Macroinvertebrate trophic responses to nutrient addition in a temperate stream in South America

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Abstract: The present continuous anthropogenic pressure has resulted in an enhancement of nutrient inputs into rivers and streams. This situation has worsened, mainly in agricultural areas, causing an accelerated eutrophication. Macroinvertebrate feeding strategies reflect the species' adaptations to environments. For a characterization of the macroinvertebrate trophic response to eutrophication, we added nitrogen and phosphorus into the La Choza stream and examined the gut contents of those taxa in order to analyze dietary alterations and assign each macroinvertebrate to a functional feeding group (FFG). Complementary C and N stable isotopes analysis was carried out. The gut contents of all taxa studied contained principally detritus, but statistical analyses indicated significant differences before and after fertilization in some taxa. At the control site, and the treatment site before fertilization, the FFGs maintained constant proportions, the gathering-collector species being dominant in all samples. After fertilization the composition of the taxa at the treatment site varied. The $\delta^{13}C$ isotopes values showed that most taxa used detritus as a basal resource. $\delta^{15}N$ values were generally coincident with gut content analyses. These results provide a prediction of the functional responses of macroinvertebrates to the environmental consequences expected from accelerated land use. The functional responses constitute a powerful tool to assess eutrophication and its consequences in temperate plain streams.

Key words: nutrients, macroinvertebrates, gut contents, stable isotopes, functional feeding groups.

Introduction

Ecosystem processes are key indicators of river health and integrity (Bunn et al. 1999). Many studies have emphasized the relevance of biotic interactions in community structure at both small and large spatial scales (e.g., Hildrew et al. 1985, Englund 1991). The interest in studying the trophic relationships among the invertebrates of rivers and streams has recently increased. However, most investigations have concerned temperate species of the Northern Hemisphere and little is known about the feeding habits of aquatic fauna in South America (Motta & Uieda 2004, Tomanova et al. 2006). Most of the work within the Neotropical region (e.g., Poi de Neiff 1990, Bonetto & Wais de Badgen 1995, Callisto et al. 2001) has been modelled on the studies of Cummins and co-workers (e.g., Cummins et al. 1966, Cummins 1973, Cummins & Klug 1979, Merritt & Cummins 1996) in the United States. However, King et al. (1988) have pointed out that the transfer of information on functional feeding groups (FFGs) between continents requires extreme caution.

Since the analysis of gut contents provides information regarding trophic relationships among the

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species in a community, feeding strategies reflect the adaptation of species to ongoing environmental conditions. According to Fenoglio et al. (2005), improving our knowledge of feeding behavior and trophic ecology is indispensable to better understand applied and basic elements of stream ecology. The same authors consider that increased human influence in aquatic ecosystems lead to changes in feeding and growth of aquatic invertebrates, altering composition and structure of benthic communities.

The present continuous anthropogenic pressure has increased nutrient inputs into rivers and streams (Rodrigues Capítulo et al. 2010), mainly in areas with agricultural potential. According to Aizen (2009), in Argentina the use of monocultivation has involved a progressively widening area, and as a result lands formerly employed for both diversified agriculture and livestock pasturing have been displaced by the intensive and exclusive culture of soybeans, a crop which has become the main agricultural product in much of the country and especially in the pampean plain. This author concludes that this shift has led to an increased use of agrochemicals (pesticides and fertilizers). In addition, traditional ranching involving free-range grazing has been supplanted by the use of feedlots containing high densities of animals for the purpose of rapid fattening, which produces a high load of organic waste matter that eventually finds its way into the environment. According to Rodrigues Capítulo et al. (2010), these nutrient increases will favor autotrophy, particularly by those species capable of strategies for surviving in more turbid and enriched environments. An elevation in the nutrient content of rivers and streams can alter biological processes by stimulating the growth of primary producers and accelerating the rates of decomposition (Elwood et al. 1981, Harvey et al. 1998), while enhanced eutrophication will encourage herbivores and detritivores (Sabater et al. 2005). The adverse effects of these environmental changes on habitats and their resident biota should therefore be minimized through appropriate management policies. For this step to be taken, as suggested by Erwin (2009), we must first understand the nature of the ecological changes that will most probably take place within each specific region before implementing the appropriate wetland management and restoration.

