7145.2 \pm 2882.7	3962.4 \pm 2023.4	8877.2 \pm 1982.5
FPOM	7277.6 \pm 2795.1	3323.7 \pm 737.1	6311.1 \pm 1696.4	2638.4 \pm 733.6	660.1 \pm 146.4	2001.8 \pm 277.3
SPOM	1.1 \pm 0.1	2.3 \pm 0.1	1.5 \pm 0.5	0.8 \pm 0.1	1.4 \pm 0.1	1.3 \pm 0.1

¹ Habitat weighted.

The highest standing stocks of vascular plants were in March at the downstream reach and in April 2002 at the upstream reach. CPOM and FPOM did not differ substantially between the two reaches over the sampling period (Table 5.1). Both, CPOM and FPOM were lower in the two reaches in June and September 2002. At the upstream reach, the CPOM:FPOM ratio was lower in November 2001 and January 2002 and higher in April 2002 (Figure 5.1). At the downstream reach, the CPOM:FPOM ratio decreased from November 2001 towards April 2002 and increased again in June and September 2002. SPOM was, on average, more than twofold below the point source input in relation to the upstream reach. At the upstream reach, SPOM concentration was quite constant but lower in November 2001 and January 2002 than in the rest of the samplings. At the downstream reach, the variability of the SPOM concentration was much higher than at the upstream reach. Furthermore, the difference in SPOM between the two reaches was significantly correlated with the relative contribution of the point source to the stream discharge ($R_s = 0.886$, $p = 0.019$, $n = 6$).

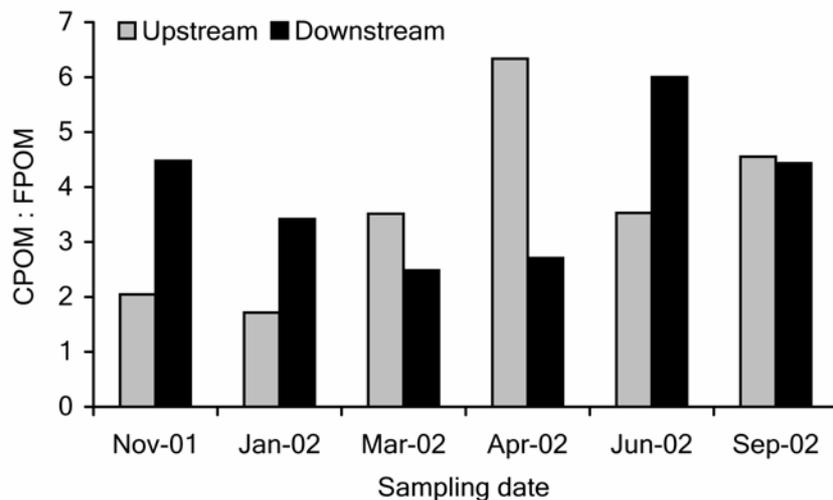


Figure 5.1 Coarse to fine particulate organic matter ratio (CPOM:FPOM) for the two reaches in La Tordera stream over the sampling period (2001-2002).

Functional organization of macroinvertebrates

In the two reaches, gatherers followed by scrapers were usually the most abundant functional feeding groups (Figure 5.2). At the upstream reach, those two functional feeding groups represented, on average, 42% and 38% of total macroinvertebrate biomass, respectively. In contrast, at the downstream reach macroinvertebrate biomass was mainly comprised by predators (38%, on average). Shredders were the least abundant macroinvertebrates in the two reaches in terms of both density (on average less than 3% at the upstream reach and 1% at the downstream reach) and biomass (on average 8% at the upstream reach and 5% at the downstream reach). Shredders density and biomass were similar in the two reaches, but relative percentage was lower at the downstream reach for both density and biomass (Table 5.2 and Figure 5.2). At the upstream reach, shredder density was dominated by the stoneflies *Leuctra geniculata* and *Capnioneura mitis* and shredder biomass by the family Tipulidae (Diptera). In contrast, at the downstream reach shredder density and biomass were dominated by the snail *Physella acuta*. Although density of scrapers was three times higher at the downstream reach than at the upstream reach, the two reaches did not differ greatly in relative density and neither biomass or relative biomass (Table 5.2 and Figure 5.2). At the upstream reach, the limpet *Ancylus fluviatilis* constituted 65% of scraper biomass, whereas at the downstream reach, biomass of scrapers was dominated by *P. acuta* and *A. fluviatilis*. Density and relative density of gatherers were much higher at the downstream reach than at the upstream reach. Contrarily to density of gatherers, relative biomass of gatherers decreased at the downstream reach. Biomass of gatherers was similar in the two reaches, indicating that individual biomass of the macroinvertebrates included in this functional feeding group was, on average, lower at the downstream reach than at the upstream reach. At the upstream reach, *Eiseniella tetraedra* (Lumbricidae) contributed 64% of

gatherer biomass, whereas at the downstream reach gatherer biomass was dominated by the chironomid tribe Tanytarsini and *P. acuta*. The downstream reach had higher density, relative density, and biomass of filterers than the upstream reach (Table 5.2 and Figure 5.2). At the upstream reach, biomass of filterers was dominated by the caddisfly *Hydropsyche instabilis* and blackflies in second term, whereas at the downstream the reach was dominated by blackflies and Tanytarsini. Differences in density and relative density of predators (mainly water mites at the upstream reach and nematodes at the downstream reach) were not substantially different between the two reaches. However, biomass of predators at the downstream reach (mainly the leech *Erpobdella* sp.) was sevenfold that at the upstream reach (mainly water mites and the stonefly *Isoperla grammica*). Relative biomass varied accordingly.

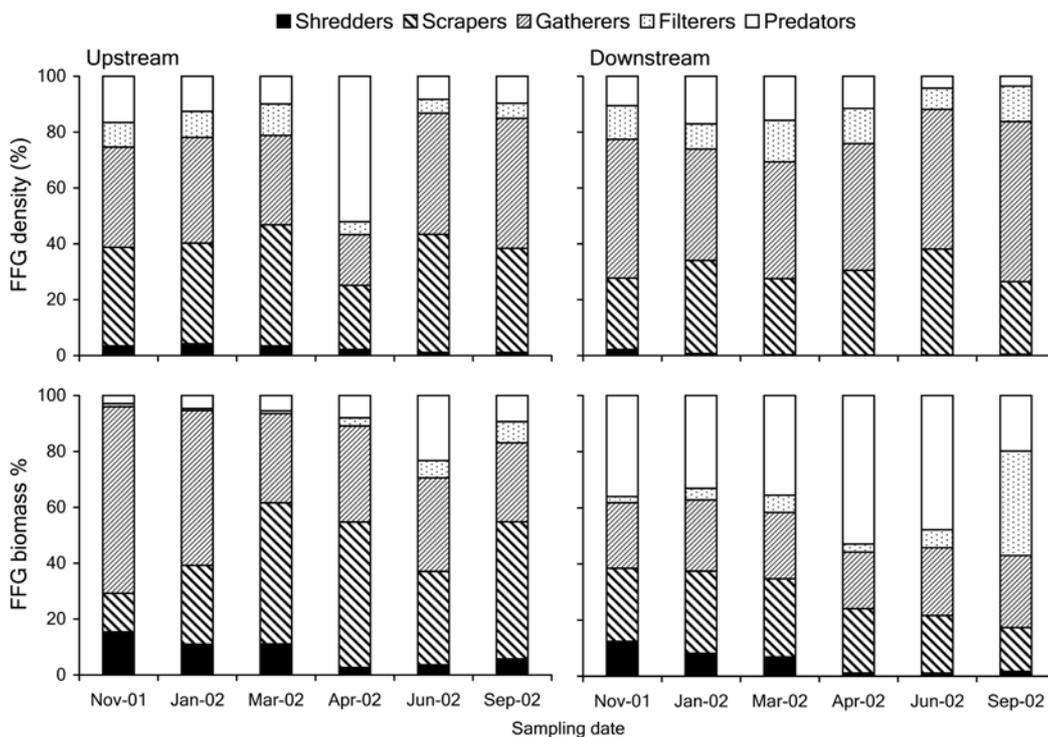


Figure 5.2 Relative contribution of density and biomass of macroinvertebrate functional feeding groups in the two reaches in La Tordera stream over the sampling period (2001-2002).

Table 5.2 Means \pm SE of functional feeding group density (ind./m²) and biomass (DW/m²) in the two reaches of La Tordera stream over the six sampling dates ($n = 6$).

	Shredders		Scrapers		Gatherers		Filterers		Predators	
	Density	Biomass	Density	Biomass	Density	Biomass	Density	Biomass	Density	Biomass
Upstream										
Nov-01	179 \pm 20	252 \pm 95	1849 \pm 425	228 \pm 51	1880 \pm 448	1096 \pm 262	462 \pm 113	18 \pm 5	870 \pm 189	47 \pm 6
Jan-02	605 \pm 54	594 \pm 96	5232 \pm 624	1533 \pm 336	5495 \pm 975	3004 \pm 550	1347 \pm 599	39 \pm 7	1832 \pm 177	253 \pm 37
Mar-02	466 \pm 65	642 \pm 259	5890 \pm 955	2919 \pm 538	4322 \pm 691	1842 \pm 522	1528 \pm 400	55 \pm 27	1360 \pm 284	319 \pm 75
Apr-02	323 \pm 80	61 \pm 27	3375 \pm 740	1238 \pm 248	2674 \pm 722	815 \pm 276	679 \pm 195	69 \pm 61	7679 \pm 3645	189 \pm 89
Jun-02	205 \pm 25	20 \pm 6	8102 \pm 1085	184 \pm 26	8289 \pm 1006	184 \pm 10	956 \pm 120	34 \pm 10	1589 \pm 425	128 \pm 65
Sep-02	190 \pm 34	90 \pm 50	6534 \pm 726	780 \pm 94	8150 \pm 936	448 \pm 59	953 \pm 99	121 \pm 55	1695 \pm 192	148 \pm 57
Downstream										
Nov-01	514 \pm 260	633 \pm 280	5985 \pm 2593	1329 \pm 574	11659 \pm 3747	1195 \pm 423	2834 \pm 1134	113 \pm 62	2477 \pm 563	1840 \pm 393
Jan-02	492 \pm 264	367 \pm 161	22014 \pm 9736	1325 \pm 587	26360 \pm 10871	1145 \pm 393	5965 \pm 2464	188 \pm 90	11300 \pm 3305	1494 \pm 390
Mar-02	192 \pm 58	401 \pm 211	17680 \pm 3049	1635 \pm 541	27184 \pm 5612	1389 \pm 361	9678 \pm 2049	360 \pm 83	10272 \pm 2559	2086 \pm 504
Apr-02	41 \pm 8	22 \pm 11	8367 \pm 1681	466 \pm 67	12509 \pm 2884	411 \pm 50	3465 \pm 748	58 \pm 14	3186 \pm 419	1076 \pm 357
Jun-02	68 \pm 20	10 \pm 4	12089 \pm 2260	187 \pm 27	15979 \pm 3737	220 \pm 42	2419 \pm 995	58 \pm 34	1375 \pm 146	435 \pm 168
Sep-02	396 \pm 80	62 \pm 30	23668 \pm 3177	593 \pm 83	52156 \pm 7123	973 \pm 140	11502 \pm 3244	1413 \pm 903	3271 \pm 445	750 \pm 91

The temporal variability of the relative density of each functional feeding group was relatively low in the two reaches. An exemption was predator density in April 2002 at the upstream reach because of very high density of water mites (Figure 5.2). Relative contribution of shredder biomass was higher from November 2001 to March 2002 in the two reaches. At the upstream reach, the relative percentage of gatherer biomass was especially high in November 2001 and January 2002. At the downstream reach, relative biomass of filterers was up to 17 times higher in September 2002 than on the other sampling dates because of blackflies. Bray-Curtis index of similarity in functional feeding group densities between the two reaches was much higher in June 2002 (73.59%) than on the rest of the sampling dates (from 32.30% to 47.08%; Table 5.3). Similarities in functional feeding group biomass between the two reaches were higher than similarities in functional feeding group densities but the highest similarity value was also in June 2002 (73.91%).

Table 5.3 Bray-Curtis index of similarity in functional feeding group densities (B-C d) and Bray-Curtis index of similarity in functional feeding group biomass (B-C b), between the upstream reach and the downstream reach.

	Nov-01	Jan-02	Mar-02	Apr-02	Jun-02	Sep-02
B-C d	36.51	35.71	33.90	47.08	73.59	32.30
B-C b	48.61	62.95	65.08	52.07	73.91	51.04

Relationships between consumers and their food resources

The only significant relationship between consumer abundance and their presumed food resources considering the two reaches combined was between abundance of filterers and SPOM concentration (power regression, $r^2 = 0.513$, $p = 0.009$, $n = 12$; Figure 5.3). The samples of the upstream reach had relatively low SPOM concentrations and densities of filterers while at the downstream reach the values were higher (Figure 5.3d). At the upstream reach, gatherer density

decreased significantly when increasing standing stock of FPOM (linear regression, $r^2 = 0.840$, $p = 0.010$, $n = 6$), but the relationship was not significant for the downstream reach ($p > 0.05$, $n = 6$; Figure 5.3c). On the other hand, regression analyses between invertebrate functional feeding group biomass and food resources were usually significant. Monthly biomass of scrapers was significantly related to chlorophyll *a* concentration (power regression, $r^2 = 0.356$, $p = 0.040$, $n = 12$; Figure 5.4a). The relationship between shredder biomass and CPOM standing stock was not significant for the two reaches combined ($p > 0.05$, $n = 12$). At the downstream reach, biomass of shredders was strongly related to CPOM (logarithmic regression, $r^2 = 0.870$, $p = 0.007$, $n = 6$), whereas at the upstream reach the relationship was not significant ($p > 0.05$, $n = 6$; Figure 5.4b). Gatherer biomass for the two reaches combined was positively related to FPOM standing stock (power regression, $r^2 = 0.483$, $p = 0.012$, $n = 12$; Figure 5.4c). Filterer density was significantly correlated to biomass of filterers ($R_s = 0.699$, $p < 0.0005$, $n = 12$) and, hence, regression analysis between filterer biomass and SPOM concentration showed similar results than for density (power regression, $r^2 = 0.358$, $p = 0.040$, $n = 12$; Figure 5.4d). The ratio predator to primary consumer biomass was on average six times higher at the downstream reach than at the upstream reach (Figure 5.4e). In consequence, the relationship between monthly predators and primary consumer biomass for the two reaches combined was not significant ($p > 0.05$, $n = 12$). However, the two reaches showed significant relationships when analyzed separately. At the upstream reach, predators were related to primary consumers through a linear regression characterized by a low slope ($r^2 = 0.738$, $p = 0.028$, $n = 6$). At the downstream reach, the relationship between predator and primary consumer biomass was not statistically significant ($p > 0.05$, $n = 6$) due to the relatively high biomass of blackflies in September

2002. However, this relationship was clearly significant excluding the sample of September 2002 (logarithmic regression, $r^2 = 0.933$, $p = 0.008$, $n = 5$).

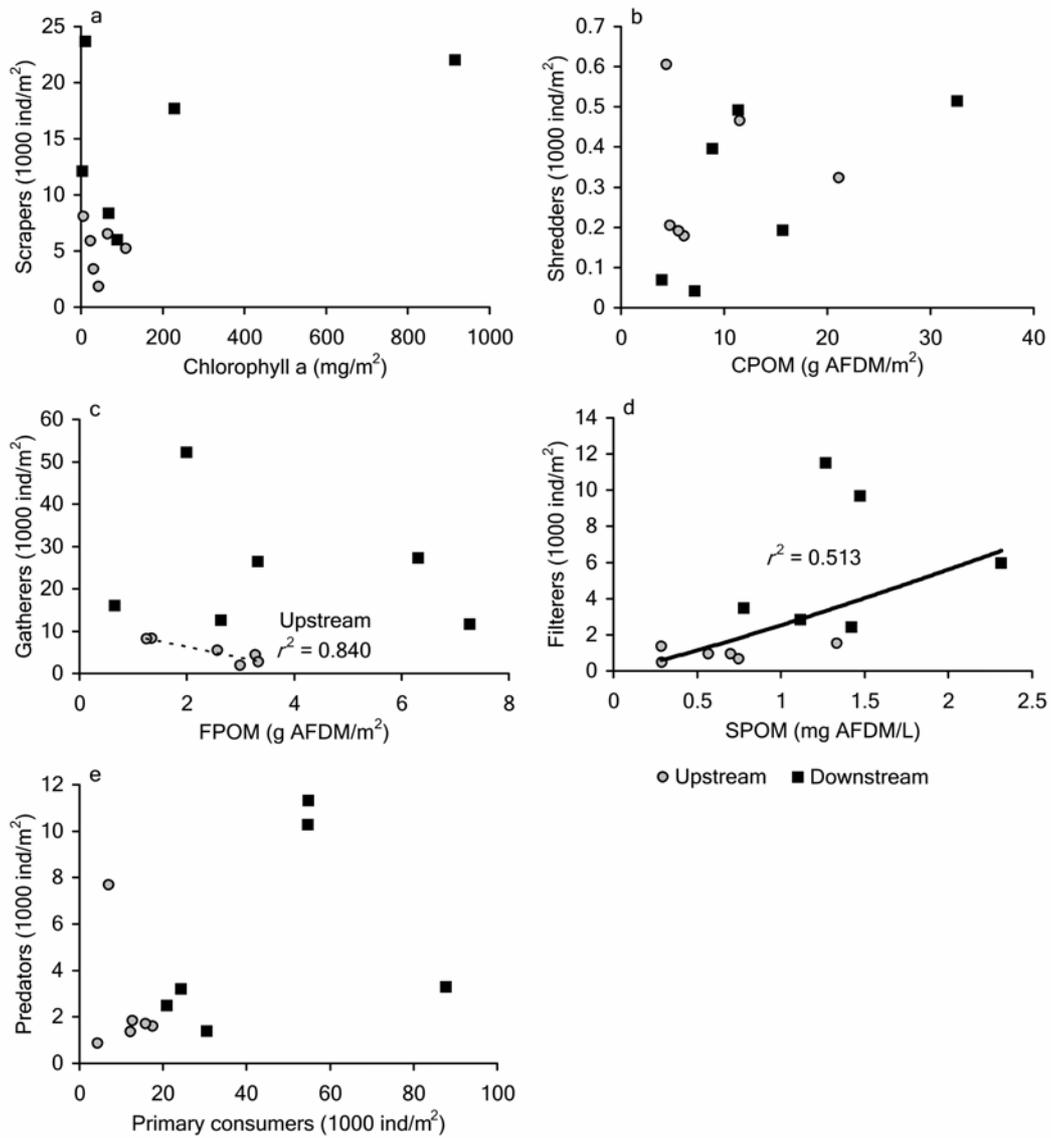


Figure 5.3 Relationship between mean densities of functional feeding groups and their presumed food resources. Regression coefficients and trend lines denote significant relationships. Dotted line = significant for the upstream reach, solid bold line = significant for the two reaches combined.