The present study is part of the GlobRio Project, whose design consists of analyzing the effects of an experimental addition of nutrients on biodiversity, food web, and fluvial-system functioning (Rodrigues Capítulo et al. 2010). The aim of the present study was to quantify changes in the diets of invertebrates in re-

sponse to a long-term input of N and P into water in the form of fertilizer, thus simulating the increase in the concentration of nutrients that would be a consequence of the continued intensification of unregulated land use. We assume here that the addition of nutrients would increase the nutritional quality (in terms of a decrease in the C:N and C:P ratios) and/or quantity (biomass) of the basal resources for the primary consumers in our study site. Slavik et al. (2004) and Sabater et al. (2011) demonstrated that algal biomass increased and epilithon stoichiometry changed after nutrient addition. An alteration in the diet of certain consumers, mainly the scrapers and detritivores, would also be expected following a change in the quality of the food resources. We predicted that the consumers would show preferences for chemically enriched algae and detritus, and their diet would accordingly contain a larger proportion of those food items. We report here the results of analyses of the gut contents and stable isotopes of the macroinvertebrates in the Argentine temperate-plains stream La Choza after the performance of an artificial-fertilization experiment. A BACI (Before-After Control-Impact) design was applied with the aim of determining significant differences attributable to nutrient addition. In the same way, the relationships with changes in basal resources (Feijóo et al. 2012) were taken into account.

Material and methods

Study area

The La Choza Stream (Fig. 1) is located in Luján, Buenos Aires province, Argentina (in the Río de la Plata basin). Pampean grassland is the typical biome here, the region being a steppe composed of gramineous grasses (Cabrera 1971). The main previous anthropogenic activities were scattered agriculture, though now that form of land use has been replaced by intensive soybean farming, along with widespread cattle raising. At present, the cultivation of this crop has increased by 82 % in the Province of Buenos Aires compared to the 1970s and in addition it now occupies more than 50 % of the arable land throughout the entire country (Aizen et al. 2009). The climate of this region is humid and temperate with a mean temperature of 20 °C and a mean rainfall of 900 mm/year. The basin's drainage area is 15,200 ha. The homogeneity of the streams in the region made possible the selection of two segments of 100 m in length in the same lotic system, located 5 km away from each other. The first reach was located upstream (control site: 34 ° 39′ 14″ S −59 ° 10′ 00″ W), and the second downstream from the experimental input of nutrients (treatment site: 34 ° 47′ 51″ S −58 ° 3′ 17″ W). The distance from the source is 2.5 km for control site and 7.5 km for the treatment site.

The stream is characterized by a low slope (< 1 %) and the width varies between 4 and 10 m in both reaches. This environment has high turbidity, abundant organic matter and high sus-
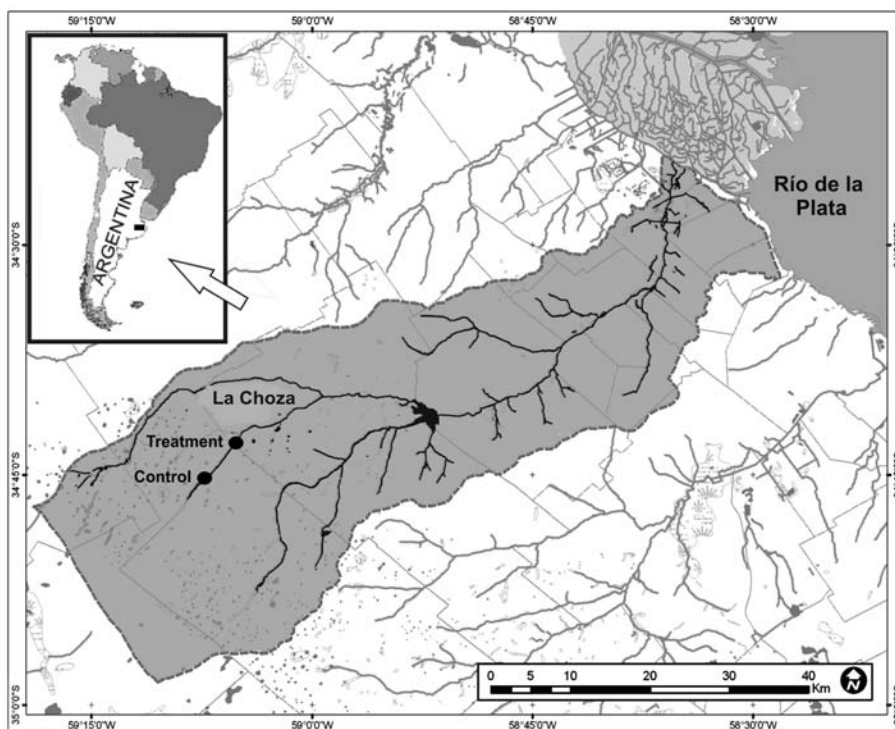
Fig. 1. Map of the study area showing the La Choza Stream with the location of the sampling stations.