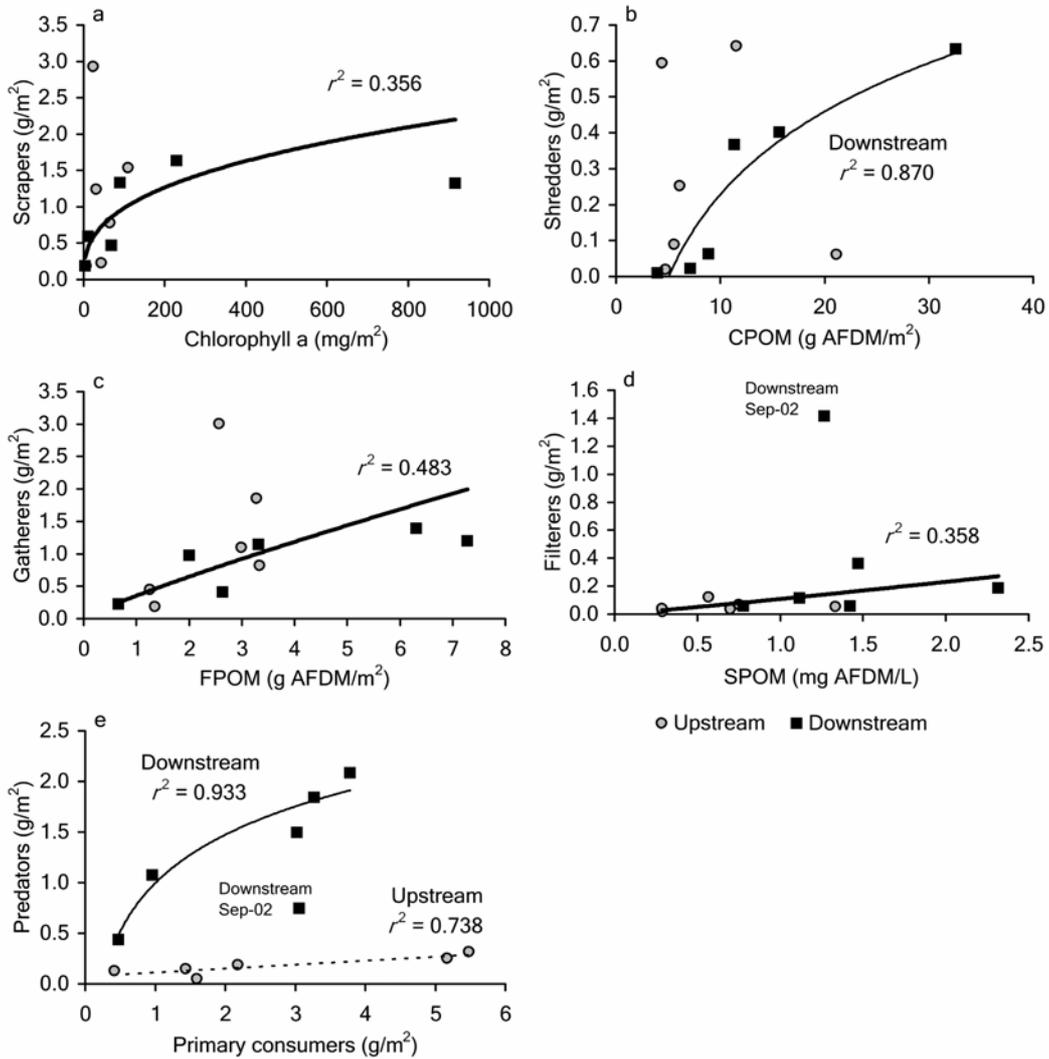


Figure 5.4 Relationship between mean biomasses of functional feeding groups and their presumed food resources. Regression coefficients and trend lines denote significant relationships. Dotted line = significant for the upstream reach, solid line = significant for the downstream reach, solid bold line = significant for the two reaches combined. The data from the downstream reach in September 2002 was not considered in the regression between predators and primary consumers.

Discussion

Primary producers and organic matter

The point source substantially increased chlorophyll *a* concentrations on most dates. We observed that chlorophyll *a* concentrations were positively related to nutrient concentrations (DIN) as seen before (Biggs 2000), but was not only affected by this factor. Our results showed that chlorophyll *a* concentrations, as well as filamentous algae and mosses, were much higher at the downstream reach than at the upstream reach in early spring, when sun irradiation was high and flood disturbance was low. A number of studies relate an increase of primary producers to moderate nutrient enrichment, including periphyton (Welch *et al.* 1992) filamentous algae (Hynes 1978) and mosses (Lee and Hershey 2000). A previous study in a small river in the east of France (Brunet *et al.* 2001) observed that chlorophyll *a* was higher below a wastewater treatment plant (WWTP) input in spring but lower in summer. They attributed such temporal variability to a lower water velocity in summer that increased sedimentation and mineralization above the WWTP input. In our study case, we think that this pattern indicated that light levels in winter might result low enough to restrict production of primary producers (Boston and Hill 1991, Hill *et al.* 1995). In addition, the extremely low values of chlorophyll *a* and biomass of all primary producers in June 2002 in the two reaches suggest that flood disturbance also played an important role (Biggs 2000). The lack of any effect of the point source on vascular plants contrast with the findings of Carr *et al.* (2003) and lends support to the opinion of Allan (1995) that rooted plants are not so dependent on water chemistry as they obtain nutrients from the substrate.

Although nutrient concentrations may influence significantly on leaf litter breakdown (Braioni *et al.* 1997, Paul and Meyer 2001), changes in CPOM standing stocks below point sources were usually attributed to changes in riparian

vegetation (Shieh *et al.* 1999, Shieh *et al.* 2003). Therefore, the similar riparian vegetation in the two reaches may explain such similarity in CPOM standing stocks over the sampling period. However, the two reaches showed clear differences in the temporal variability of CPOM standing stock. At the downstream reach, the standing stock of CPOM was higher in November 2001 and decreased towards the summer, reflecting the typical seasonal pattern of riparian vegetation dominated by deciduous trees (Kaushik and Hynes 1971, Webster and Benfield 1986, Pozo *et al.* 1997). At the upstream reach, in contrast, the temporal variability of CPOM most likely fitted with that of an evergreen forest (Campbell and Fuchshuber 1994), although riparian vegetation was so similar in the two reaches. In this sense, differences between the two reaches in hydrologic regime could pay to modify the typical pattern of riparian CPOM standing stocks (Boulton and Lake 1992, Gasith and Resh 1999). The stream was affected by a drought from July to middle October 2001 in which the upstream reach was completely dry whereas the downstream reach was feed by the point source. This meant that at the upstream reach most leaves felt in a dry streambed. Therefore, leaf litter was exposed to wind transport and breakdown. In addition, the first storms are also known to imply a higher CPOM export on dry channels as a pulse (Boulton and Lake 1992). The increase of CPOM standing stock at the upstream reach in April 2002 may be partially explained by an increase of lateral inputs induced by rainfall (Pozo *et al.* 1997). Of major relevance was the higher abundance of vascular plants that may act as retention structures (Koetsier and McArthur 2000). As argued by Gasith and Resh (1999), flooding may also play an important role on CPOM dynamics by increasing transport and reducing standing stocks as observed in June 2002 in the two reaches.

Similar to CPOM, standing stocks of FPOM did not differ between the two reaches either. It is well known that point sources can increase dissolved and

particulate organic carbon concentrations (Paul and Meyer 2001). However, the complexity of FPOM dynamics (Anderson and Sedell 1979, Allan 1995) and the scarcity of published studies on that topic (Paul and Meyer 2001) makes difficult to identify factors controlling FPOM standing stocks. A study in a north central Colorado stream (Shieh *et al.* 1999) found an increase of FPOM below a WWTP input. The higher FPOM standing stock could not be directly related to the point source but to the increase in retention capacity provided by vascular plants and macroalgae. In agreement with a number of studies (e.g., Petersen *et al.* 1989, Boulton and Lake 1992), FPOM standing stocks were rather constant over the sampling period at the upstream reach. We believed that only flow (Wallace *et al.* 1991, Martinez *et al.* 1998) and retention structures such as vascular plants, filamentous algae, and mosses (Stream Bryophyte Group 1999, Koetsier and McArthur 2000) were in charge of temporal changes in FPOM standing stocks.

We could not infer clear temporal patterns of the CPOM:FPOM ratio as reported by Wallace *et al.* (1982). However, a previous study in this stream during a dry year (Ortiz *et al.*, in press) found a higher CPOM:FPOM ratio below the WWTP input, presumably because of a decrease on shredder abundance. Many studies reported a significant increase of the conversion rate of CPOM:FPOM by shredders (Petersen and Cummins 1974, Cuffney *et al.* 1990). This lends support to the hypothesis of Ortiz *et al.*, (in press), because in the present study shredder density and biomass did not differ between the two reaches.

Either from this study or from surveys in France (Brunet *et al.* 2001) and in Georgia (Roy *et al.* 2003) it is demonstrated that WWTP effluents can significantly increase SPOM concentrations. As Wallace *et al.* (1991) pointed out, SPOM concentrations were poorly related to discharge. In contrast with the findings of Wallace *et al.* (1982), SPOM concentration was not influenced by the trend of the hydrograph in any of the two reaches, probably because of differences

in hydromorphology. However, as have been seen with nutrient concentrations (Gasith and Resh 1999), higher stream discharges can reduce SPOM concentration by increasing dilution capacity.

Effects of the point source on functional organization

Overall, our results lent support to the expectations of Kerans and Karr (1994). Relative density of shredders decreased while that of gatherers and filterers increased. A similar response has been seen in related studies (Ortiz *et al.*, in press, Hachmöller *et al.* 1991, Shieh *et al.* 1999, Roy *et al.* 2003). In our study case, the decrease of shredder relative density below the point source was not due to a decrease of shredder density but to the effect of the increase of gatherers and filterers. The density of scrapers was also higher at the downstream reach, but it was not reflected on relative density. In fact, the most important change in taxa composition below the point source we could appreciate was an increase in density of chironomids, as reported by studies from decades ago (Hynes 1978). Most chironomids are not only pollution resistant but are also able to reach high densities in nutrient enriched ecosystems (Hynes 1978, Paul and Meyer 2001). Non-Tanytopodinae chironomids are considered mainly gatherers and/or scrapers (Moog 2002), and hence the observed increase of density of gatherers and scrapers below the point source might be merely due to an increase in chironomid abundance. The density of scrapers was consistently higher at the downstream reach than at the upstream reach although chlorophyll *a* was similar in the two reaches on some sampling dates. Similarly, the density of gatherers was also consistently higher below the point source even though FPOM standing stock did not differ between the two reaches. In this sense, we found the use of biomass more reliable than density for bioassessments focused on functional aspects of stream ecosystems. Nutrient enrichment may lead to morphological and

behavioral adaptations that not necessarily reflect changes on resource availability but have energetic implications (Benke *et al.* 1988). However, our results showed that the hypotheses of Kerans and Karr (1994) could not easily be applied to relative biomass. At the downstream reach, relative biomass of shredders was lower than at the upstream reach, akin to relative density. On the contrary, relative biomass of gatherers decreased and relative biomass of predators augmented below the point source input. The decrease of shredder and gatherer relative biomass below the point source was a consequence of the high increase of predator biomass. The unexpected increase in relative biomass of predators may be explained by the fact that leeches and Tanypodinae midges were not regarded as “strict” predators by Kerans and Karr (1994) because they are considered resistant to pollution (Moog 2002). At the downstream reach, the contribution of leeches and midges represented the 94% of the biomass of predators whereas at the upstream reach this group hardly reached the 16%. Changes on relative biomass of a given functional feeding group did not always reflect a change on its biomass but on the biomass of other groups, as seen for shredders and gatherers. On the other hand, biomass of filterers was higher at the downstream reach, but was not translated in a higher relative biomass. Changes on resources availability leads to changes on functional organization (Cummins 1973) as a response of the benthic community trend to obtain the greatest energy balance (Vannote *et al.* 1980, Hall *et al.* 1992). Thus, similarities in chlorophyll *a*, CPOM and FPOM between the two reaches were translated in similar biomasses of scrapers, shredders, and gatherers, respectively. The increase in SPOM concentrations at the downstream reach relative to the upstream reach enhanced filterers, as has been reported before (Wallace and Webster 1996). In contrast, predator biomass was higher at the downstream reach than at the upstream reach although primary consumers biomass was similar between the two reaches. The explanation of such

incongruence might lie on changes in the characteristics of primary consumers. At the downstream reach, chironomids comprised five times more biomass of primary consumers than at the upstream reach. The higher relevance of chironomids at the downstream reach meant that primary consumers not only had higher biomass turnovers (Benke 1998) but also higher susceptibility to predation (Wallace and Webster 1996), probably because of small body sizes and low motility (Tachet *et al.* 2000).

Many studies showed that point sources might alter the functional organization of the receiving ecosystems by modifying the relative importance of feeding strategies, but of most relevance to the present study is that relationships between consumers and resources are also susceptible to change. A similar study performed on a north central Colorado stream (Shieh *et al.* 1999) found weak but significant correlations for scrapers with periphyton biomass, shredders with CPOM, and gatherers with FPOM. However, in pristine streams these relationships are often not significant (Martinez *et al.* 1998, Doisy and Rabeni 2001) or habitat dependent (Barmuta 1988, Boulton and Lake 1992). Contrary to the opinion of (Hawkins and Sedell 1981), relationships between density of functional feeding groups and their presumed resources were generally not significant in the present study, while biomass offered best relationships. The wide range of body sizes within the taxa of each functional feeding group and especially the high mortality of young individuals in natural population makes biomass more reliable than density to explain the dependence of macroinvertebrates to their presumed food resources. However, production is supposed to provide more reliable information because it includes all components of success of macroinvertebrate communities (Benke 1993). In the two reaches, biomass of scrapers, gatherers, and filterers were higher with increasing chlorophyll *a* concentration, FPOM standing stock, and SPOM concentration,

respectively. These relationships were suitable for the two reaches combined, indicating that the response of consumer biomass to their presumed resources was not significantly affected by the point source. However, the weakness of these relationships might indicate: 1) improper functional feeding group classification or inadequate resolution in characterization and/or quantification of food availability (Hawkins and Sedell 1981), 2) variability of resource quality, 3) a delayed response of macroinvertebrates due to colonization dynamics (Boulton and Lake 1992), or 4) over-abundance of resources (Boulton and Lake 1992, Martinez *et al.* 1998). At the downstream reach, shredders biomass was strongly related to CPOM standing stock, whereas at the upstream reach this relationship was not significant. Previous research showed that the relationship between shredders and CPOM is not so consistent in non-altered streams (Martinez *et al.* 1998, Doisy and Rabeni 2001), although high shredder densities are usually related to high BOM standing crops (Richardson 1991, Dobson and Hildrew 1992, Wallace *et al.* 1999). At the downstream reach, shredders biomass peaked in November 2001, when most leaf fall occurred, and then decreased towards the summer. In contrast, at the upstream reach the lack of flow in the summer of 2001 altered the typical seasonal pattern of CPOM. In consequence, shredders showed a delay relative to the expected synchronization of their live cycles to the typical seasonal pattern of CPOM (Cummins *et al.* 1989). The relationship between predators and consumers was also different between the two reaches. Not all taxa and body sizes are equally susceptible to predation, small individuals may be not considered depending on the used mesh-size in sample collection (Hawkins and Sedell 1981), and cannibalism is not uncommon within macroinvertebrates (Wallace *et al.* 1999). Regarding the difficulty to obtain realistic estimates, the generalization of considering all primary consumers as potential prey usually provides satisfactory relationships (Hawkins and Sedell 1981, Wallace *et al.*

1999). Our results showed that the relationship between predators and primary consumers could change according to the morphobehavior of prey. The point source favored taxa that were best adapted to the mediated conditions according to the energy balances theory of Hall *et al.* (1992). This translated in an increase in chironomid biomass, which had a higher success under these conditions through an *R* strategy (i.e., short live spans and high reproduction rates). The change of the dominant prey also resulted in favoring those predators that could obtain the lower cost-benefit ratio.

Conclusions

The point source moderately increased nutrient concentrations that enhanced primary producers under sufficient light exposure and stable flow conditions. Dynamics of benthic organic matter were also affected by the point source through changes on flow regime. Changes on functional feeding group biomass were probably more reliable for bioassessment because in this stream they were best explained by the effects of the point source than density or relative contributions. Our results demonstrated that the relationships between consumers and resources might also be altered by point sources. Concretely, we detected that shredder biomass was not related to CPOM standing stock above the point source whereas below the point source the relationship was significant, apparently because of changes in hydrologic regime. The relationship between predators and primary consumers was steeper at the downstream reach than at the upstream reach, presumably because of higher biomass turnover rates and susceptibility to predation of primary consumers at the downstream reach.

Summary

We monitored benthic macroinvertebrates and their potential food resources for one year (2001-2002) to assess the effect of a point source input on the functional organization in La Tordera stream (Catalonia, NE Spain). The two reaches did not differ in standing stocks of periphyton, vascular plants, CPOM and FPOM. Chlorophyll *a* concentrations, filamentous algae, mosses, and SPOM concentrations were increased by the point source. Relative density of shredders was reduced at the downstream reach, whereas gatherers and filterers were enhanced. However, density of shredders was similar in the two reaches. Relative biomass of shredders decreased below the point source as well, but gatherers diminished and predators increased. Shredder biomass was similar in the two reaches and only filterer and predator biomass increased below the point source. Bray-Curtis similarities between the two reaches in terms of density and biomass of functional feeding groups were higher in June 2002, after the spate of May 2002. The relationships between density of functional feeding groups and their presumed food resources were rarely significant. The relation between functional feeding groups and food resources was best explained through macroinvertebrate biomass. The two reaches had the same relationship for scrapers, gatherers, and filterers. At the downstream reach, shredders biomass was strongly related to CPOM standing stock whereas at the upstream reach this relationship was not significant. The relationship between predator and primary consumer biomass was significant for the two reaches, but at the upstream reach had a low slope whereas at the downstream reach was more steep.

6. Microhabitat changes and the distribution of benthic macroinvertebrates below the point source

Introduction

Human activities may alter the microhabitat conditions and have severe implications on the structure and function of stream ecosystems (Hart and Finelli 1999, Harrison *et al.* 2004). The increase of nutrient concentrations and oxygen demand induced by wastewater treatment plant (WWTP) effluents into stream ecosystems generally enhance primary producers (Welch *et al.* 1992, Suren 2000, Lee and Hershey 2000) and, subsequently, increase retention of particulate organic matter (Stream Bryophyte Group 1999, Koetsier and McArthur 2000). The distribution of macroinvertebrates in stream ecosystems is determined by the energy balance resulting from the costs and benefits of food and oxygen acquisition in their hydraulic environment (Benke *et al.* 1988, Hall *et al.* 1992, Quinn and Hickey 1994, Doisy and Rabeni 2001). Therefore, changes in the microhabitat conditions will result in adjustments of taxa composition and of the subsequent functioning of the biological compartments.

The works of Percival and Whitehead (1929) symbolized the first significant contribution to the study of macroinvertebrate-substratum relationships and paved the way for posterior studies on invertebrate microdistribution in flowing ecosystems. The relevance of microhabitat conditions has long been studied in stream ecology (see reviews by Ward 1992, Minshall 1984, and Death 2000). Results from earlier studies concluded that although certain taxa may only occur in particular substrata sizes, inorganic substrate exerted a lesser influence on macroinvertebrate distribution (Statzner *et al.* 1988, Death 2000). Water velocity is considered by far the best indicator of macroinvertebrate distribution in a number of studies (Quinn and Hickey 1994, Bouckaert and Davis 1998, Fairchild

and Holomuzki 2002). However, either water velocity itself or integrated in complex hydraulic parameters remains insufficient to describe the distribution of most taxa and it is usually confounded with other interacting variables. Studies at reach and catchment scales also considered other environmental variables such as nutrient and ionic concentrations, salinity, acidity, temperature, dissolved oxygen, light exposure, riparian vegetation, geology, and pollutants (e.g., Rabeni and Doisy 2000, Burgherr and Ward 2001, Parsons *et al.* 2003). Most of these factors may also play an important role at the scale perceived by stream organisms, but they are hardly considered in microdistribution studies given the actual methodological constraints (Pringle *et al.* 1988).

Some studies examined the effect of pollution on macroinvertebrate behavior, including mobility and feeding (Johnson *et al.* 1993). However, few authors have stressed the importance of macroinvertebrate-microhabitat relationships in human altered streams and only a small number of microhabitat variables were contemplated. A study in a north central Colorado stream found that total macroinvertebrate density was positively related to macroalgae, benthic organic matter, and chlorophyll *a*, while taxa richness was negatively related to the mentioned variables (Shieh *et al.* 1999). The correlations obtained by this study derived from the combined results from reference and altered sites and could not determine the most relevant variables in each site. In addition, only a few studies dealt with relationships between community parameters and microhabitat variables and patterns are less certain (Doisy and Rabeni 2001).

We collected macroinvertebrate samples and determined microhabitat variables (including organic matter, primary producers, and hydraulics) on six sampling dates over the hydrologic year 2001-2002 in one reach upstream and one reach downstream of a point source in La Tordera stream (Catalonia, NE Spain). Our objectives were to: 1) determine the microhabitat variables of major relevance

for the macroinvertebrate community characteristics and macroinvertebrate distribution, 2) assess the effect of the point source input on the dependence of macroinvertebrates to microhabitat variables, and 3) compare the information obtained from density data to that of biomass in taxa-microhabitat analyses. We hypothesize that changes on taxa composition will result in a lower dependence of macroinvertebrates to microhabitat conditions because of lower taxa richness and higher dominance of generalist taxa below the point source input. However, the study of the effects of nutrient enrichment on macroinvertebrate distribution is beyond the scope of this research. Macroinvertebrate biomass is hardly used in macroinvertebrate-microhabitat studies; we believe that biomass may provide information of higher functional significance relative to macroinvertebrate density.

Results

Benthic macroinvertebrates

From the 91 taxa that were recorded over the sampling period in the two reaches, 24 taxa were only found in the upstream reach (most of them mayflies, stoneflies and caddisflies) and four in the downstream reach (e.g., *Pisidium casertanum* and *Glossiphonia* sp.; Appendix 6.1). Sixteen taxa were present on all samplings and in at least a 50% of the samples considering either all sampling dates or just the four samplings before the spate of May 2002 in the two reaches (e.g., *Baetis rhodani*, Orthocladinae, Simuliidae, *Ancyclus fluviatilis*, *Physella acuta*). On the other hand, 21 and 13 taxa appeared only in one sample at the upstream reach and at the downstream reach, respectively (e.g., *Coelostoma* sp., *Dixa* sp., *Dryops* sp.).

Microhabitat variables

Most considered microhabitat variables showed a similar range of values in the two reaches (Table 6.1). FPOM standing stock showed a narrower range than CPOM as well as a lower variability in the two reaches. Chlorophyll *a* concentration, filamentous algae and, especially, mosses standing stock had higher mean and maximum values at the downstream reach than at the upstream reach. Mean and maximum water velocity were slightly higher at the downstream reach (0.29 m/s and 0.56 m/s, respectively) than at the upstream reach (0.23 m/s and 0.45 m/s, respectively). Minimum water velocity was close to 0 m/s in most samples of the two reaches. The range of water depth was quite similar between the two reaches over the sampling period and varied between 2.0 cm and 37.5 cm. We estimated mean water depth from the average between maximum and minimum water depth because these two variables were strongly correlated ($r = 0.970$, $p < 0.0005$, $n = 72$). The dominant substrata types were cobbles and pebbles in the samples of the two reaches.

The two-tailed Spearman's rank correlation performed for microhabitat data from November 2001 to April 2002 revealed significant correlations between certain microhabitat variables (Appendix 6.2 and 6.3). CPOM and FPOM were positively correlated in the two reaches. Chlorophyll *a* was negatively correlated to water velocity and Reynolds number in the two reaches. There was a positive correlation among water velocities (V_{\max} , V_{\min} , and V_{mean}) and complex hydraulic parameters (Fr , Re , and V_*). At the upstream reach, large substrata sizes were positively correlated with minimum water velocity while small substrata sizes were negatively correlated with maximum and mean water velocity. At the downstream reach, substratum type was not significantly correlated with any water velocity measurement.

Table 6.1 Mean \pm SE, minimum (min) and maximum (max) values for the measured microhabitat variables from November 2001 to September 2002 in the two reaches ($n = 36$). Units and acronyms of the variables are also shown.

Variable	Units	Acronym	Upstream			Downstream		
			mean	min	max	mean	min	max
CPOM	mg AFDM/m ²	CPOM	8375 \pm 2532	233	92245	13267 \pm 3619	209	131995
FPOM	mg AFDM/m ²	FPOM	2338 \pm 316	329	8132	3702 \pm 663	382	19110
Chlorophyll <i>a</i>	mg Chl <i>a</i> /m ²	Chl <i>a</i>	37.98 \pm 5.14	0.72	110.03	199.47 \pm 54.37	1.49	915.94
Filamentous algae	mg AFDM/m ²	Fil	114 \pm 28	0	599	1587 \pm 456	0	11585
Moss	mg AFDM/m ²	Moss	57 \pm 20	0	536	4274 \pm 1427	0	35315
Vascular plants	mg AFDM/m ²	Vasc	73 \pm 46	0	1616	87 \pm 47	0	1597
Maximum water velocity	m/s	V max	0.45 \pm 0.03	0.15	0.87	0.56 \pm 0.04	0.17	1.17
Minimum water velocity	m/s	V min	0.06 \pm 0.01	0.00	0.32	0.05 \pm 0.01	0.00	0.28
Mean water velocity	m/s	V mean	0.23 \pm 0.02	0.05	0.61	0.29 \pm 0.03	0.09	0.83
Maximum water depth	cm	D max	22.1 \pm 1.1	8.0	37.0	23.0 \pm 1.3	9.5	37.5
Minimum water depth	cm	D min	16.7 \pm 1.2	3.0	32.0	17.3 \pm 1.4	2.0	33.0
Mean water depth	cm	D	19.4 \pm 1.2	5.5	34.5	20.2 \pm 1.3	5.8	35.0
Boulder	%	Bou	8 \pm 4	0	95	4 \pm 2	0	74
Cobble	%	Cob	49 \pm 5	0	100	51 \pm 5	0	100
Pebble	%	Peb	25 \pm 4	0	71	31 \pm 4	0	81
Gravel	%	Gra	14 \pm 3	0	76	9 \pm 2	0	57
Sand	%	San	4 \pm 2	0	51	5 \pm 2	0	34
Substrate roughness	-	k _v	2.35 \pm 0.07	1.56	3.44	2.40 \pm 0.06	1.78	3.22
Froude number	-	Fr	0.02 \pm 0.00	0.00	0.05	0.02 \pm 0.00	0.01	0.06
Reynolds number	-	Re	4898040 \pm 516221	947603	11438127	5993280 \pm 640191	2132107	19894091
Roughness shear velocity	-	V _s	0.02 \pm 0.00	0.00	0.06	0.03 \pm 0.00	0.01	0.07

Community parameters in relation to microhabitat variables

Correlations of macroinvertebrate density, biomass, and taxa richness with the microhabitat variables showed similar results for the two reaches (Table 6.2). At the two reaches combined and per separate, density and biomass were significantly, positively correlated with CPOM, FPOM, chlorophyll *a*, filamentous algae, moss, water velocity, Froude number, Reynolds number, and roughness shear velocity. Taxa richness was negatively correlated with water velocity. At the two reaches combined, taxa richness was negatively correlated to chlorophyll *a*, filamentous algae, and moss. However, at the upstream reach taxa richness was positively correlated with chlorophyll *a*. Community parameters were generally weakly correlated to any substrata type and to substrate roughness.

Taxa distribution in relation to microhabitat variables

The explained variance by the first three axes of the PCAs performed for the macroinvertebrate data of the two reaches combined were a 49% for density and a 50% for biomass (Table 6.3). In the PCAs performed for each reach separately, the explained variance was slightly higher at the downstream reach (54% and 53% for density and biomass, respectively) than at the upstream reach (48% for density and 48% for biomass). The percentage of variance of taxa explained by the three first axes of direct gradient analyses (RDA) were quite low for the two reaches separately (between 24% and 25%) and, especially, combined (17% for density and 18% for biomass, respectively). However, if data from June and September 2002 (after the spate of May 2002) were excluded from the analyses, the explained variance was higher (between 5% and 11% of increase). The increase of the explained variance was mainly due to an increase of the eigenvalues of the second axis at the upstream reach and of the first axis at the downstream reach. In all RDA analyses, axis III explained a low percentage of the

Table 6.2 Two tailed Spearman's rank correlations of total density, total biomass, and taxa richness of macroinvertebrates with the 19 microhabitat variables from November 2001 to April 2002 at the two reaches combined ($n = 48$) and per separate ($n = 24$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See Table 6.1 for acronyms.

	Upstream and downstream ($n = 48$)						Upstream ($n = 24$)						Downstream ($n = 24$)						
	Density		Biomass		Richness		Density		Biomass		Richness		Density		Biomass		Richness		
CPOM	0.413**	0.399**	-0.096	0.323	0.260	0.003	0.375	0.413*	0.093	0.500***	0.523***	0.051	0.393	0.438*	0.336	0.547***	0.590**	0.270	
FPOM	0.520***	0.203	-0.329*	-0.007	-0.055	0.466*	0.287	0.241	-0.189	0.752***	0.449**	-0.412**	0.530**	0.657***	0.234	0.633***	0.491*	-0.281	
Chl a	0.640***	0.300*	-0.373**	-0.039	0.062	0.045	0.718***	0.574**	0.271	0.131	0.095	0.018	0.141	-0.046	0.053	0.125	0.221	-0.046	
V asc	0.101	-0.226	-0.467***	-0.260	-0.368	-0.534**	-0.028	-0.206	-0.189	-0.074	-0.208	-0.294*	-0.068	-0.077	-0.385	-0.306	-0.343	-0.093	
V max	0.060	-0.192	-0.461***	-0.100	-0.163	-0.442*	-0.193	-0.270	-0.259	0.060	-0.192	-0.461***	-0.100	-0.163	-0.442*	-0.193	-0.270	-0.259	
D	-0.110	-0.035	-0.127	-0.491*	-0.158	-0.342	-0.165	0.053	0.117	0.061	0.058	-0.136	-0.262	-0.344	-0.400	0.478*	0.480*	0.099	
Bou	-0.102	0.015	0.084	-0.023	0.124	0.055	-0.107	-0.110	-0.122	0.020	-0.095	-0.087	0.274	0.134	0.218	-0.263	-0.232	-0.052	
Cob	0.017	0.063	0.276	0.289	0.205	0.267	0.047	-0.010	0.166	0.311*	0.066	-0.283	0.087	0.086	0.181	0.058	0.099	-0.087	
Peb	-0.059	-0.139	-0.180	-0.225	-0.236	-0.226	-0.006	-0.052	-0.135	0.066	-0.161	-0.333*	0.105	-0.092	-0.273	-0.129	-0.255	-0.251	
Gra	0.066	-0.172	-0.374**	-0.263	-0.214	-0.493*	-0.283	-0.159	-0.103	-0.063	-0.172	-0.374**	-0.263	-0.214	-0.493*	-0.283	-0.159	-0.103	
San	0.054	-0.189	-0.408**	-0.027	-0.162	-0.391	-0.154	-0.261	-0.271	0.054	-0.189	-0.408**	-0.027	-0.162	-0.391	-0.154	-0.261	-0.271	
k_v																			
Fr																			
Re																			
V_s																			

Table 6.3 Length of gradient resulting from DCAs, eigenvalues of the first three axes of combined and separate PCAs and RDAs of density and biomass of macroinvertebrates from November 2001 to September 2002 at the two reaches (N-S), and eigenvalues of the two first axes of RDAs of density and biomass of macroinvertebrates from November 2001 to April 2002 at the two reaches (N-A).

Variable	Method	Upstream and downstream						Upstream			Downstream		
		Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
Density	Length of gradient N-S	1.57	1.52	1.19	1.59	1.17	0.90	1.27	1.04	0.72			
	PCA N-S	0.27	0.14	0.09	0.25	0.12	0.10	0.26	0.19	0.10			
	RDA N-S	0.09	0.06	0.03	0.13	0.07	0.06	0.11	0.09	0.04			
	RDA N-A	0.15	0.06	0.04	0.12	0.11	0.07	0.13	0.09	0.07			
Biomass	Length of gradient N-S	2.12	1.82	1.36	1.86	1.70	1.19	1.58	1.26	1.19			
	PCA N-S	0.31	0.11	0.09	0.24	0.16	0.08	0.23	0.20	0.10			
	RDA N-S	0.10	0.05	0.03	0.12	0.06	0.05	0.13	0.08	0.05			
	RDA N-A	0.16	0.05	0.04	0.15	0.12	0.08	0.15	0.09	0.07			

Table 6.4 Summary statistics for the RDA relating macroinvertebrate densities and biomasses to the microhabitat variables from November 2001 to April 2002 at the upstream reach and the downstream reach combined. See Table 6.1 for acronyms.

Variable	Density				Biomass			
	Forward selection		Canonical coefficients		Forward selection		Canonical coefficients	
	Extra-fit	<i>p</i> -value	Axis 1	Axis 2	Extra-fit	<i>p</i> -value	Axis 1	Axis 2
Chl <i>a</i>	0.11	< 0.0005	0.67	0.23	0.12	< 0.0005	0.69	0.35
Moss	0.06	< 0.0005	0.37	0.50	0.06	< 0.0005	0.40	-0.42
V max	0.04	0.001	0.08	-1.08	0.04	0.002	0.00	0.87
CPOM	0.04	0.002	0.05	0.57	0.04	0.003	0.09	-0.79
D	0.03	0.004	0.28	-0.31	0.04	0.001	0.27	0.33
Fil	0.03	0.005	0.20	-0.54	0.03	0.004	0.16	0.06

variance (< 8%). The forward selection procedure of the RDA performed for the two reaches from November 2001 to April 2002 retained six microhabitat variables (chlorophyll *a*, moss, maximum water velocity, CPOM, water depth, and filamentous algae; Table 6.4). The retained variables were the same for density and biomass but the canonical coefficients were slightly different. The explained inertia (extra-fit) was quite low for most variables.

In the RDA performed for the upstream reach from November 2001 to April 2002, the forward selection procedure retained five microhabitat variables (chlorophyll *a*, filamentous algae, CPOM, maximum water velocity, and sand%; Table 6.5). Although the explained inertia and the canonical coefficients of the microhabitat variables were slightly different, the selected variables were the same for density and biomass. In the RDA performed on taxa density of the upstream reach, maximum water velocity was positively related to axis I, and chlorophyll *a* concentration was negatively related to axis I (Figure 6.1a). CPOM standing stock, filamentous algae, and sand% were positively related to axis II. In the RDA performed on taxa biomass of the upstream reach, CPOM, filamentous algae, and sand% were positively related to axis I, whereas chlorophyll *a* and maximum

Table 6.5 Summary statistics for the RDA relating macroinvertebrate densities and biomasses to the microhabitat variables from November 2001 to April 2002 at the upstream reach. See Table 6.1 for acronyms.

Variable	Density				Biomass			
	Forward selection		Canonical coefficients		Forward selection		Canonical coefficients	
	Extra-fit	<i>p</i> -value	Axis 1	Axis 2	Extra-fit	<i>p</i> -value	Axis 1	Axis 2
Chl <i>a</i>	0.11	< 0.0005	-0.73	0.46	0.13	< 0.0005	-0.30	1.03
Fil	0.08	0.002	-0.05	0.62	0.10	< 0.0005	0.58	0.67
CPOM	0.08	0.006	-0.09	0.63	0.08	0.035	0.27	0.48
V max	0.06	0.015	0.44	-0.30	0.05	0.021	0.02	-0.31
San	0.05	0.033	0.12	0.48	0.06	0.010	0.28	0.16

water velocity were respectively related to the positive and to the negative sides of axis II (Figure 6.1b). In the two RDAs performed on taxa of the upstream reach (density and biomass), few taxa were positively correlated to maximum water velocity (*Leuctra geniculata*, Psychodidae, Pediciini) and the correlations were weak in all cases. On the other hand, the strongest correlations of taxa with maximum water velocity were negative (Digenea, *Caenis luctuosa*, *Physella acuta*, *Habrophlebia fusca*, *Potamopyrgus antipodarum*, *Chaetogaster* spp., and *Agabus* sp.). Most of the taxa that were negatively correlated to maximum water velocity were at the same time positively correlated to chlorophyll *a* (*Chaetogaster* spp., *Agabus* sp., and Digenea) and vice versa (*Leuctra geniculata*). Most of the taxa that were positively correlated to CPOM were also positively correlated to sand% (*Hydropsyche instabilis*, *Oecetis* sp., and pupae of *Sericostoma personatum*, *Hemerodromiinae*, and *Tanypodinae*). Although the correlation with canonical axes was similar for CPOM, sand% and filamentous algae, the correlation was higher between CPOM and sand% ($r = 0.447$, $p = 0.028$) than between any of them and filamentous algae ($r = 0.196$, $p = 0.360$ and $r = -0.002$, $p = 0.994$, respectively). *Serratella ignita*, *Ecdyonurus angeli* and Lumbriculidae density and biomass were positively correlated to filamentous algae but several taxa were

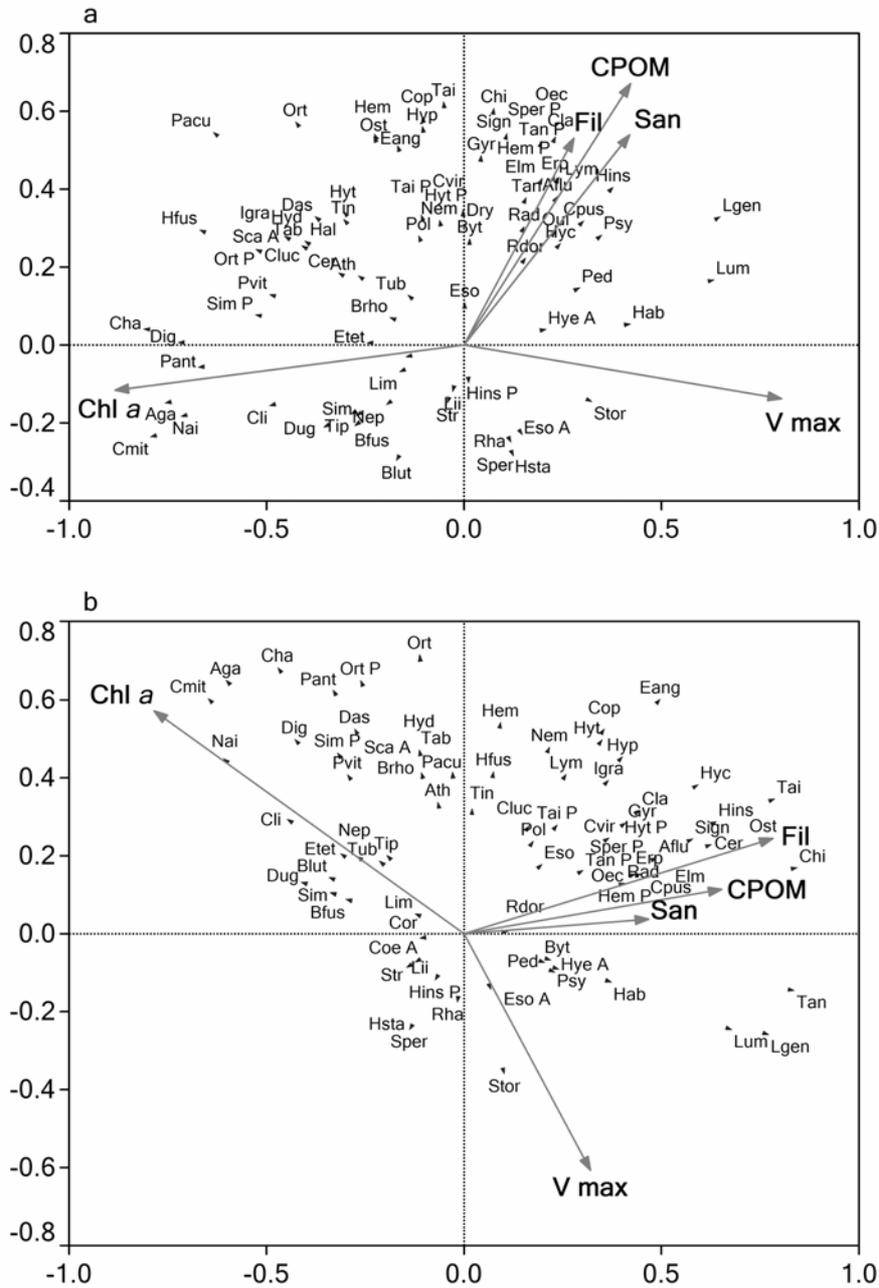


Figure 6.1 RDAs performed on macroinvertebrate density (a) and biomass (b) for the upstream reach. See Appendix 6.1 for taxon codes and Table 6.1 for acronyms.

correlated to this variable only for density (*Ancylus fluviatilis*, Cladocera) or only for biomass (Ostracoda, Ceratopogoninae, Chironomini, Tanytarsini).

In the RDA performed for the downstream reach from November 2001 to April 2002, the forward selection procedure retained five microhabitat variables (Table 6.6). Four of these variables were common with the analyses performed on the upstream data (maximum water velocity, CPOM, filamentous algae, and chlorophyll *a*) but moss standing stock was selected instead of sand. Similar to the analyses performed for the upstream reach, the retained microhabitat variables were the same between density and biomass and the explained inertia differed little. In the two RDAs performed on taxa of the downstream reach (density and biomass), maximum water velocity was positively related to axis I and negatively related to axis II (Figure 6.2). CPOM, moss, filamentous algae, and chlorophyll *a* were negatively related to axis I. Chlorophyll *a* was also positively related to axis II, whereas moss and filamentous algae were negatively related to axis II. Only *Hydropsyche instabilis* was positively correlated to maximum water velocity whereas other taxa as *Pisidium casertanum* were negatively correlated to this variable. Ostracoda, Simuliidae, and *Erpobdella* sp. were positively correlated to CPOM standing stock. Similar to the upstream reach, the oligochaete family Naididae, including the genus *Chaetogaster* spp., was positively correlated to chlorophyll *a*. At the downstream reach, *Hydra* sp. and Chironomini were also positively correlated to chlorophyll *a*, whereas *Serratella ignita* was negatively correlated to this variable. Filamentous algae and moss showed a similar correlation with canonical axes but were not significantly correlated ($r = 0.275$, $p = 0.193$). However, Cladocera and the midges Tanytarsini and Orthocladiinae (larvae or pupae) were strongly correlated with these two microhabitat variables.