pended solids; furthermore the occurrence of high nutrient concentrations is an essential feature of these streams (Feijóo et al. 2012). The bed comprises consolidated carbonates ("caliche") covered by fine sediments (mud and silt). Aquatic vegetation was represented by *Ludwigia peploides* and *Bacopa monnieri*. Mean water velocity is $0.04 \, \text{m s}^{-1}$ in the control site and $0.08 \, \text{m s}^{-1}$ in the treatment site. Mean water flow is $9.63 \, \text{L s}^{-1}$ in the control and $14.83 \, \text{L s}^{-1}$ in the treatment. Mean dissolved oxygen concentration is $9.61 \, \text{mg l}^{-1}$ in the control site and $9.09 \, \text{mg l}^{-1}$ in treatment, conductivity is $1110 \, \mu\text{S cm}^{-1}$ and $1513 \, \mu\text{S cm}^{-1}$ respectively. Mean water temperature is $17.6 \, ^\circ\text{C}$ in the control site and $17.1 \, ^\circ\text{C}$ in the treatment site.

Experimental design

Sampling was carried out bimonthly over a period of two years (from March 2007 to December 2008).

From March 2007 through October 2007 both selected reaches in La Choza stream were studied before nutrient addition. From November 2007 until the end of the experiment in December 2008, a fertilizer (Nitrofoska®) rich in nitrogen (in the form of nitrates and ammonium salts) and phosphorus (as phosphoric anhydride) was added continuously upstream from the treatment site placing 12 bags in the water to give a total of 750 g of the fertilizer and 250 g of urea, thus attaining a concentration threefold higher than the average original levels in the water. The bags were located along three transects separated by 20 m at the beginning of the treatment reach (4 bags per transect). The proportion of added nutrients was calculated to maintain the N/P ratio in the water. The nutrient bags were replaced 2 or 3 times a week during the fertilization period to keep the added nutrients at a constant concentration (Feijóo et

al. 2012). The water samples were collected and analyzed daily by the staff of the Universidad Nacional de Luján, who determined values of dissolved inorganic nitrogen (DIN) and total phosphorus (TP) (APHA 1998).

Macroinvertebrates sampling

Macroinvertebrates were collected following Rodrigues Capítulo et al. (2009). Three replicates of benthic macroinvertebrates were removed with an Ekman grab ($100 \, \text{cm}^2$) for the analysis of the FFGs. The macroinvertebrates associated with the macrophytes were collected with sieves ($250 \, \mu\text{m}$) within the area subsumed by a $1{,}300 \, \text{cm}^2$ Plexiglas square. In both reaches the same microhabitats were sampled. The samples were fixed *in situ* with 5 % formaldehyde and the organisms sorted, identified and counted under a stereomicroscope using the taxonomic keys of Fernández & Domínguez (2001).

Analyses of gut contents and FFG determination

Only taxa found at both sites (except in the case of bivalves) were selected for the analysis of gut contents: *Perithemis* sp. 1, *Orthemis nodiplaga*, *Aeshna bonariensis*, *Phyllogomphoides joaquini*, Coenagrionidae sp. 1 (Odonata), Chironomidae (Diptera), *Diplodon delodontus* (Mollusca, Bivalvia), and *Pomacea canaliculata* (Mollusca, Gastropoda) for the control site and all of the above taxa but with *Corbicula fluminea* (Mollusca, Bivalvia) instead of *D. delodontus* for the treatment site (because the latter species was absent at this site). For each species, 10 individuals (at the same instar) per site were collected on all sampling dates and fixed in the field with 5 % formaldehyde.

In the laboratory, the invertebrates were dissected under a stereoscopic microscope and the foregut separated and placed in vials with Bengal's rose colorant for 24 h to stain their contents. These contents were removed, homogenized in distilled water, and filtered through a 0.45-µm membrane. The filters were clarified by the addition of immersion oil and 15 randomly selected fields per slide counted under an optical microscope at a magnification of 100× to 400×. The area covered by each item of gut contents was estimated with a graduated eyepiece. The size of consumed particles was also measured using the same method. The quantification of a given ingested item was based on the fraction its area covered relative to the area subsumed by the total gut contents, expressed as the relative frequency of each food item (following Winterbourn et al. 1984, Jaarsma et al. 1998, Diaz Villanueva & Albariño 1999). We classified the gut contents according to five categories: detritus (unidentifiable organic mater), diatoms, vascular plants, animal matter and mineral material. The gut content of the predators was furthermore identified whenever possible (identifiable animal remains).