Table 6.6 Summary statistics for the RDA relating macroinvertebrate densities and biomasses to the microhabitat variables from November 2001 to April 2002 at the downstream reach. See Table 6.1 for acronyms.

Variable	Density				Biomass			
	Forward selection		Canonical coefficients		Forward selection		Canonical coefficients	
	Extra-fit	<i>p</i> -value	Axis 1	Axis 2	Extra-fit	<i>p</i> -value	Axis 1	Axis 2
Moss	0.09	0.002	-0.65	-0.36	0.10	0.001	-0.61	0.35
V max	0.07	0.019	0.72	-0.50	0.07	0.018	0.61	0.52
CPOM	0.07	0.033	-0.58	0.58	0.07	0.017	-0.49	-0.66
Fil	0.07	0.011	0.02	-0.79	0.07	0.016	-0.11	0.83
Chl a	0.07	0.008	-0.19	0.62	0.08	0.005	-0.34	-0.55

The upstream reach showed a higher number of significant correlations between macroinvertebrate taxa and microhabitat variables than the downstream reach (Table 6.7). The difference of significant correlations between the two reaches was first explained by higher taxa richness at the upstream reach than at the downstream reach (51% and 66% of difference explained for density and biomass, respectively). The remaining difference was explained by differences on the response of macroinvertebrate distribution to microhabitat variables between the two reaches (49% and 34% of difference explained for density and biomass, respectively). In the two reaches, the number of positive significant correlations was two times higher than the number of negative significant correlations either for density or for biomass. Most of the positive significant correlations of macroinvertebrates occurred with variables related to food resources or structure (CPOM, FPOM, filamentous algae, and chlorophyll *a* in the two reaches and moss in the downstream reach). Tanytarsini, Cladocera, Copepoda, and Ostracoda showed the strongest correlations with these microhabitat variables. Nearly all negative significant correlations of macroinvertebrates with microhabitat variables occurred with water velocity and complex hydraulic parameters in the two reaches (e.g., *Caenis luctuosa*, *Physella acuta*). All the significant correlations between

Response of benthic macroinvertebrates to a point source

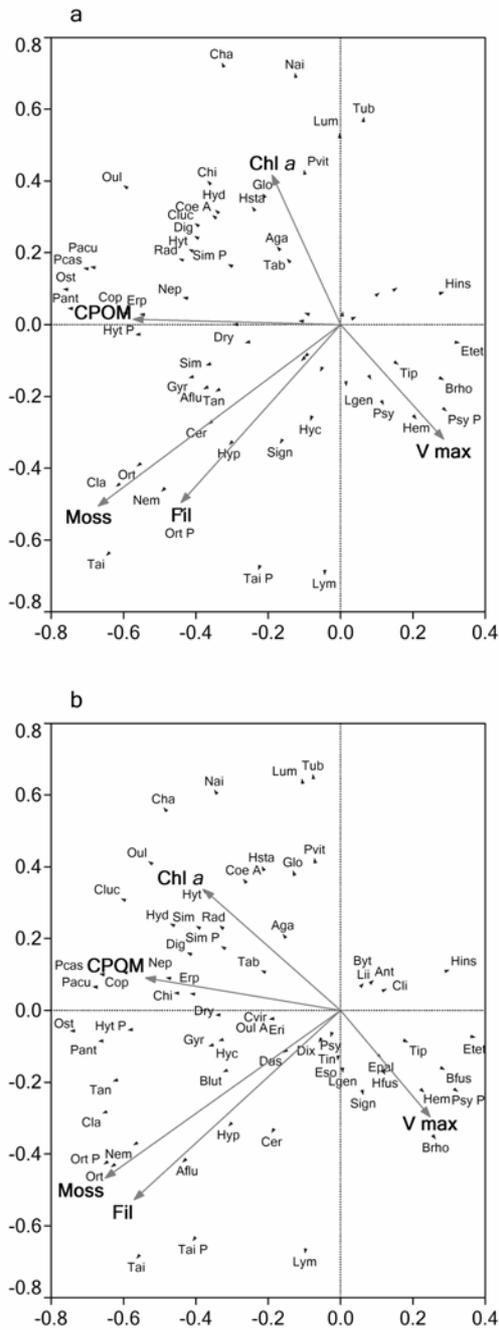


Figure 6.2 RDAs performed on macroinvertebrate density (a) and biomass (b) for the downstream reach. See Appendix 6.1 for taxon codes and Table 6.1 for acronyms.

Table 6.7 Number of positive (+), negative (-) and total significant correlations of macroinvertebrate densities and biomasses with microhabitat variables for each microhabitat variable from November 2001 to April 2002 at the two reaches. Forward selected variables in RDA analyses were highlighted in bold. See Table 6.1 for acronyms.

Variable	Density						Biomass					
	Upstream			Downstream			Upstream			Downstream		
	+	-	Total	+	-	Total	+	-	Total	+	-	Total
CPOM	13	3	16	9	1	10	15	3	18	8	1	9
FPOM	13	0	13	8	0	8	9	0	9	7	0	7
Chl <i>a</i>	13	2	15	5	2	7	12	7	19	6	1	7
Fil	16	3	19	7	1	8	21	0	21	10	0	10
Moss	5	1	6	16	0	16	5	1	6	13	0	13
Vasc	2	0	2	1	0	1	2	0	2	1	0	1
V max	4	8	12	1	3	4	3	10	13	1	3	4
V min	4	8	12	4	3	7	3	3	6	3	5	8
V mean	6	9	15	2	3	5	4	8	12	2	4	6
D	0	9	9	0	2	2	0	10	10	0	4	4
Bou	1	1	2	4	0	4	1	1	2	11	0	11
Cob	2	0	2	0	1	1	2	0	2	0	0	0
Peb	0	0	0	0	1	1	2	0	2	1	2	3
Gra	6	1	7	2	0	2	4	0	4	3	0	3
San	9	1	10	2	2	4	11	1	12	2	1	3
<i>k_v</i>	3	1	4	0	2	2	1	1	2	0	2	2
Fr	5	9	14	1	3	4	3	7	10	1	3	4
Re	4	10	14	2	3	5	4	9	13	2	4	6
V*	3	1	4	1	3	4	1	0	1	1	2	3
Total	109	67	176	65	30	95	103	61	164	72	32	104

macroinvertebrates and water depth were negative and were especially important at the upstream reach (e.g., Orthocladiinae, *Physella acuta*). Overall, the number of significant correlations between macroinvertebrates and substrata types and substrate roughness was relatively low (Table 6.7). However, sand% was especially relevant at the upstream reach mainly because of correlations of taxa that were absent at the downstream reach (*Caenis pusilla*, *Sericostoma*

personatum, *Oecetis* sp.). Naididae was significantly negatively correlated with sand% in the two reaches for density and biomass. Boulder% was positively correlated with a number of taxa in terms of biomass at the downstream reach (e.g., Hydracarina, *Baetis lutheri*, *Gyraulus* sp.).

From the significant correlations between taxa present in the two reaches and microhabitat variables (200 for density and 196 for biomass), a relatively low percentage showed the same response in the two reaches (7% for density and 6% for biomass). The percentage of correlations that were significant only at the upstream reach (57% for density and 52% for biomass) was higher than those that were significant only at the downstream reach (37% for density and 41% for biomass). The correlation of density of *Baetis fuscatus* and substrate roughness was positive at the upstream reach and negative at the downstream reach. The correlations of biomass of Simuliidae, Orthocladiinae, and Chironomini with CPOM, boulder% and chlorophyll *a*, respectively, were negative at the upstream reach and positive at the downstream reach.

At the upstream reach, density and biomass showed a 55% of coincidence in significant correlations between taxa and microhabitat variables. There were no significant correlations with opposite response to microhabitat variables between density and biomass of taxa. On the other hand, a 45% of the taxa were significantly correlated to microhabitat variables only for density (e.g., *Habrophlebia fusca*, *Potamopyrgus antipodarum*) or only for biomass (e.g., Orthocladiinae, Tanypodinae). Similarly, at the downstream reach, there was a 55% of coincidence between significant correlations of density and biomass with microhabitat variables. A 45% of correlations were significant only for density (e.g., *Caenis luctuosa*) or only for biomass (e.g., Tanypodinae, Hydracarina) but any taxa showed an opposite response for density and biomass to microhabitat variables. The RDAs show that different life stages of certain taxa had similar

microhabitat preferences (e.g., *Hydroptila* sp., Tanytarsini; Figures 6.1 and 6.2). However, differences between life stages of most insect taxa could not be established because of the low density and biomass of pupae and adults (Appendix 6.1).

Discussion

Microhabitat variables of relevance for macroinvertebrates

Flow has been usually considered the most important structuring factor in flowing ecosystems (Ward 1992, Allan 1995, Hart and Finelli 1999). The hydrodynamic power of flow act as a direct mechanism that influences the distribution of living organisms, but may also have indirect implications through altering the structure and distribution of intermediate microhabitat variables (Hart and Finelli 1999). At the same time, irregularities of the streambed (substrate, BOM, vascular plants, etc.) alter current patterns and lead to complex interactions. Many studies have been focused in the distribution of taxa according to their environment, but much less is known about community parameters (Doisy and Rabeni 2001). We found water velocity, chlorophyll *a*, CPOM, filamentous algae, and mosses as the most relevant microhabitat variables for community parameters (density, biomass, and taxa richness) and macroinvertebrate distribution. Our results contrast with previous research that found complex hydraulic characteristics (i.e., k_v , Fr, Re, V^*) more feasible than conventional ones (V , D) to explain macroinvertebrate distribution (Statzner *et al.* 1988, Mérigoux and Dolédec 2004). In our study, we found only negative correlations of depth with total macroinvertebrate density and individual taxa. Many studies highlighted the relevance of water depth and also found negative correlations with macroinvertebrate density (Chutter 1969, Orth and Maughan 1983, Lencioni and Rossaro 2005, Brooks *et al.* 2005). However, they did not offered any mechanistic

explanation of its potential effects on stream organisms. Depth may influence complex hydraulic parameters, but it is still closely related to water velocity (Allan 1995). Therefore, unless water was turbid enough to reduce photosynthetic production (Quinn and Hickey 1994, Masseret *et al.* 1998), significant relationships may arise from the covariance with water velocity. Invertebrates can also find refuges from flow in areas of high water velocity given the microhabitat complexity of stream ecosystems, whereas only slowly moving water occurs in areas of low water velocity (Ward 1992). Stutzner (1981) suggested that, in spite of this, water velocity restrict the distribution of certain taxa because during their movements they have to pass through fast waters. Mérioux and Dolédec (2004) expected a dome-shaped relationship between taxa richness and water velocity based on the capacity of invertebrates to resist hydraulic forces and low oxygen supply. Their results showed a negative relationship between taxa richness and flow in spring, but the opposite pattern in autumn. We found that taxa richness decreased with increasing water velocity at the two reaches combined and at the upstream reach, but did not at the downstream reach. In addition, our results show that, although several taxa were positively related with flow, many were limited by this factor. Our results contrast with the findings of Brooks *et al.* (2005) and lend support to studies that found that total macroinvertebrate density was not correlated with water velocity (Downes *et al.* 1995, Doisy and Rabeni 2001). However, a study in a southern England stream found that density was higher in flow refugia after periods of high and fluctuating flow (Lancaster and Hildrew 1993). The moderate increase of the explained variance when data collected after the spate of May 2002 was excluded from the analyses suggest that flood disturbance can affect the relationships between benthic macroinvertebrates and their environment. Individual taxa are known to depend on current velocity given their respiratory mechanisms (Allan 1995) or their resource acquisition (Wallace

and Merritt 1980). We found that few taxa were positively related to water velocity, from which only *Hydropsyche instabilis* can be easily justified. On the other hand, a number of taxa, such as *Caenis luctuosa*, *Physella acuta*, *Agabus* sp., or *Pisidium casertanum*, were negatively correlated with water velocity. Our results, together with previous studies, seem indicate that community parameters are not directly driven by microhabitat variables. Furthermore, the overall response of macroinvertebrate communities seems to be determined by the response of individual taxa and, therefore, vary according to the spatial and temporal distribution of taxa.

Either from this study or from previous research, results show that high resources availability lead to high macroinvertebrate densities and biomass (Culp *et al.* 1983, Suren and Winterbourn 1992, Shieh *et al.* 1999). In addition, several food resources may also act as structures and increase the complexity of microhabitat architecture (Sheldon and Haick 1981, Robson and Barmuta 1998). Vascular plants and mosses are rarely used for food by macroinvertebrates (Ward 1992), but provide them a colonizable surface (Suren 1991) and a source of epiphytes and BOM (Minshall 1984, Humphries 1996, Ságová-Marecková 2002, Fairchild and Holomuzki 2002). On the other hand, leaf litter acts not just as substrate but as food source too (Winterbourn 1978, Linklater 1995). In any case, the discrimination between purely physical and biotic influences is still a difficult task for stream ecologists. In the two reaches combined and per separate, moss and filamentous algae increased density and biomass of macroinvertebrates accordingly, as said before. We also found a decrease in taxa richness in the two reaches combined as found in a similar study in north central Colorado (Shieh *et al.* 1999). However, this relationship was not significant if the two reaches were analyzed per separate, suggesting that it becomes an artifact because at the downstream reach moss and filamentous algae were more abundant than at the

upstream reach while taxa richness was lower. At the upstream reach, taxa distribution was more influenced by BOM, chlorophyll *a*, and filamentous algae, whereas at the downstream reach the pervasive effect of mosses prevailed over the other biotic variables.

Despite some taxa only occur on particular substrate types, specificity among stream invertebrates is usually weak (Death 2000). Reice (1980) established that macroinvertebrates have strong substratum preferences in absence of current variability, but posterior studies lessen the relevance of substrate particle size for macroinvertebrates (Statzner *et al.* 1988, Doisy and Rabeni 2001, Mérioux and Dolédec 2004). Apparent correlations of macroinvertebrate density with substratum may be explained by the different retention capacity of detritus of each substrata size (Culp *et al.* 1983). Large substrates seem to be more productive than small-sized ones because larger substrates are more stable and accumulates more periphyton and BOM (Death 2000). However, this pattern is not consistent (Minshall 1984). In this sense, we found that boulders supported higher macroinvertebrate densities and biomasses and that a number of taxa were positively correlated with this substratum type, but only at the downstream reach. Few invertebrates are able to dwell in sandy substrates because its instability and low BOM retention (Ward 1992). However, this substrata size may become of special relevance for certain psammophilous taxa, such as *Oecetis* sp. and *Sericostoma personatum*, as seen at the upstream reach. Hydraulic and food requirements may vary through the different life stages of the same species (Rabeni and Minshall 1977, Hart and Finelli 1999). Our results show that certain taxa had a similar distribution for their different life stages, but firm conclusions cannot be drawn because of low density and biomass of certain live stages.

Effects of the point source on macroinvertebrate distribution

Little attention has been paid to the effects of nutrient enrichment on the dependence of macroinvertebrate distribution to microhabitat variables. Improper analysis of taxa-microhabitat relationships considering reference and altered sites combined may lead to confusing conclusions. Shieh *et al.* (1999) found positive significant correlations of macroinvertebrate density with macroalgae, CPOM, FPOM, and chlorophyll *a*. Our research in La Tordera stream demonstrates that the microhabitat variables overriding the macroinvertebrate community may differ among reaches. Certain community descriptors and microhabitat variables are susceptible to covariate and therefore, provide casual significant correlations. In our study, most of the retained microhabitat variables by forward selection procedures in RDAs were the same in the two reaches (CPOM, chlorophyll *a*, filamentous algae, and V max). However, sand coverage was only retained at the upstream reach while moss was only retained at the downstream reach. The range of sand% was slightly higher at the upstream reach, but of major relevance was the presence of psammophilous taxa, such as *Oecetis* sp. and *Sericostoma personatum*, both also considered sensitive taxa. Mosses, conversely, were relatively unimportant at the upstream reach but dominated the streambed at the downstream reach because of high nutrient concentrations (Lee and Hershey 2000). Mosses increased the complexity of habitat architecture and provided shelter and food (Glime and Clemons 1972, Suren 1991) to a number of macroinvertebrates, prevailing over the other variables. The differences in taxa-microhabitat relationships between the two reaches were mainly explained by differences in taxa composition. The higher taxa richness at the upstream reach was undoubtedly the major difference in charge of the higher number the significant correlations. However, important differences were also observed between taxa present in the two reaches. Such differences in taxa-microhabitat

relationships may be explained by: 1) different ranges of macroinvertebrate densities and biomasses, 2) different ranges of microhabitat variables, 3) behavioral changes that lead to different responses of macroinvertebrates to microhabitat variables (Statzner *et al.* 1988, Hart and Finelli 1999), 4) differences on species composition of higher taxonomic groups, and 5) increase of relative abundance of generalist taxa. Controlled laboratory studies will be necessary to determine better the effect of nutrient enrichment on taxa-microhabitat relationships and to identify factors that may indicate potential pathways by which it affects macroinvertebrate distribution.

Differences and similarities between macroinvertebrate density and biomass

Given the relevance of the distribution and adaptations of invertebrates on the functioning of lotic ecosystems (Allan 1995), special effort has been focused to the study of taxa-microhabitat relationships (e.g., Percival and Whitehead 1929, Glime and Clemons 1972, Statzner *et al.* 1988, Suren and Winterbourn 1992, Doisy and Rabeni 2001). However, little attention has been paid to macroinvertebrate biomass while most studies only considered density of individuals. In our study, density and biomass supplied similar information. The explained variability by the studied microhabitat variables was slightly higher for macroinvertebrate biomass. Similarly, the explained inertia of the retained microhabitat variables in RDAs varied little between density and biomass. These observations imply that the extra effort made to obtain macroinvertebrate biomass was not balanced by additional information. The convenience of using macroinvertebrate biomass has been established in a study relating functional organization of macroinvertebrates to their presumed food resources (see Chapter 5). Therefore, we believe that under certain conditions biomass could provide

more reliable relationships as it incorporates information about body size thought hydraulic and food requirements may shift through life stages (Rabeni and Minshall 1977, Hart and Finelli 1999).

Conclusions

Either from our research or from previous studies, results indicate that microhabitat conditions are not directly influencing the macroinvertebrate community but individual taxa. Of the considered microhabitat variables, water velocity, chlorophyll *a*, CPOM, filamentous algae, and moss had the greatest influence on macroinvertebrate distribution in our study reaches. Most macroinvertebrates preferred areas with high complexity of habitat architecture, availability of food resources and low current velocity. Substrate and complex hydraulic parameters exerted a lesser influence on macroinvertebrate distribution. The microhabitat variables of relevance for macroinvertebrate distribution were similar in the two reaches. However, our study demonstrated that the microhabitat variables prevailing over macroinvertebrate distribution might be modified by human disturbance. At the upstream reach, substrate was relatively relevant whereas at the downstream reach the high abundance of mosses overwhelmed the other variables. In La Tordera stream, the point source input altered taxa-microhabitat relationships by changing taxa composition and microhabitat conditions. Clearly, there is a need for more comprehensive studies on microhabitat distribution of macroinvertebrates influenced by human disturbance before firm conclusions can be drawn. In our study case, the results obtained from macroinvertebrate biomass were not appreciably different from those obtained from macroinvertebrate density.

Summary

We examined how community measures (density, biomass, and richness) and community composition were related to microhabitat variables (including hydraulic parameters, substrate, and food resources) in La Tordera stream in Catalonia (NE Spain). We collected macroinvertebrate samples on six dates between November 2001 and September 2002 upstream and downstream of a point source input. Macroinvertebrate density and biomass were positively correlated with food resources and complexity of habitat architecture (benthic organic matter, chlorophyll *a*, vascular plants, and mosses) while taxa richness was negatively correlated with conventional (water velocity and depth) and complex hydraulic parameters (Froude number, Reynolds number, roughness shear velocity). Inorganic substrate exerted a lesser influence on macroinvertebrate distribution. Ordination analysis revealed that the microhabitat variables of major relevance at the two reaches were CPOM, chlorophyll *a*, filamentous algae, and maximum water velocity. Sand coverage was only retained at the upstream reach and moss, at the downstream reach. The number of significant correlations between macroinvertebrates and microhabitat variables was higher at the upstream reach than at the downstream reach mainly because of higher taxa richness. We also demonstrated that macroinvertebrate biomass provided similar information to that obtained from density in our study case.

Appendix 6.1 Taxa list, taxa codes, presence of taxa in samplings ($n = 6$) and samples ($n = 36$), mean \pm ES, minimum and maximum density (individuals/m²) and biomass (mg DM/m²) of benthic macroinvertebrates recorded in the samples of the two reaches over the sampling period. The 16 taxa that were present in all samplings (S) and more than 50 % of the samples (s) were highlighted in bold. A = adult, P = pupae.

Taxa	Codes		Presence						Density						Biomass					
			Upstream			Downstream			Upstream			Downstream			Upstream			Downstream		
			S	s	S	S	s	S	mean \pm SE	min.	max.	mean \pm SE	min.	max.	mean \pm SE	min.	max.	mean \pm SE	min.	max.
<i>Ecdyonurus angulifer</i>	Eang	6	33	2	9	286 \pm 45	0	1184	17 \pm 6	0	128	92 \pm 25	0	825	1 \pm 0	0	5			
<i>Epeorus torrentium</i>	Etor	1	4	0	0	53 \pm 31	0	912	0 \pm 0	0	0	40 \pm 26	0	863	0 \pm 0	0	0			
<i>Baetis fuscatus</i>	Bfus	5	12	5	19	230 \pm 116	0	3619	715 \pm 282	0	8996	7 \pm 3	0	69	7 \pm 2	0	49			
<i>Baetis lutheri</i>	Blut	5	9	6	28	21 \pm 8	0	247	2750 \pm 1070	0	28150	1 \pm 1	0	14	46 \pm 14	0	323			
<i>Baetis rhodani</i>	Brho	6	34	6	27	2849 \pm 725	0	14381	2801 \pm 853	0	19460	212 \pm 41	0	953	65 \pm 15	0	327			
<i>Serratella ignita</i>	Sign	6	33	5	13	460 \pm 111	0	2880	21 \pm 6	0	123	71 \pm 19	0	625	6 \pm 2	0	51			
<i>Caenis luctuosa</i>	Cluc	6	36	6	27	305 \pm 135	0	4492	2140 \pm 834	0	19621	3 \pm 1	0	25	24 \pm 7	0	158			
<i>Caenis pusilla</i>	Cpus	5	17	0	0	6 \pm 3	0	88	0 \pm 0	0	0	0 \pm 0	0	9	0 \pm 0	0	0			
<i>Habropleptoides</i> sp.	Hab	2	8	1	1	77 \pm 35	0	816	1 \pm 1	0	53	4 \pm 2	0	42	0 \pm 0	0	2			
<i>Habrophlebia fusca</i>	Hfus	5	26	5	11	336 \pm 74	0	2032	8 \pm 3	0	53	10 \pm 2	0	46	0 \pm 0	0	2			
<i>Amphinemura</i> sp.	Amp	1	3	0	0	3 \pm 2	0	64	0 \pm 0	0	0	0 \pm 0	0	1	0 \pm 0	0	0			
<i>Nemoura</i> sp.	Neu	2	6	0	0	5 \pm 2	0	48	0 \pm 0	0	0	0 \pm 0	0	0	0 \pm 0	0	0			
<i>Protonemura</i> sp.	Pro	1	6	0	0	10 \pm 5	0	144	0 \pm 0	0	0	0 \pm 0	0	1	0 \pm 0	0	0			
<i>Leuctra geniculata</i>	Lgen	6	28	2	3	282 \pm 51	0	992	4 \pm 3	0	107	36 \pm 6	0	111	1 \pm 1	0	13			
<i>Capnioneura mitis</i>	Cmit	2	12	0	0	101 \pm 32	0	640	0 \pm 0	0	0	24 \pm 10	0	236	0 \pm 0	0	0			
<i>Siphonoperla torrentium</i>	Stor	5	14	0	0	12 \pm 3	0	80	0 \pm 0	0	0	4 \pm 1	0	36	0 \pm 0	0	0			
<i>Isoperla grammatica</i>	Igra	5	25	0	0	231 \pm 59	0	1632	0 \pm 0	0	0	34 \pm 8	0	200	0 \pm 0	0	0			
<i>Calopteryx virgo</i>	Cvir	1	1	2	5	0 \pm 0	0	16	6 \pm 3	0	64	0 \pm 0	0	6	3 \pm 2	0	69			
<i>Onychogomphus</i> sp.	Ony	1	4	1	1	3 \pm 2	0	64	1 \pm 1	0	53	0 \pm 0	0	3	0 \pm 0	0	3			
<i>Cordulegaster</i> sp.	Cor	1	1	0	0	0 \pm 0	0	16	0 \pm 0	0	0	0 \pm 0	0	1	0 \pm 0	0	0			
<i>Haliplus</i> sp.	Hal	1	1	0	0	0 \pm 0	0	16	0 \pm 0	0	0	0 \pm 0	0	0	0 \pm 0	0	0			
<i>Hydropsyche</i> sp.	Hyp	4	18	2	2	23 \pm 5	0	107	2 \pm 2	0	53	7 \pm 2	0	32	1 \pm 0	0	16			
<i>Scarabaeus</i> sp. A	Sca A	1	1	0	0	1 \pm 1	0	32	0 \pm 0	0	0	4 \pm 4	0	141	0 \pm 0	0	0			
<i>Deronectes</i> sp.	Der	1	2	1	1	1 \pm 1	0	32	1 \pm 1	0	32	0 \pm 0	0	6	0 \pm 0	0	6			
<i>Agabus</i> sp. L	Aga	2	11	2	2	8 \pm 2	0	64	1 \pm 1	0	16	14 \pm 6	0	133	0 \pm 0	0	3			
<i>Coelostoma</i> sp. A	Coe A	1	1	1	1	0 \pm 0	0	16	1 \pm 1	0	53	0 \pm 0	0	2	1 \pm 1	0	19			

Appendix 6.1 Continued

<i>Hydraena</i> sp. A	Hye A	6	11	1	1	1	1	8 ± 2	0	48	0 ± 0	0	16	2 ± 1	0	14	0 ± 0	5
<i>Dryops</i> sp.	Dry	1	1	1	1	1	1	0 ± 0	0	16	1 ± 1	0	53	0 ± 0	0	0	0 ± 0	2
<i>Elmisp</i> sp.	Elm	5	16	1	1	1	1	27 ± 9	0	240	4 ± 4	0	160	7 ± 2	0	37	1 ± 1	32
<i>Esolus</i> sp.	Eso	6	31	4	6	6	6	124 ± 26	0	576	14 ± 6	0	160	2 ± 0	0	9	0 ± 0	4
<i>Esolus</i> sp. A	Eso A	5	14	0	0	0	0	14 ± 5	0	128	0 ± 0	0	0	1 ± 1	0	16	0 ± 0	0
<i>Limnius</i> sp.	Lis	1	1	0	0	0	0	0 ± 0	0	16	0 ± 0	0	0	0 ± 0	0	0	0 ± 0	0
<i>Oulimnius</i> sp.	Oul	3	8	3	6	6	6	6 ± 2	0	64	14 ± 6	0	160	0 ± 0	0	1	2 ± 1	16
<i>Oulimnius</i> sp. A	Oul A	0	0	2	4	4	4	0 ± 0	0	0	6 ± 4	0	107	0 ± 0	0	0	3 ± 1	43
<i>Eubria pallustris</i>	Epal	0	0	1	1	1	1	0 ± 0	0	0	0 ± 0	0	16	0 ± 0	0	0	0 ± 0	0
<i>Elodes</i> sp.	Elo	1	1	0	0	0	0	0 ± 0	0	16	0 ± 0	0	0	0 ± 0	0	7	0 ± 0	0
<i>Hydropsyche instabilis</i>	Hins	5	14	2	5	5	5	78 ± 38	0	1104	6 ± 3	0	53	59 ± 28	0	701	1 ± 0	6
<i>Hydropsyche instabilis</i> P	Hins P	3	3	0	0	0	0	2 ± 1	0	32	0 ± 0	0	0	18 ± 11	0	333	0 ± 0	0
<i>Polycentropus</i> sp.	Pol	4	7	1	1	1	1	4 ± 1	0	32	0 ± 0	0	16	0 ± 0	0	10	0 ± 0	5
<i>Tinodes</i> sp.	Tin	4	11	2	4	4	4	7 ± 2	0	48	3 ± 1	0	32	1 ± 1	0	14	0 ± 0	6
<i>Rhyacophila dorsalis</i>	Rdor	4	11	0	0	0	0	18 ± 6	0	144	0 ± 0	0	0	14 ± 11	0	382	0 ± 0	0
<i>Rhyacophila dorsalis</i> P	Rdor P	1	1	0	0	0	0	1 ± 1	0	32	0 ± 0	0	0	9 ± 9	0	333	0 ± 0	0
<i>Hydroptila</i> sp.	Hyt	3	14	5	17	17	17	15 ± 5	0	144	180 ± 61	0	1493	2 ± 1	0	25	20 ± 8	208
<i>Hydroptila</i> sp. P	Hyt P	1	1	5	12	12	12	0 ± 0	0	16	49 ± 16	0	373	0 ± 0	0	4	11 ± 4	87
Limnephilidae	Lim	2	2	0	0	0	0	1 ± 1	0	16	0 ± 0	0	0	7 ± 5	0	126	0 ± 0	0
<i>Mystacides azurea</i>	Mazu	1	1	0	0	0	0	4 ± 4	0	144	0 ± 0	0	0	0 ± 0	0	7	0 ± 0	0
<i>Oecetis</i> sp.	Oee	1	1	0	0	0	0	0 ± 0	0	16	0 ± 0	0	0	0 ± 0	0	1	0 ± 0	0
<i>Sericostoma personatum</i>	Sper	3	9	0	0	0	0	8 ± 4	0	144	0 ± 0	0	0	3 ± 2	0	78	0 ± 0	0
<i>Sericostoma personatum</i> P	Sper P	1	1	0	0	0	0	0 ± 0	0	16	0 ± 0	0	0	5 ± 5	0	166	0 ± 0	0
Psychodidae	Psy	2	3	6	15	15	15	1 ± 1	0	16	30 ± 10	0	288	0 ± 0	0	2	5 ± 2	43
Psychodidae P	Psy P	2	7	3	7	7	7	8 ± 5	0	160	6 ± 2	0	53	1 ± 0	0	10	0 ± 0	4
<i>Dixa</i> sp.	Dix	1	1	1	1	1	1	0 ± 0	0	16	0 ± 0	0	16	0 ± 0	0	0	0 ± 0	2
Simuliidae	Sim	6	32	6	28	28	28	146 ± 35	0	976	486 ± 217	0	7232	13 ± 3	0	60	221 ± 154	5520
Simuliidae P	Sim P	3	6	3	10	10	10	4 ± 2	0	48	45 ± 19	0	592	2 ± 1	0	21	19 ± 8	255
Ceratopogoninae	Cer	6	32	6	32	32	32	66 ± 10	0	224	115 ± 18	0	373	9 ± 3	0	64	10 ± 2	39
Dasyheleinae	Das	3	5	2	2	2	2	4 ± 2	0	64	3 ± 2	0	53	0 ± 0	0	3	0 ± 0	3
Tanypodinae	Tan	6	34	6	29	29	29	228 ± 41	0	1040	350 ± 110	0	2981	21 ± 4	0	109	12 ± 3	77
Tanypodinae P	Tan P	3	4	1	2	2	2	2 ± 1	0	16	1 ± 1	0	32	0 ± 0	0	2	1 ± 0	16

Appendix 6.1 Continued

Naididae	Nai	6	32	5	27	231 ± 71	0	2224	2325 ± 1044	0	33867	2 ± 1	0	22	37 ± 16	0	515
<i>Chaetogaster</i> spp.	Cha	2	8	3	14	55 ± 25	0	672	2128 ± 1024	0	27840	1 ± 0	0	8	16 ± 8	0	205
Tubificidae	Tub	4	6	6	36	7 ± 3	0	64	1467 ± 279	53	5744	0 ± 0	0	3	49 ± 10	2	210
Lumbriculidae	Lum	6	20	6	20	39 ± 12	0	384	92 ± 29	0	720	20 ± 9	0	299	75 ± 30	0	767
<i>Eiseniella tetraedra</i>	Etet	6	28	3	7	89 ± 14	0	352	5 ± 2	0	64	794 ± 180	0	4730	32 ± 13	0	275
<i>Glossiphonia</i> sp.	Glo	0	0	3	6	0 ± 0	0	0	4 ± 2	0	48	0 ± 0	0	0	49 ± 25	0	773
<i>Helobdella stagnalis</i>	Hsta	1	1	6	32	1 ± 1	0	32	177 ± 41	0	960	0 ± 0	0	13	98 ± 23	0	498
<i>Erpobdella</i> sp.	Erp	3	4	6	35	2 ± 1	0	32	208 ± 32	0	784	9 ± 6	0	165	1049 ± 148	0	3406
Nematoda	Nem	6	33	6	36	85 ± 13	0	352	2759 ± 658	160	20693	2 ± 0	0	7	18 ± 3	2	91
Nematomorpha	Nep	5	11	6	35	10 ± 3	0	80	223 ± 34	0	832	1 ± 0	0	15	11 ± 2	0	60
<i>Hydra</i> sp.	Hyd	1	1	2	4	0 ± 0	0	16	6 ± 3	0	107	0 ± 0	0	1	0 ± 0	0	5
<i>Phagocata vitta</i>	Pvit	2	3	2	2	2 ± 1	0	32	1 ± 1	0	16	0 ± 0	0	1	0 ± 0	0	1
<i>Dugesia</i> sp.	Dug	2	7	0	0	50 ± 25	0	848	0 ± 0	0	0	5 ± 2	0	58	0 ± 0	0	0
Digenea	Dig	2	7	2	3	8 ± 3	0	80	8 ± 6	0	213	0 ± 0	0	3	0 ± 0	0	4

Appendix 6.2 Spearman's 2-tailed rank correlation coefficients among the 19 microhabitat variables from November 2001 to April 2002 at the upstream reach ($n = 24$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See Table 6.1 for acronyms.

	CPOM	FPOM	Chl <i>a</i>	Fil	Moss	Vasc	Vmax	Vmin	Vmean	D	Bou	Cob	Peb	Gra	San	kv	Fr	Re	V*
CPOM	1.000																		
FPOM	0.426*	1.000																	
Chl <i>a</i>	-0.460*	-0.163	1.000																
Fil	0.178	0.104	-0.334	1.000															
Moss	0.369	0.214	-0.200	-0.190	1.000														
Vasc	0.112	0.132	0.305	-0.164	-0.014	1.000													
V max	0.243	-0.238	-0.372	-0.245	0.267	-0.215	1.000												
V min	0.118	0.071	-0.497*	0.088	0.198	-0.226	0.613**	1.000											
V mean	0.242	0.047	-0.463*	-0.050	0.102	-0.231	.863***	.799**	1.000										
D	0.136	0.040	-0.385	-0.234	0.251	-0.498*	0.458*	0.434*	0.486*	1.000									
Bou	-0.194	-0.206	-0.105	-0.207	-0.394	-0.172	0.215	0.445*	0.396	0.26	1.000								
Cob	0.353	0.049	-0.067	0.036	0.317	-0.073	0.120	-0.129	0.015	0.361	-0.459*	1.000							
Peb	-0.212	-0.011	0.183	-0.032	0.190	0.103	-0.093	-0.082	-0.141	-0.458*	-0.135	-0.606**	1.000						
Gra	-0.194	0.046	0.107	0.351	-0.397	0.087	-0.449*	-0.371	-0.452*	-0.599**	-0.290	-0.439*	0.299	1.000					
San	0.244	0.122	-0.234	-0.116	0.555**	-0.215	0.448*	0.401	0.455*	0.306	-0.114	0.248	-0.021	-0.432*	1.000				
kv	0.126	-0.078	0.005	-0.350	0.102	-0.025	0.289	0.230	0.356	0.415*	0.466*	0.401	-0.427*	0.875**	0.227	1.000			
Fr	0.130	0.029	-0.283	-0.047	0.061	-0.030	.738**	.660**	.878**	.0124	0.366	-0.114	0.071	-0.321	0.394	0.353	1.000		
Re	0.239	0.004	-0.518**	-0.129	0.177	-0.402	.843**	.767**	.918**	.758**	0.375	0.138	-0.255	-0.539**	0.444*	0.354	.651**	1.000	
V*	0.196	0.037	-0.369	-0.072	0.045	-0.110	.810**	.752**	.969**	.329	0.459*	-0.064	-0.079	-0.435*	0.411*	0.420*	.958**	.810**	1.000

Appendix 6.3 Spearman's 2-tailed rank correlation coefficients among 19 microhabitat variables from November 2001 to April 2002 at the downstream reach (n = 24). * p < 0.05, ** p < 0.01, *** p < 0.001. See Table 6.1 for acronyms.

	CPOM	FPOM	Chl <i>a</i>	Fil	Moss	Vasc	Vmax	Vmin	Vmean	D	Bou	Cob	Peb	Gra	San	kv	Fr	Re	V*
CPOM	1.000																		
FPOM	0.547**	1.000																	
Chl <i>a</i>	0.246	0.114	1.000																
Fil	0.240	0.174	0.450*	1.000															
Moss	0.571**	0.530**	0.120	0.260	1.000														
Vasc	0.102	-0.095	0.465*	0.304	0.022	1.000													
V max	0.488*	0.048	-0.028	-0.041	0.445*	0.005	1.000												
V min	-0.005	-0.132	-0.524**	-0.460*	0.047	-0.402	0.560**	1.000											
V mean	0.408*	0.030	-0.186	-0.202	0.288	-0.122	0.930**	0.728**	1.000										
D	-0.256	-0.167	-0.428*	-0.297	-0.142	-0.337	-0.418*	0.037	-0.347	1.000									
Bou	0.198	0.311	0.216	0.311	0.389	0.234	0.159	-0.144	0.137	-0.389	1.000								
Cob	0.094	-0.122	-0.080	-0.178	0.082	-0.222	0.040	0.269	0.131	0.297	-0.333	1.000							
Peb	-0.104	-0.027	0.038	-0.029	-0.420*	-0.067	-0.077	-0.058	-0.117	-0.101	-0.305	0.647**	1.000						
Gra	-0.163	-0.264	-0.042	0.004	0.077	0.193	0.045	-0.098	0.025	-0.297	0.385	-0.442*	-0.048	1.000					
San	-0.298	0.036	-0.374	0.255	-0.067	0.004	-0.195	-0.198	-0.208	0.302	0.175	-0.142	-0.206	-0.087	1.000				
kv	0.336	0.118	0.147	-0.169	0.163	-0.195	0.147	0.326	0.219	0.143	-0.269	0.774**	-0.321	0.630**	-0.450*	1.000			
Fr	0.425*	0.010	-0.081	-0.109	0.334	-0.003	0.956**	0.618**	0.972**	-0.478*	0.215	0.069	-0.140	0.098	-0.204	0.153	1.000		
Re	0.158	0.005	-0.554**	-0.469*	0.143	-0.438*	0.441*	0.746**	0.596**	0.490*	-0.194	0.281	-0.125	-0.188	0.020	0.290	0.463*	1.000	
V*	0.445*	0.043	-0.142	-0.165	0.320	-0.064	0.951**	0.687**	0.993**	-0.371	0.171	0.138	-0.144	0.012	-0.202	0.239	0.979**	0.559**	1.000

7. Variability of consumer-resource stoichiometry below the point source

Introduction

*There is no coming into being of aught that perishes, nor any end for it...
but only mingling, and separation of what has been mingled.*

Empedocles of Acragas (492-432 B.C.)

Ecological stoichiometry is a conceptual framework that provides an integrative approach for the analysis of the balance among chemical elements in ecological interactions and links their cycling in ecosystems (Reiners 1986, Elser *et al.* 1996, Sterner and Elser 2002, Frost *et al.* 2002, Moe *et al.* 2005). Elements cannot be synthesized or interconverted by organisms, and must therefore be acquired in quantities sufficient to attain their requirements for maintenance, growth, and reproduction (Anderson *et al.* 2004, Frost *et al.* 2005). In opposition to autotrophic organisms, metazoans are often realized as being stoichiometrically homeostatic, namely having a relatively constant body nutrient proportions regardless of the chemical composition in their food (Elser *et al.* 1996). This implies that differences in nutrient proportions between consumers and their food resources may lead to stoichiometric constraints. Recent research have shown that elemental imbalances between consumers and their food resources can impinge on ecosystem processes, such as population dynamics (Burkhardt and Lehman 1994, Loladze *et al.* 2000), trophic interactions (Sterner *et al.* 1997, Elser *et al.* 1998), and community structure (DeMott and Gulati 1999, Grover 2002).

Frost *et al.* (2003) presented a pioneer research examining the elemental composition of lake benthic macroinvertebrates across a wide range of dissolved

nutrient concentrations that lent support to the hypothesis of homeostatic regulation. However, in a likewise interesting study, Cross *et al.* (2003) found that some stream insects did not exhibit strict homeostasis in a detritus-based stream. Homeostatic regulation has been established for lake zooplankton (Hessen and Lyche 1991) and *Elimia* snails (Stelzer and Lamberti 2002) through manipulations of food elemental composition. On the other hand, an experiment where mayfly nymphs were grown under different quantities and qualities of food supply demonstrated that body stoichiometry is susceptible to change under high elemental mismatches between consumers and their food resources (Frost and Elser 2002a). Similar results have been also obtained in lake planktonic ecosystems for *Daphnia* (DeMott *et al.* 1998).

After the works of Redfield (1958), who demonstrated that biogeochemical cycles of C, N, and P are strongly coupled in pelagic oceanic ecosystems, ecological stoichiometry has been extensively studied in marine and lake planktonic ecosystems (e.g., Stauffer 1985, Andersen and Hessen 1991, Sterner *et al.* 1997, Elser and Urabe 1999, Urabe *et al.* 2002). These works provided valuable outcomes that supposed the starting point for further development of stoichiometric theory. The establishment of a more comprehensive framework encouraged researchers to set up the study of biological systems with increasing degrees of complexity, from benthic and terrestrial autotrophs (Enríquez *et al.* 1993, Kahlert 1998) to terrestrial insects (Schade *et al.* 2003) and vertebrates (Schindler and Eby 1997, Sterner and George 2000, Vanni *et al.* 2002). Simultaneously, the implementation of laboratory and field experiments (e.g., Urabe and Watanabe 1992, Sterner 1997, Elser *et al.* 1998, Stelzer and Lamberti 2002, Frost and Elser 2002a) increased the understanding of processes and patterns involving nutrient cycling in ecosystems and generated valuable hypotheses for future research addressing elemental stoichiometry.

However, there is yet little empirical support for stoichiometric theory in benthic ecosystems, because the elemental content of many biomass compartments (i.e., autotrophs, organic matter, macroinvertebrates, etc.) remain largely unknown.

Within this framework, we determined the C, N, and P contents in coarse, fine, and suspended particulate organic matter (CPOM, FPOM, and SPOM), periphyton, filamentous algae, mosses, and benthic macroinvertebrates upstream and downstream of a point source input in La Tordera stream. Our objectives were to: 1) examine potential differences in stoichiometric ratios of autotrophs and organic matter, with a special insight on potential food resources for macroinvertebrates, 2) determine the degree of homeostatic regulation in stream macroinvertebrates, 3) contrast the elemental stoichiometry between consumers and their potential food resources, 4) assess the variability in stoichiometric relationships among macroinvertebrate taxa, and 5) identify major food resources for heterotrophic production in the stream ecosystem.

Results

Food resources

Elemental contents for periphyton and mosses were similar in the two reaches (Figure 7.1). Periphyton contained less C than filamentous algae and mosses, which had in turn the highest N and P contents. Filamentous algae had similar %C and %N in the two reaches but at the downstream reach %P was, on average, two times higher than at the upstream reach. The differences in elemental content of autotrophs between the two reaches were reflected in the respective elemental ratios (Figure 7.1). CPOM and FPOM contained less C and more P below the point source than in the upstream reach. In consequence, C:N, C:P, and N:P ratios of CPOM and FPOM were substantially higher in the upstream reach than in the downstream reach. We observed no important differences in elemental

contents for SPOM between the two reaches, but C:P and N:P ratios for the upstream reach were almost twofold those for the downstream reach. The P content for SPOM was between three and seven times lower than that observed for CPOM or FPOM.

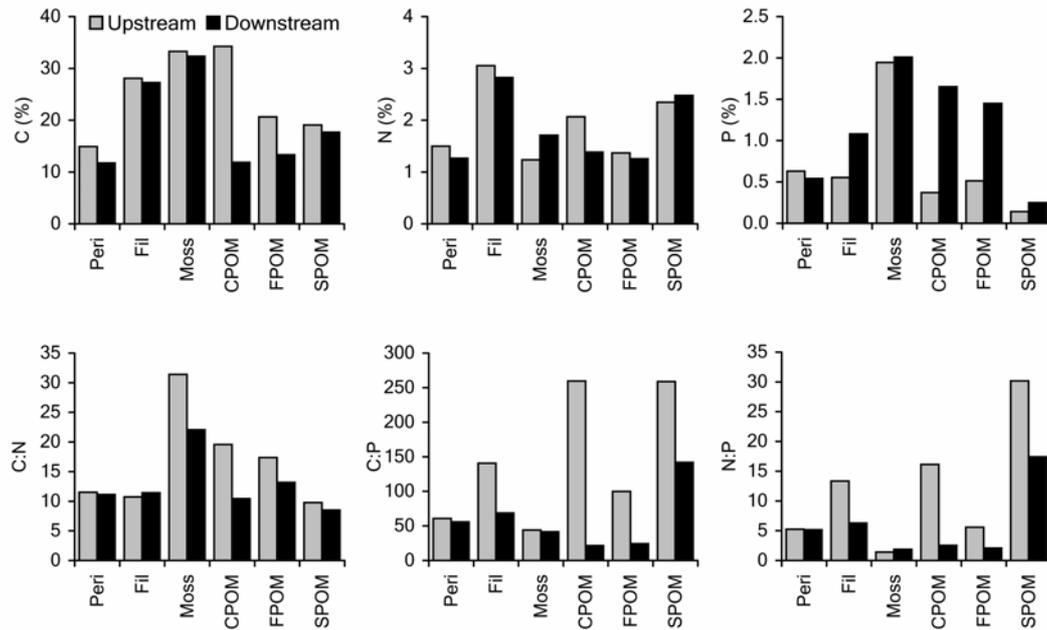


Figure 7.1 Mean elemental contents of C, N, and P (% of dry mass) and their molar elemental ratios C:N, C:P, and N:P of primary producers and organic matter in the upstream reach and the downstream reach in La Tordera stream. Peri = periphyton, Fil = filamentous algae.

Benthic macroinvertebrates

We analyzed the nutrient content of 58 and 46 macroinvertebrate taxa from the upstream reach and the downstream reach, respectively. Three taxa from the upstream reach and five from the downstream reach were analyzed as larvae and as pupae. At the upstream reach, Anthomyidae was analyzed only as pupae. All beetles were analyzed as larvae but *Scarodytes* sp., which was analyzed as

adult at the upstream reach. At the upstream reach, *Esolus* sp. was analyzed as larvae and as adult.

Elemental contents varied considerably among the macroinvertebrates sampled in the two reaches (Table 7.1 and Figure 7.2). Macroinvertebrate C content varied more than 15-fold from 16.43% in *Ancylus fluviatilis* at the downstream reach to 58.99% in *Elmis* sp. and *Esolus* sp. at the upstream reach. The macroinvertebrate %N was also highly variable and ranged from 1.06% in *Pisidium casertanum* at the downstream reach and 14.65% in *Erpobdella* sp. at the upstream reach. Macroinvertebrate P varied from 0.18% in *Ancylus fluviatilis* at the downstream reach to 2.76% in *Polycentropus* sp. at the upstream reach. Mean elemental contents of macroinvertebrates were slightly higher at the upstream reach than at the downstream reach. The macroinvertebrate %C distribution was considerably different between the two reaches. In contrast, the distribution of macroinvertebrate N and P contents and elemental ratios differed little between the two reaches. The coefficient of variation for %P was higher than those for %N and %C, but did not differ greatly between the two reaches. High variability of %P was reflected in elemental ratios, where the coefficient of variation was much higher in C:P and N:P ratios than in C:N ratios for the two reaches, but especially for the upstream reach. The mean ratios for macroinvertebrates found in our study were slightly higher than those previously reported for stream and lake macroinvertebrates and were more similar to those for terrestrial invertebrates or lake zooplankton (Table 7.1).

Variation in macroinvertebrate nutrient contents was relatively high among taxa phylogenetically related (Figure 7.3). However, mollusks generally had the lowest C, N, and P contents. Leeches were among the taxa with highest %N while elmids had a very low P content. Paired comparisons between the two reaches revealed that most taxa had a similar elemental content in the two reaches.

Table 7.1 Mean, median, standard deviation (SD), and coefficient of variation (CV) of macroinvertebrate C, N, P, C : N, C : P, and N : P at the upstream reach (up, $n = 62$) and the downstream reach (dw, $n = 51$) in La Tordera stream. See also Figure 7.2.

	Mean		Median		SD		CV (%)	
	up	dw	up	dw	up	dw	up	dw
C (%)	46.14	41.88	48.83	43.24	11.46	10.48	25	25
N (%)	8.69	7.96	9.16	8.54	2.93	2.66	34	33
P (%)	1.37	1.35	1.26	1.31	0.63	0.56	46	41
C : N	6.6	6.7	6.1	5.9	1.5	2.2	23	33
C : P	113	100	88	83	76	60	68	59
N : P	17.86	15.74	13.36	13.88	12.54	8.77	70	56
Stream macroinvertebrates (Cross <i>et al.</i> 2003)								
C : N	5.99		-		0.88		15	
C : P	268.25		-		158.33		60	
N : P	44.25		-		22.29		51	
Lake macroinvertebrates (Frost <i>et al.</i> 2003)								
C : N	5.6		5.5		0.75		13	
C : P	148		141		51.2		34	
N : P	27.3		25.8		9.92		36	
Terrestrial invertebrates (Elser <i>et al.</i> 2000)								
C : N	6.5		6.4		1.9		29	
C : P	116		73.2		72.4		62	
N : P	26.4		22.6		10.1		38	
Lake zooplankton (Elser <i>et al.</i> 2000)								
C : N	6.3		6.0		1.3		21	
C : P	124		114		48.0		38	
N : P	22.3		18.5		10.5		47	

Macroinvertebrate C, N, and P contents were not significantly different between the two reaches (paired T -test, $p = 0.092$, $p = 0.267$, $p = 0.778$, respectively). However, the elemental content of some macroinvertebrates, such as *Serratella ignita*, *Calopteryx virgo*, *Helobdella stagnalis*, *Eiseniella tetraedra*, and *Leuctra geniculata*, varied greatly between the two reaches. The C, N and P content of the snails *Physella acuta* and *Radix* sp. reach were higher at the downstream than

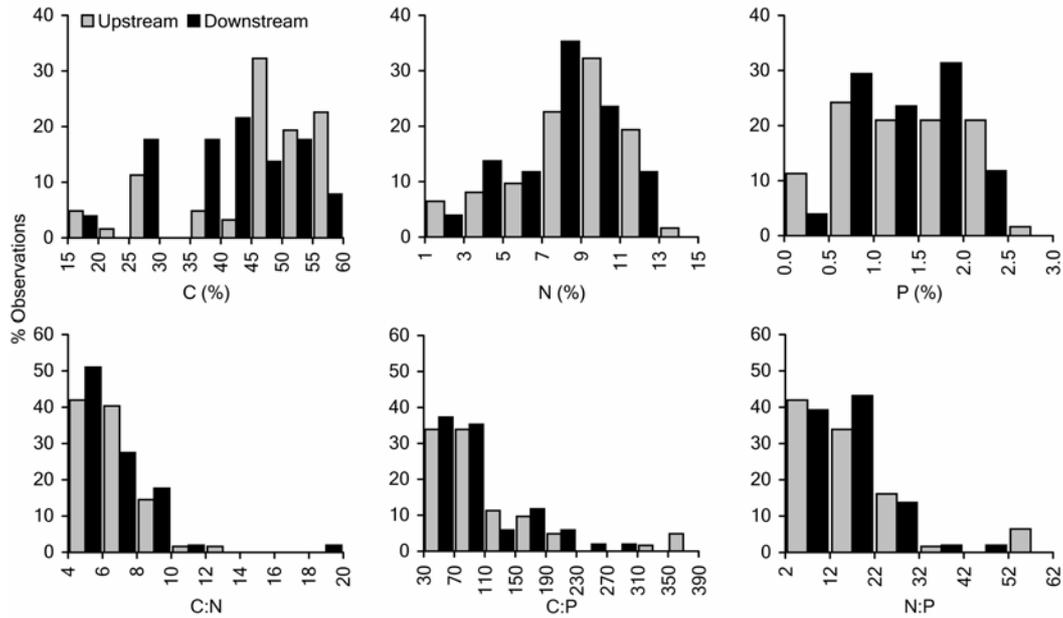


Figure 7.2 Frequency histograms of body C, N, and P content (% of dry mass) and their molar elemental ratios C:N, C:P, and N:P of macroinvertebrates in the upstream reach and the downstream reach in La Tordera stream.

at the upstream reach, whereas the %P of Orthoclaudiinae, *C. virgo*, and *S. ignita* were much higher at the upstream reach than at the downstream reach. Overall, mollusks showed the highest C:N ratios while midges and microcrustaceans generally had the lowest C:P and N:P ratios (Figure 7.4). Although several taxa differed greatly in C:N, C:P, and N:P ratios in the two reaches, differences were not significant among paired taxa between the two reaches (paired *T*-test, $p = 0.408$, $p = 0.637$, $p = 0.866$, respectively).

We found no significant differences in any elemental content or elemental ratio between the two reaches with regard to the dominant taxonomic groups (two-way ANOVA, reach and reach \times taxonomic group interaction: $p > 0.05$; Figure 7.5). However, differences among major taxonomic groups were

Response of benthic macroinvertebrates to a point source

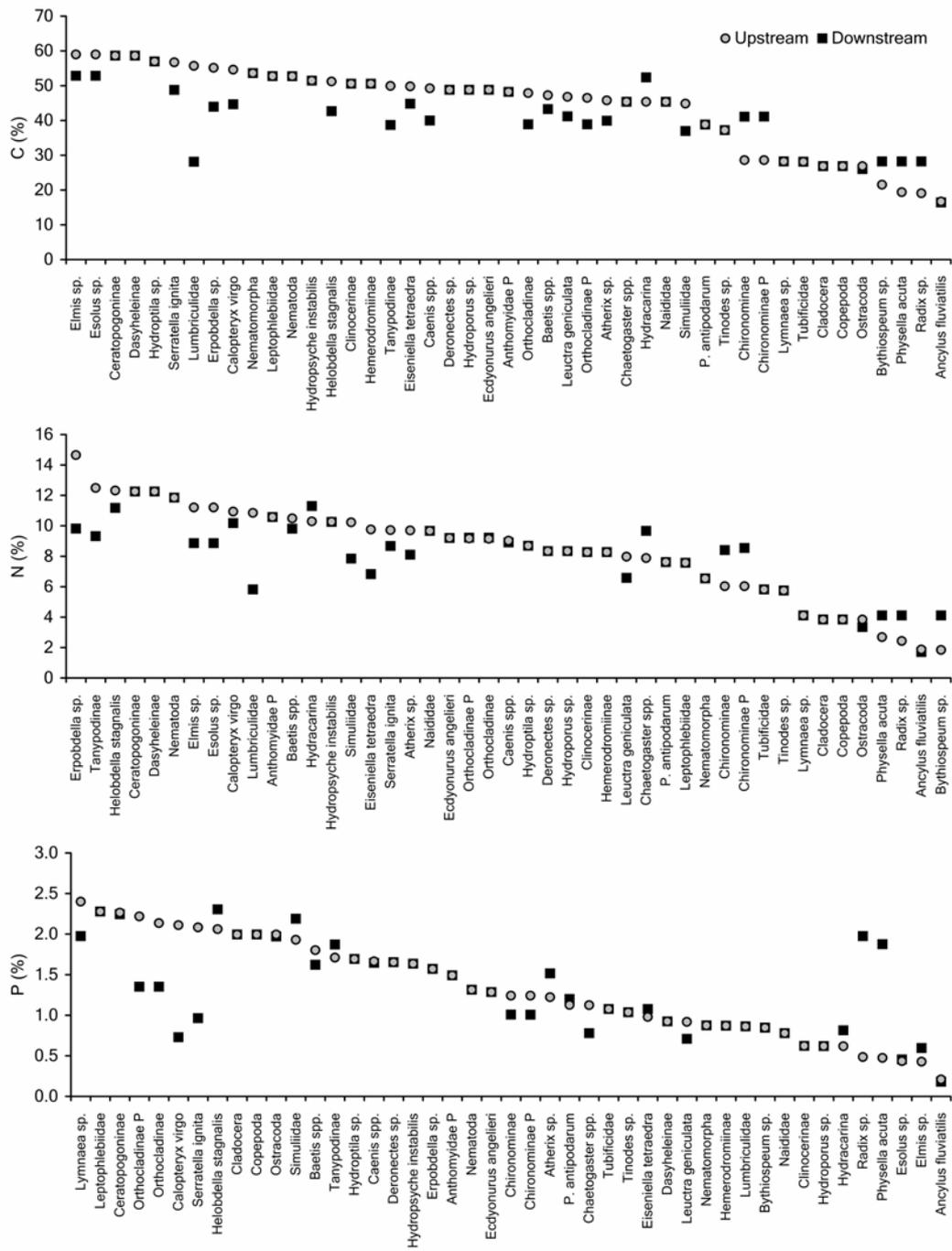


Figure 7.3 Mean %C, %N, and %P of paired macroinvertebrate taxa from the upstream reach and the downstream reach in La Tordera stream. *P. antipodarum* = *Potamopyrgus antipodarum*, P = pupae.

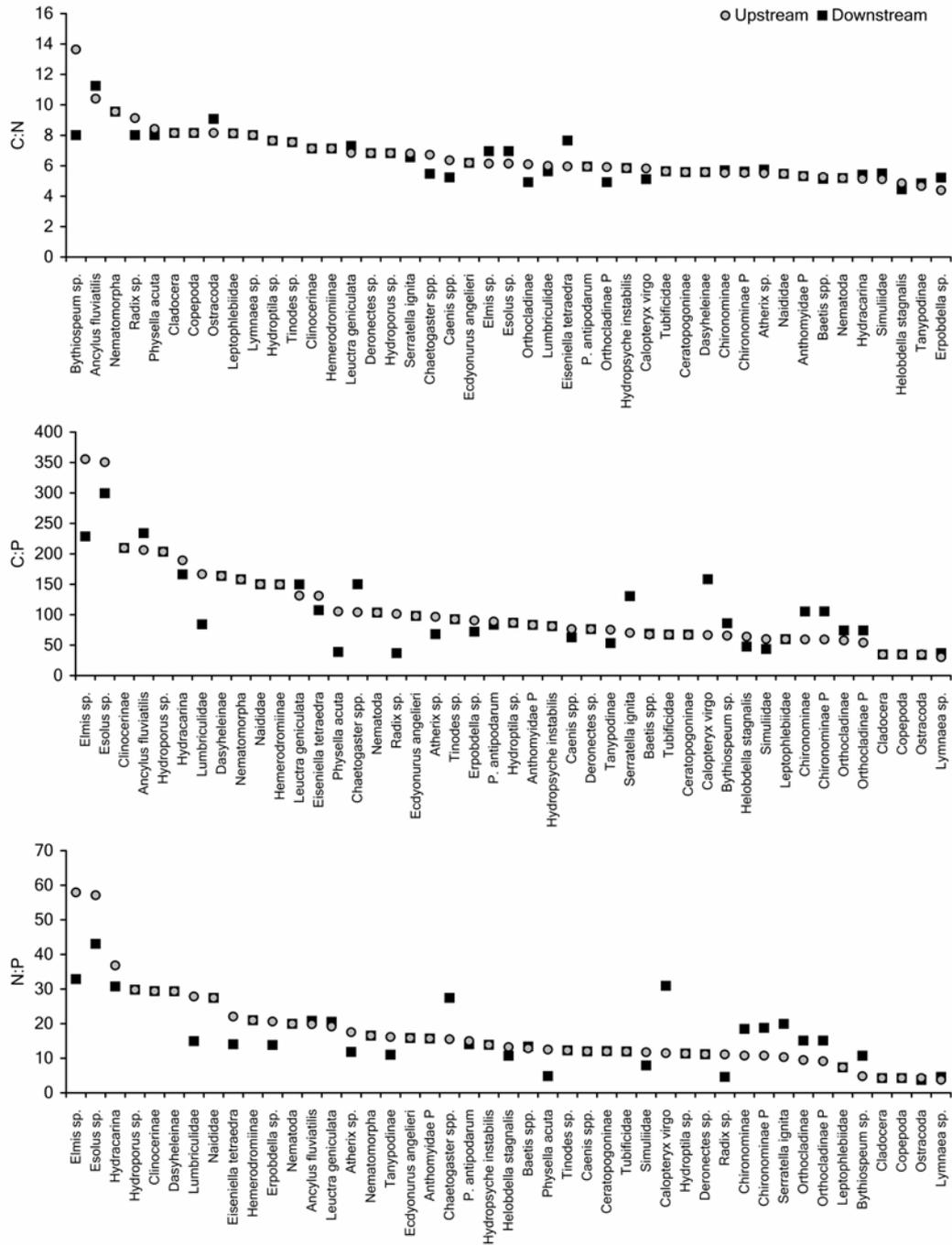


Figure 7.4 Mean C:N, C:P, and N:P ratios of paired macroinvertebrate taxa from the upstream reach and the downstream reach in La Tordera stream. P. antipodarum = *Potamopyrgus antipodarum*, P = pupae.

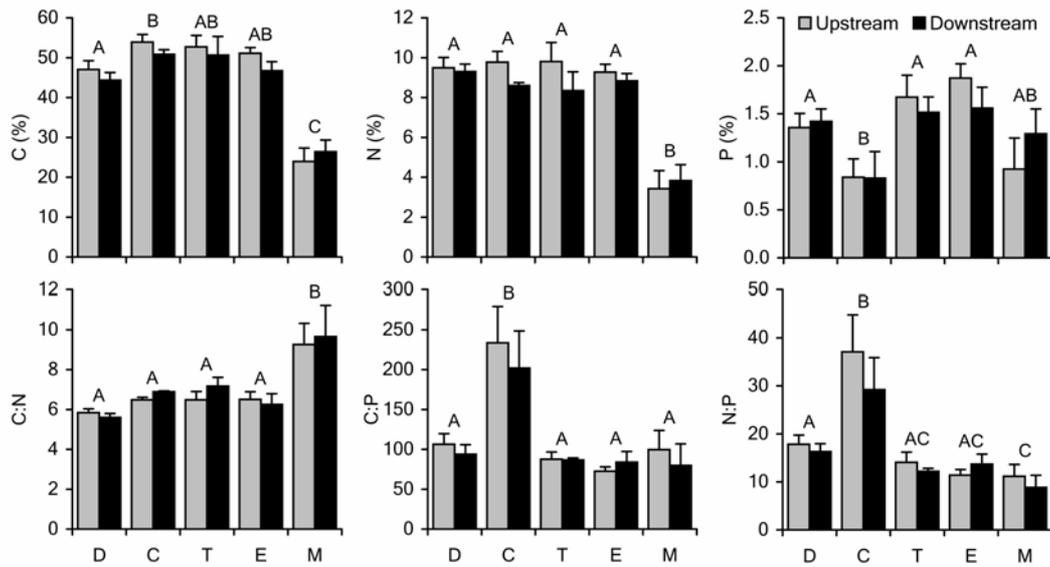


Figure 7.5 Mean %C, %N, and %P (\pm SE) of dominant taxonomic groups in the upstream reach and the downstream reach in La Tordera stream. D = Diptera, C = Coleoptera, T = Trichoptera, E = Ephemeroptera, M = Mollusca. Results of two-way ANOVA: taxonomic group factor significant for all variables (%C, $p < 0.005$; %N, $p < 0.005$; %P, $p = 0.001$; C:N, $p < 0.005$; C:P, $p < 0.005$; N:P, $p < 0.005$), reach factor not significant for any variable ($p > 0.05$), taxonomic group \times reach not significant for any variable ($p > 0.05$). Different capital letters indicate significant differences (Tukey's HSD) based on data from the two reaches combined. For all elemental contents and ratios in the upstream reach and the downstream reach, respectively: D ($n = 15, 15$), C ($n = 8, 4$), T ($n = 7, 4$), E ($n = 6, 5$), and M ($n = 6, 7$).

significant when data from the two reaches were combined (two-way ANOVA, taxonomic group: $p < 0.001$). Dipterans, caddisflies, and mayflies had similar nutrient contents and stoichiometry. Mollusks had the lowest C and N content and, consequently, the highest C:N ratio. The %P of beetles was significantly lower than that of the other insect orders and resulted in significantly higher C:P and N:P ratios than those for the other groups.

Similar to taxonomic groups, functional feeding groups (FFG) did neither show significant differences between the two reaches (two-way ANOVA, reach and reach \times FFG interaction: $p > 0.05$) but, differences among FFGs were significant when data from the two reaches were combined (two-way ANOVA,

FFG: $p < 0.009$; Figure 7.6). The C and N content harbored by predators were significantly higher than those for the other FFGs. On average, filterers had the highest %P, but was significantly higher only to that for scrapers (Tukey's HSD, $p = 0.009$). Consequently, filterers had the lowest C:P and N:P ratios.

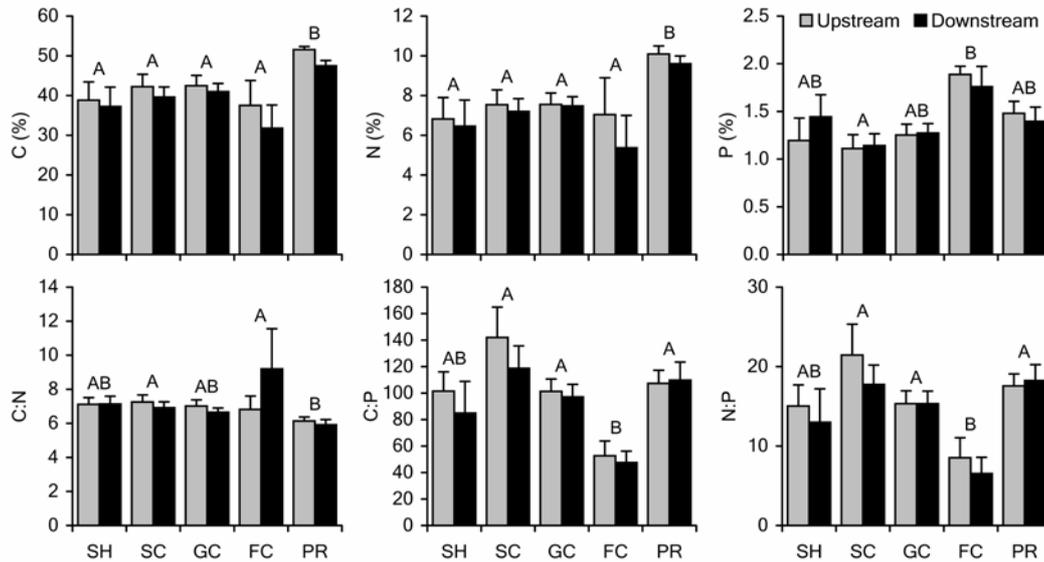


Figure 7.6 Mean %C, %N, and %P (\pm SE) of functional feeding groups in the upstream reach and the downstream reach in La Tordera stream. SH = shredders, SC = scrapers, GC = gatherers, FC = filterers, PR = predators. Results of two-way ANOVA: functional feeding group factor significant for all variables (%C, $p < 0.005$; %N, $p < 0.005$; %P, $p = 0.009$; C:N, $p = 0.004$; C:P, $p = 0.003$; N:P, $p = 0.001$), reach factor not significant for any variable ($p > 0.05$), taxonomic group \times reach not significant for any variable ($p > 0.05$). Different capital letters indicate significant differences (Tukey's HSD) based on data from the two reaches combined. For all elemental contents and ratios in the upstream reach and the downstream reach, respectively: SH ($n = 9, 6$), SC ($n = 22, 19$), GC ($n = 24, 24$), FC ($n = 4, 5$), and PR ($n = 24, 17$).

Consumer-resource stoichiometry

Overall, elemental imbalances between consumers and their presumed food resources were higher at the upstream reach than at the downstream reach (Figure 7.7). All FFG tended to have a lower C:N ratio than their presumed food

resources, indicating that consumers accumulated N. Predators and filterers had C:N ratios close to the 1:1 line that represents identical stoichiometry in consumers and resources, indicating that their C:N ratios were similar to those for prey and SPOM, respectively. At the upstream reach, in contrast, shredders and gatherers were far out of balance from their food resources for C:N ratios. Filterers had a positive imbalance between their C:P and N:P ratios and those for their food resources (i.e., consumer ratio lower than the ratio for its food resource), indicating that their relative P content was higher than that in their food resources. At the upstream reach, shredders showed the same pattern as filterers for the C:P ratio. Predators were generally closer to be stoichiometrically balanced with their food, based on consumption of N and P-rich prey. All other consumer-resource relationships for C:P and N:P ratios were located above the 1:1 line, indicating that the relative P contents in food resources were higher than in their respective consumers.

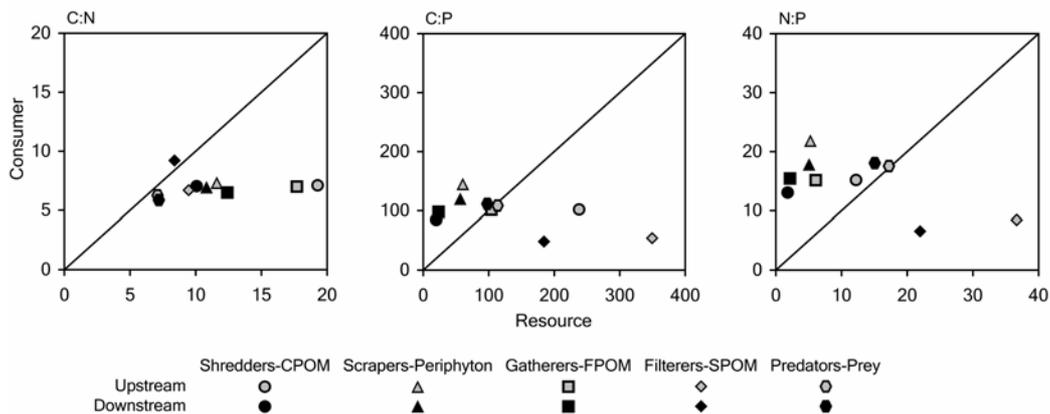


Figure 7.7 Stoichiometric relationships between consumers and their presumed food resources for the two reaches in La Tordera stream. Points on the 1:1 line (slope 1, intercept 0) represent identical stoichiometry in consumers and resources. All ratios are molar.

Elemental distribution among biomass compartments

Although standing stocks of all biomass compartments tended to be higher at the downstream reach than at the upstream reach on all sampling dates (see Chapter 5), the differences in benthic elemental content between the two reaches were not consistent through time (Table 7.2). The nutrient contents of all biomass compartments in the benthos were higher at the downstream reach than at the upstream reach in November 2001 and March 2002, but the opposite in January and April 2002. Total nutrient standing stocks for all benthic biomass compartments together were, on average, 17528 mg C/m², 1453 mg N/m², and 470 mg P/m² at the upstream reach, and 16937 mg C/m², 1808 mg N/m², and 1436 mg P/m² at the downstream reach. This indicates that while C and N standing stocks were similar in the two reaches, P was more than threefold at the downstream reach.

Overall, C and P storage in benthic macroinvertebrates relative to nutrient budgets retained in the ecosystem was rather low in the two reaches (on average, 7% and 9% for C and 8% and 3% for P at the upstream reach and the downstream reach, respectively; Table 7.2). However, this biomass compartment showed a relatively high N retention within the ecosystem (on average, 15% and 16% of the ecosystem at the upstream reach and at the downstream reach, respectively). Nutrients stored on macroinvertebrates were lower in June 2002 in the two reaches, according to their biomass standing stock. C and N stored in macroinvertebrates were slightly higher at the downstream reach, while P budget in macroinvertebrates was more important at the upstream reach. Similar to biomass (see Chapter 5), the major pool of nutrients were not evenly distributed among functional feeding groups. At the upstream reach, scrapers and gatherers stored the higher amount of nutrients while filterers were irrelevant. At the

downstream reach, predators were by far the most important functional feeding group in nutrient storage followed by scrapers, and gatherers.

On average, the contribution of in-stream primary producers (periphyton, filamentous algae, and mosses) to C and N bulks of the ecosystem was slightly higher than that for CPOM in the two reaches (Table 7.2). The trend was similar for P at the upstream reach but opposite at the downstream reach, where the P budget was higher for CPOM than for the other biomass compartments. This indicates that at the upstream reach, autochthonous production represented, on average, the major input of C, N, and P into the stream food web. At the downstream reach, the main source of C and N to the ecosystem was also derived from in-stream production but, in contrast, most P entered into the ecosystem via allochthonous sources. Temporal variability of standing stocks of CPOM and periphyton revealed that those two biomass compartments were alternated over time in the two reaches. The C, N, and P standing stocks of CPOM (and FPOM) were substantially higher than for periphyton in November 2001 and January 2002, after major leaf fall occurred. On the other hand, periphyton was the main potential resource for macroinvertebrate production from March to September 2002 when, although foliage was denser, sun irradiation was more intense because channel width was enough to avoid light limitation (see Table 4.1 in Chapter 4). Filamentous algae and mosses were especially abundant from November 2001 to March 2002 but overall, nutrient budgets on them were relatively low in the two reaches. Similar to AFDM, the nutrient content in SPOM was higher at the downstream reach than at the upstream reach on all sampling dates (see Chapter 5). However, differences were particularly relevant for P because %P for SPOM at the downstream reach was twofold that at the upstream reach.

Table 7.2 Elemental standing stocks of biological compartments in the upstream reach (up) and in the downstream reach (dw) over the sampling period in La Tordera stream. Values are expressed in mg/m² for all biological compartments except for SPOM, which was in mg/L.

	Nov-01		Jan-02		Mar-02		Apr-02		Jun-02		Sep-02	
	up	dw	up	dw	up	dw	up	dw	up	dw	up	dw
Shredders												
C	97.83	235.69	230.46	136.49	249.19	149.40	23.72	8.28	7.61	3.54	34.88	23.26
N	17.19	40.93	40.49	23.71	43.78	25.95	4.17	1.44	1.34	0.62	6.13	4.04
P	3.01	9.14	7.10	5.29	7.67	5.79	0.73	0.32	0.23	0.14	1.07	0.90
Scrapers												
C	96.10	526.50	647.54	524.88	1237.48	647.49	522.56	184.69	77.71	73.99	329.55	234.99
N	17.16	95.65	115.65	95.35	221.01	117.63	93.33	33.55	13.88	13.44	58.86	42.69
P	2.53	15.19	17.03	15.14	32.54	18.68	13.74	5.33	2.04	2.13	8.67	6.78
Gatherers												
C	465.33	490.14	1275.81	469.78	787.78	569.25	346.27	168.43	78.07	90.30	190.13	398.92
N	82.78	89.34	226.95	85.63	140.14	103.76	61.60	30.70	13.89	16.46	33.82	72.71
P	13.74	15.23	37.66	14.59	23.25	17.68	10.22	5.23	2.30	2.81	5.61	12.39
Filterers												
C	6.88	36.04	14.77	59.61	20.57	114.53	25.97	18.58	12.76	18.49	45.27	448.75
N	1.29	6.09	2.77	10.08	3.86	19.36	4.88	3.14	2.40	3.13	8.50	75.87
P	0.35	2.00	0.74	3.30	1.04	6.35	1.31	1.03	0.64	1.02	2.28	24.87
Predators												
C	24.49	873.87	130.21	709.70	164.33	990.57	97.59	510.89	66.00	206.75	76.54	355.96
N	4.79	176.75	25.50	143.55	32.18	200.36	19.11	103.34	12.92	41.82	14.99	72.00
P	0.70	25.68	3.74	20.86	4.72	29.11	2.80	15.01	1.90	6.08	2.20	10.46
Periphyton												
C	2049.43	527.85	1013.54	2744.82	10681.25	9608.70	5486.13	2392.53	5213.25	6436.37	6375.51	3911.10
N	206.25	56.93	102.00	296.01	1074.93	1036.23	552.11	258.02	524.65	694.12	641.62	421.78
P	86.76	24.37	42.91	126.71	452.19	443.57	232.25	110.45	220.70	297.13	269.91	180.55
Filamentous algae												
C	-	125.30	-	1136.55	190.65	1982.47	4.46	285.37	2.12	3.67	100.32	288.43
N	-	12.98	-	117.76	20.72	205.41	0.49	29.57	0.23	0.38	10.90	29.88
P	-	4.97	-	45.06	3.75	78.60	0.09	11.31	0.04	0.15	1.97	11.44

Table 7.2 Continued

Mosses													
	C	31.47	1920.33	31.47	2991.74	21.68	5926.93	84.53	1714.14	5.26	200.30	4.92	649.43
	N	1.17	101.52	1.17	158.17	0.80	313.34	3.14	90.62	0.20	10.59	0.18	34.33
	P	1.84	119.33	1.84	185.91	1.27	368.31	4.94	106.52	0.31	12.45	0.29	40.36
	CPOM												
	C	7179.89	11498.53	1894.28	2155.36	4979.87	5652.16	8879.85	2080.27	2042.37	848.25	1092.82	3024.66
	N	433.57	1342.60	114.39	251.67	300.72	659.96	536.23	242.90	123.33	99.04	65.99	353.17
	P	78.01	1601.42	20.58	300.18	54.11	787.19	96.48	289.72	22.19	118.14	11.87	421.25
	FPOM												
	C	18402.02	16659.08	18402.02	1339.97	1409.91	3465.84	1242.29	1175.78	647.25	459.54	316.05	1133.80
	N	1219.77	1568.62	1219.77	126.17	93.45	326.34	82.34	110.71	42.90	43.27	20.95	106.76
	P	456.86	1811.11	456.86	145.68	35.00	376.79	30.84	127.83	16.07	49.96	7.85	123.26
	SPOM												
	C	0.125	0.369	0.097	1.150	0.382	0.582	0.111	0.169	0.189	0.498	0.154	0.373
	N	0.015	0.052	0.012	0.161	0.047	0.082	0.014	0.024	0.023	0.070	0.019	0.052
	P	0.001	0.005	0.001	0.016	0.003	0.008	0.001	0.002	0.001	0.007	0.001	0.005

Discussion

Differential susceptibility in the stoichiometry of food resources to the point source

Overall, food resources in La Tordera stream tended to be P-rich both upstream and downstream of the point source input. Based on studies from a wide range of freshwater ecosystems from worldwide, Kahlert (1998) proposed the $C_{158}:N_{18}:P_1$ ratio as the optimal median value and for freshwater algae in opposition, but close, to the Redfield theoretical ratio of $C_{106}:N_{16}:P_1$ based on oceanic systems. In addition, she concluded that the optimal ratio may largely vary among algal communities and provided optimal ranges for C:N (8-11), C:P (99-369), and N:P ratios (11-32). The C, N, and P contents for periphyton were similar in the two reaches, indicating a potential homeostatic regulation (Sturner and Elser 2002). This result disagrees with the findings of Stelzer and Lamberti (2001) and Cross *et al.* (2003), who found that %N and %P in stream periphyton increased after an addition of N and P. In the two reaches, periphyton C:N ratio was within the Kahlert optimal range for freshwater algae. However, C:P and N:P ratios in periphyton were extremely low compared to previous studies (Kahlert 1998, Stelzer and Lamberti 2002, Kahlert *et al.* 2002, Cross *et al.* 2003), indicating P accumulation. At the downstream reach, C:N:P ratios for filamentous algae were similar to those for periphyton. At the upstream reach, in contrast, C:P and N:P ratios for filamentous algae were twofold those at the downstream reach and were located within the optimal range proposed by Kahlert (1998). The higher P content relative to C and N indicates that filamentous algae were potentially N-limited at the downstream reach even though such differences may also be due to differences on species composition (Sturner and Elser 2002) or growth rates (Agren 2004). Although nutrient concentrations were much higher at the downstream reach than at the upstream reach, the low DIN:SRP ratios at the

downstream reach (see Table 4.1 in Chapter 4), especially from April to September 2002, lends support to the assumption that autotrophs were N-limited. The C:x ratios (where x is either N or P) in mosses was much higher than in periphyton or filamentous algae because of a higher content in phenolic compounds (Stream Bryophyte Group 1999). The C:N ratios for mosses were slightly lower than those previously reported in a forested stream in eastern Tennessee (Mulholland *et al.* 2000). In our study, however, N and P contents for mosses were, respectively, two and 40 times higher than those previously reported for aquatic bryophytes in North America (Bedford *et al.* 1999). We expected that N:P ratios should be higher at the downstream reach than at the upstream reach, since aquatic mosses are known to take up P rapidly (Meyer 1979, Steinman 1994). However, elemental contents, and consequently elemental ratios, in mosses were similar above and below the point source in discrepancy with the findings of Christmas and Whitton (1998), probably because nutrient concentrations in the two reaches were much higher than those considered in previous studies.

CPOM and FPOM represent the major C pathway in most ecosystems (Wallace *et al.* 1997), and were the dominant food resources in La Tordera stream in November 2001 and January 2002. Below the point source, C:N, C:P, and N:P ratios for CPOM and FPOM decreased relative to those at the upstream reach. Cross *et al.* (2003) and Kaushik and Hynes (1971) obtained similar results, apparently because microbes growing on BOM can uptake N and P from dissolved pools (Suberkropp 1998, Robinson and Gessner 2000, Frost *et al.* 2002). In addition, CPOM and FPOM were higher in N content at the upstream reach than at the downstream reach, but the contrary occurred for P. This pattern was closely related with differences in DIN:SRP ratios between the two reaches. The C:N content for CPOM and FPOM in La Tordera stream were lower than those previously reported (Mulholland *et al.* 2000, Cross *et al.* 2003), but the C:P ratio

was one order of magnitude lower than those provided by Cross *et al.* (2003), probably because of differences in stream water concentrations. Research from a ^{15}N tracer addition in a forested stream (Mulholland *et al.* 2000) found that SPOM was not generated only by entrainment of FPOM but also from other biomass compartments such as periphyton or consumer feces. This may explain the observed differences in nutrient contents between FPOM and SPOM in La Tordera stream. Differences in nutrient contents of SPOM between the two reaches were not as high as the observed for FPOM or CPOM. However, N and P were slightly higher at the downstream reach and, combined with a lower C content, resulted in C:P and N:P ratios almost two times lower at the downstream reach than at the upstream reach. Similar to CPOM and FPOM, such differences may also be explained by differences in water chemistry. Few publications supplied elemental analysis for SPOM, among them the C:N ratio provided by Mulholland *et al.* (2000) was twofold those found in our study, probably because of differences in material composition.

Homeostatic regulation of consumer stoichiometry

Although stoichiometric ratios may largely vary in autotrophic organisms (Kahlert 1998, Bedford *et al.* 1999), metazoans have been seen as having relatively invariant elemental ratios (Sterner and Elser 2002). The number of published studies is scarce, but stoichiometric homeostasis has been demonstrated for freshwater and marine zooplankton (Andersen and Hessen 1991, Anderson *et al.* 2004) and also for macroinvertebrates (Stelzer and Lamberti 2002, Frost *et al.* 2003). We found no significant differences in elemental content between the taxa collected above and below of the point source. However, certain taxa showed important differences in nutrient content between the two reaches. Indeed, variability in elemental contents and stoichiometry within high taxonomic groups

(i.e., family or higher) between the two reaches may have been due to differences on species composition in the two reaches. Cross *et al.* (2003) found that stream macroinvertebrates might not be strictly homeostatic in a detritus-based stream. They suggested that their study streams were strongly limited by P and that their fauna were adapted to such nutritional constraints as seen before (Fagan *et al.* 2002). This may indicate that stream macroinvertebrates have varying degrees of homeostasis according to the stoichiometry of their food resources (Sterner and Elser 2002). In natural ecosystems, macroinvertebrates may experience nutritional constraints that lead to suboptimal growth conditions (Elser *et al.* 1996). An increase of the DIN:SRP ratio in stream water translated in higher N:P ratios in food resources that could increase consumer P accumulation (Woods *et al.* 2002) and growth rates (Frost and Elser 2002a).

Elemental imbalances between consumers and resources

The calculation of elemental imbalances provides a measure of the dissimilarity in relative supply of an element between consumers and their presumed resources (Sterner and Elser 2002) and has direct effects on nutrient cycling (Vanni 2002) and growth (Söderström 1988, Stelzer and Lamberti 2002, Frost and Elser 2002a). The elemental imbalances in C:N between scrapers and periphyton were similar to those previously reported for lake zooplankton-phytoplankton (Sterner and Hessen 1994, Elser *et al.* 2000) and lake benthic invertebrates-algae (Frost and Elser 2002b), but stand out against those found between terrestrial insects and leaves (Elser *et al.* 2000). Overall, our results lends support to the findings of Cross *et al.* (2003) in a detritus-based headwater stream that consumer-resource elemental imbalances were more relevant for shredders, less severe for scrapers and gatherers while predators were the least out of balance with their food. However, they found that imbalances in elemental composition

between consumers and resources were much higher than for La Tordera stream, probably because of very low SRP concentrations in their stream. Overall, nutrient enrichment below the point source tend to amend elemental imbalances between the requirements of consumers and the consumed food in agreement with previous research (Stelzer and Lamberti 2002, Frost and Elser 2002a, Cross *et al.* 2003). Such changes may have severe consequences on stream processes (Newbold *et al.* 1982, Fisher *et al.* 1998) through changes on population dynamics (Burkhardt and Lehman 1994, Loladze *et al.* 2000), trophic interactions (Sterner *et al.* 1997, Elser *et al.* 1998), and community structure (DeMott and Gulati 1999, Grover 2002).

Variability in stoichiometric relationships among macroinvertebrate taxa

Mean stoichiometric ratios of macroinvertebrates were slightly higher than those previously reported for stream invertebrates (Cross *et al.* 2003) and lake littoral invertebrates (Frost *et al.* 2003), and similar to those found for terrestrial invertebrates or zooplankton (Elser *et al.* 2000). The differences in C, N, and P content among macroinvertebrate taxa were noticeable. The high content in crystalline calcium carbonate of mollusks shell translates in a lower C, N, and P content relative to insect orders. The high N content in leeches has been attributed to high concentrations of structural proteins (Frost *et al.* 2003). Similarly, the low P content in beetles may result from their hard chitin-rich exoskeleton. On the other hand, the high P content in midges or microcrustaceans may be explained by their high growth rates (Main *et al.* 1997, Benke 1998) and consequent high content in ribosomal RNA (Vrede *et al.* 2004, Sterner and Elser 2002). Although differences in nutrient contents were considerable among insect taxa, we found no significant differences among insect orders. The number of studies considering stoichiometric differences among taxonomic groups is scarce and patterns are inconsistent. For example, Cross *et al.* (2003) found that dipterans harbored the

highest amount of P, while Ephemeroptera was the most P-poor insect order. In contrast, Frost *et al.* (2003) found mayflies among the taxa with higher P content. Such incongruence may be merely due to the high intra-group variability explained by differences in species composition and ontogeny, lessen the sense of making comparisons among high taxonomic groups.

Trophic basis for heterotrophic production

Although macroinvertebrates often represent little biomass storage relative to the ecosystem, their rapid growth rates (Huryn and Wallace 2000) may make them an important component of nutrient cycling (Grimm 1988, Mulholland 1992, Vanni 2002). The percentage of N stored in macroinvertebrates relative to the ecosystem was similar to that previously reported in a forest stream in eastern Tennessee (Mulholland *et al.* 2000), although only common taxa was considered in their study. In contrast, we found that the %C stored in macroinvertebrates was more than four times higher. Unfortunately, we found no published work regarding P distribution among biomass compartments.

The alternation between leaf litter and periphyton likely controlled the functional organization of benthic macroinvertebrates as seen before (e.g., Hall *et al.* 2001). The relative importance of basal food resources in streams with riparian vegetation dominated by deciduous forests may vary seasonally because of differences in light attenuation derived from tree phenology (Hill *et al.* 2001). However, in the two reaches channel width and latitude were enough to permit higher sun irradiation in summer than in winter. Therefore, in the two reaches CPOM and FPOM were the main basis for heterotrophic production in autumn and winter whereas periphyton was more important in spring and summer. The alternation from light-repletion to light-limitation combined with the processes of leaf abscission and breakdown, and flow disturbance in La Tordera stream caused

important changes in trophic interactions (as seen in Chapter 5). Such changes may have implications in trophic relationships (Benke and Wallace 1997, Wallace *et al.* 1999, Huryn and Wallace 2000) and, therefore, in nutrient cycling (Mulholland 1992, Vanni 2002).

Overall, our results show that the point source input did not alter to a great extent the total C and N standing stock of the benthic ecosystem in La Tordera stream, whereas P standing stock was more than threefold. Elemental contents of the biomass compartments presented in this study derive from averaged values of samples collected over the sampling period and, therefore, temporal patterns could not be examined. Future research addressing the variability in the relative importance of basal food resources will significantly contribute towards a better understanding of the effects of stoichiometric constraints on in-stream processes and will provide valuable information that could pay for future management plans.

Conclusions

Our results show that the point source altered the elemental composition of several food resources for macroinvertebrates, including CPOM, FPOM, and SPOM, by increasing the storage of phosphorus. Nutrient content and elemental ratios of macroinvertebrates were not significantly influenced by the point source input in La Tordera stream, providing support to the hypothesis that homeostatic regulation can be extended to stream macroinvertebrates. In contrast, elemental imbalances between consumers and their presumed resources were amended below the point source. Major differences among macroinvertebrate taxa can be explained through differences in taxonomy and food intake.

Overall, storage of C and N in the benthic ecosystem was similar between the two reaches, but P storage was higher below the point source. In La Tordera stream, macroinvertebrates represented little C, N, and P storage relative to the

nutrient bulk in the whole ecosystem but N retention was especially important. CPOM and periphyton represented the main food resources for heterotrophic production in the two reaches. These two basal resources were alternated according to seasonal variability of leaf abscission and light attenuation.

Summary

Patterns regarding elemental stoichiometry in stream ecosystems remain largely unknown. We analyzed C, N, and P contents in benthic macroinvertebrates and their potential food resources in two reaches located upstream and downstream a point source in La Tordera stream (Catalonia, NE Spain). Periphyton and mosses had similar nutrient contents in the two reaches. The %C and %N filamentous algae was also similar in the two reaches, but %P below the point source was two times higher than that at the upstream reach. Stoichiometric ratios for CPOM, FPOM, and SPOM decreased considerably below the point source. Elemental contents and ratios were highly variable among macroinvertebrate taxa but did not differ significantly between the two reaches, indicating homeostatic regulation. Dipterans, caddisflies, and mayflies had similar elemental contents and stoichiometry, whereas C and N were lower in mollusks and P in beetles. Predators had the higher C and N contents, while %P was higher in filterers and lower in scrapers. Elemental imbalances between consumers and resources were amended at the downstream reach relative to the upstream reach. Macroinvertebrates represented, on average, relatively low nutrient storage in the ecosystem. At the upstream reach, autochthonous production represented the major input of nutrients into the ecosystem food web, whereas the major source of P for heterotrophic production at the downstream reach was represented by allochthonous inputs.

8. Concluding remarks

Effects of the point source

The wastewater treatment plant (WWTP) effluent of Sta. Maria de Palautordera and related outflows substantially increased nutrient concentrations, organic matter, and discharge of La Tordera stream. The increase of nutrient concentrations and organic matter enhanced autotrophs, including periphyton, filamentous algae, and mosses, under sufficient light exposure and stable flow conditions. The increase of production driven by the higher nutrient availability downstream of the point source also caused depletion of dissolved oxygen in stream water. During the sampling period, however, the reduction in dissolved oxygen concentrations was not as prominent to cause oxygen limitation for living organisms at reach scale. The point source input also increased SPOM concentrations but did not alter standing stocks of benthic organic matter (i.e., CPOM and FPOM), which seemed to be conditioned mainly by riparian vegetation. However, nutrient enrichment led to higher N and P contents in CPOM, FPOM, and SPOM, probably because nutrients uptake from dissolved pools by the associated microorganisms. The cited changes in primary producers and organic matter meant that quantity and quality of food resources, and streambed microhabitat conditions were considerably modified by the point source. Undoubtedly, these changes favored certain macroinvertebrate taxa in detriment of some other. The community composition was adjusted by the conditions derived from the point source towards a less diverse and structured community. Tolerant taxa, such as midges, blackflies, leeches, and snails, were enhanced below the point source while sensitive taxa, especially mayflies, stoneflies, and caddisflies, were reduced or even eliminated. Indeed, the conditions at the downstream reach allowed the colonization of several taxa, such

as the clam *Pisidium casertanum* or the leech *Glossiphonia* sp., but the balance was rather negative as losses in taxa richness were much higher than gains. At the downstream reach, midges were especially enhanced because the large amounts of filamentous algae and mosses provided shelter to their small body sizes and their generalist feeding behavior allowed them to exploit the high quantities of periphyton and nutrient-rich detritus. These macroinvertebrates were characterized by having small body sizes and high turnover rates that can have consequences on the ecosystem nutrient cycling through nutrient export derived from emergence. The success of midges was evidenced by their high densities, which were noticeably reflected in the total density of the macroinvertebrate community. However, where there is something that can be eaten, someone will eat it and as midges became dominant, their predators, mainly leeches, had increasing possibilities. Although total macroinvertebrate density was substantially increased below the point source, total biomass was similar between the two reaches over the sampling period. This indicates that total macroinvertebrate biomass was constrained by factors other than resources availability. C and N standing stocks stored in the benthos, including macroinvertebrates, autotrophs, and benthic organic matter, were quite similar between the two reaches. However, P standing stock was much higher at the downstream reach than at the upstream reach, mainly because P-enrichment of CPOM and FPOM. Nutrient storage in macroinvertebrates was slightly higher at the downstream reach than at the upstream reach, but the increase of nutrient storage in macroinvertebrates at the downstream reach relative to the upstream reach was substantially higher for P than for C or N.

The changes in flow regime driven by the discharge of the point source into the stream ecosystem also had implications for the benthic macroinvertebrate community. The variable but continuous release of water from the WWTP

effluent and related outflows into the stream increased base flow discharge, especially during low flow periods. The direct effects of increasing discharge on community dynamics are obvious, especially in the Mediterranean region where temporary streams can be converted into permanent ones. However, additional water supply can also have indirect implications for macroinvertebrates through alteration of their food resources. Our results showed how at the upstream reach the dynamics of leaf litter could be affected by summer drought and the subsequent decoupling with shredders. However, the continuous discharge of the point source allowed shredders to be synchronized with CPOM standing stock at the downstream reach.

Interaction between the effects of the point source, seasonal variability, and flood disturbance

The effects of point sources on macroinvertebrate communities have been discussed extensively in the literature, but they can also be influenced by factors that are not necessarily inherent to such disturbance. Our results show how the seasonal alternation between CPOM and periphyton as main basal food resources can control the functional organization of benthic macroinvertebrates. In this sense, the seasonal alternation between leaf abscission and light exposure in combination with flow disturbance prevailed over the continuous discharge of the point source in determining the food base for heterotrophic production. In the two reaches, CPOM and FPOM were the main basis for heterotrophic production in autumn and winter whereas periphyton was more important in spring and summer.

The flooding events occurring during the sampling period decreased macroinvertebrate density and biomass in the two reaches, but did not represent losses of richness or changes in functional organization. The decrease of density was more accentuated at the downstream reach than at the upstream reach, where

macroinvertebrate density was usually higher. In contrast, the effect of flooding on macroinvertebrate biomass was similar for the two reaches. However, the recovery was faster at the downstream reach than at the upstream reach, presumably because of higher nutrient supply and the reproductive strategy of the dominant taxa.

Our results revealed that flow disturbance could substantially contribute to amelioration of water quality in human altered streams. Flooding events increased the resemblance between the two reaches in terms of primary producers, benthic organic matter, and macroinvertebrates, including community composition, structure, and functional organization. These results highlight the need of considering potential effects of seasonal variability and natural disturbances in biomonitoring plans to obtain valuable assessments. Moreover, flow disturbance can act as a reset mechanism in human altered streams and play an important role against eutrophication. Therefore, the development of future management plans should consider the use of flow disturbance manipulations for stream restoration purposes.

Acknowledgements

This work would not have been possible without the support of a number of people.

Si algú ha estat sempre fent-me costat, suportant els meus daltabaixos, el mal humor, l'estrès i les obsessions que he patit, tot contagiant-me les seves ganes de viure i de complir somnis, aquesta persona ha estat la Gora. La seva immesurable ajuda tant en el treball de camp com al laboratori i, especialment en la revisió i edició d'aquesta tesi i altres treballs, ha estat indispensable per poder haver aconseguit aquest el meu primer gran objectiu. Tampoc no vull deixar passar per alt que ella ha estat l'encarregada de bona part dels anàlisis químics que presento en aquest treball.

Sin mis padres no habría llegado ni aquí ni a ningún sitio, y no sólo porque me hayan traído a este mundo sino por todo el esfuerzo y empeño que han invertido para que yo, y cuatro bocas más, pudiéramos llegar donde hemos querido llegar. También quiero mencionar aquí el soporte incondicional que he recibido por parte de toda mi familia sin exclusión alguna, desde mi otra madre Gina, a todos mis hermanos, tíos, primos i abuelos (los de Riudoms, los de Terrassa i los de Navata). En especial quiero agradecer a Dani todos los conocimientos de diseño gráfico que me ha transmitido y a mi tío Fede, por venir hasta La Tordera para hacerme unas fotos. A Pepi, l'Àngela, el Roger, el Jordi i la Doris, els dec també la meva gratitud per haver-me fet entendre que la vida es pot prendre d'una altra manera.

Vull fer constar el meu agraïment a la M. Àngels Puig, que, com directora de tesi, ha revisat la meva feina i també per haver-se encarregat de bona part dels anàlisis elementals. L'Eugènia ha estat per a mi com una segona directora no oficial, per haver contribuït de manera prou significativa tant en la forma com en

el contingut d'aquest treball. El Quico ha constituït un gran pilar durant la meua formació com a científic tot aportant-me conceptes fonamentals en matèries de ciència en general, i d'ecologia en particular. No quiero olvidar-me de nombrar a Nico, mi incansable compañero de campo, lupa y teclado, maestro en taxonomía y en cosas de la vida. Ni tampoc tots els estudiants que van ajudar en el treball de camp.

També vull agrair el suport incondicional dels companys del CEAB, i de fora, que d'una o altra manera m'han ajudat a fer més passables aquests quatre anys i escaig de patiment, en especial a la Susana, la Raquel, l'Alba, Jara, Juanma, el Fede, Guillermo, el Marc, la Teresa, Dani, l'Esperança, el Joan Lluís i el Victor. No puc oblidar esmentar les bibliotecàries, primer la Xus i ara la Maika, sense les quals la qualitat d'aquest treball s'hauria vist clarament ressentida. Així com l'Anselm, per la seva implicació en l'elaboració d'inversions de mostreig, o el Ramon, per la cura que ha tingut del meu estimat portàtil i els seus continus consells sobre informàtica.

The participation in the STREAMES European project allowed me to meet and exchange knowledge with scientists from Portugal, France, Italy, Austria, Germany, Grece and Israel. I am particularly appreciative to Martin Pusch for his suggestions, Gabriel Singer for his help with some portions of this project, Angelo Solimini and Antonio Ruggiero for their warm welcome at the University of Rome *Tor Vergata*, and Catherina Voreadou, at the National History Museum of Crete, sense oblidar els companys i amics de Barcelona i de Girona.

I am grateful to the staff at the Flathead Lake Biological Station in general, and Jack Stanford in particular for generously sharing their equipment and expertise during my stays in Montana. I am indebted to him for his kindness and will always be thankful for the opportunity to work with his research team. Ric Hauer gave me valuable lessons about data analysis. Bob Newell provided me

useful comments about taxonomy. I greatly appreciate the valuable suggestions of Charlie Hall about how to write scientific documents. Ethan and Krista for the welcome distraction they provided to Gora and me during our stays in Montana, especially during the icy winter. Brian Reid introduced me to the fascinating world of the hyporheos.

Thanks also to the stream dwelling bugs, for holding out there although our continuous attacks.

This study was supported by funding of the STREAMES European project (EVK1-CT-2000-00081). També he d'agrair el suport del Departament d'Universitats, Recerca i Societat de la Informació de la Generalitat de Catalunya, per la concessió de la beca FPI de la qual he gaudit i sense la qual aquest treball no hauria estat possible. Els anàlisis dels ions majoritaris i de continguts elementals es van realitzar als Serveis Científico-Tècnics de la Universitat de Barcelona.

Espero no haver-me descuidat de mencionar ningú que hauria d'haver-ho estat.

Who knows the way, will understand the outcome.

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