Invertebrates were partitioned into FFGs using the complete list of taxa and their densities (as relative-frequency values) obtained at each site and on each sampling date based on the literature (Merritt & Cummins 1996, Cummins et al. 2005). In the case of taxa whose gut contents were analysed the dominant food was considered (more than 60 % of the gut contents). In both cases the nomenclature established by Merritt and Cummins (1996) was used. These FFGs were: (1) shredders (feeding on coarse particulate organic matter > 1 mm in size), (2) filtering collectors (sifting fine particulates of 0.45 µm to 1 mm from the flowing water column), (3) gathering collectors (gathering fine particulates of organic matter from the debris and sediments on the bed of the stream), (4) scrapers (scraping off and consuming the organic layer of algae, microorganisms, and dead organic matter attached to stones and other substrates), and (5) predators (feeding on other animals).

Isotope analysis

In addition, an analysis of the stable N (δ^{15}N) and C (δ^{13}C) isotopes was carried out following Muñoz et al. (2009) to establish the diet (in terms of assimilation of nutrients) and the trophic level of the species under study. Several individuals of each selected species were collected in numbers depending on their weights (10 for Odonata, 3 for Mollusca) once before (October 2007) and once after fertilization (December 2008). Next, in the laboratory, the macroinvertebrates were stored for 24 h to let the gut contents evacuate and then, the organisms were dried at 60 °C to constant weight and ground into a powder to ensure their homogeneity. The resulting samples were analyzed by a Carlo Erba elemental analyzer coupled to a mass spectrophotometer (University of Barcelona). The isotopic compositions were quantified through the use of international standard reference materials (Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were expressed as the relative difference in parts per thousand between the sample and the conventional standard. The stable isotopes and stoichiometry ratios of basal resources (detritus, periphyton, epipelon, macrophytes and seston) were obtained from Feijóo et al. (2012). The mixing model SIAR V4 (Stable Isotope Analysis in R) was used to determine the contribution of the different food items and basal resources to the diet of the studied taxa.

Statistical analysis

BACI (Before-After Control-Impact) design was applied following Stewart-Oaten & Bence (2001) with the aim of establishing whether significant differences between the sites had occurred as a result of the experimental fertilization. With respect to the FFGs this approach was performed through the use of the relative-abundance values. Differences in gut contents for each taxon were tested by means of the same method using percentages of each food item (arc sin transformation of values). The data analyses were performed using SPSS software Version 12.0 (2003). An additional Student t test was applied for each analysed taxa to compare the isotope data before and after fertilization at both sites. The same test was applied to analyse the gut contents of *P. joaquini* before and after fertilization at both sites because this species showed apparent differences not demonstrated for BACI analyses.

Results

Water phosphorus and nitrogen concentrations before and after fertilization

At the control reach, the respective nutrient concentrations before and after October 2007 (date of addition of fertilizer in treatment site) were 205.6 ± 47.3 and 93.4 ± 3 mg l^{-1} for soluble reactive phosphorus and 601.6 ± 102 and 492.4 ± 110 mg l^{-1} for dissolved inorganic nitrogen (mean values ± SD). In contrast, in the treatment stretch, after the addition of the fertilizer the respective soluble-reactive-phosphorus levels increased from 247.6 ± 37.7 to 431.6 ± 29 mg l^{-1}, while the dissolved-inorganic-nitrogen values decreased from 1331 ± 214 to 828 ± 199 mg l^{-1}. In both reaches N level decreased after the date of addition of nutrients. This decrease is caused by denitrification processes, principally during the night (Acuña et al. 2011). The N values, however, were higher in the treatment site (approximately twofold both before and after treatment). The mean ratios between the dissolved-inorganic-nitrogen and the soluble-reactive-phosphorus values were 5.4 and 2.3 for control and treatment reaches, respectively, during the enrichment period.

Macroinvertebrate FFGs

The FFG was obtained for all taxa determined (Table 1). The control site exhibited consistent proportions among the FFGs analyzed throughout the study period. The gathering collectors were dominant on all sampling dates, but an increase in predators was observed in May and December 2008 (Fig. 2a). Before fertilization, the treatment site likewise exhibited a high number of gathering collectors; but unlike the control site, the filtering collectors were a more abundant group.

Table 1. FFG for all the taxa determined in the reaches studied in the La Choza stream.

Taxa	Control reach		Treatment reach		Habitat		FFG
	Before	After	Before	After	Before	After	
Dugesiidae	x	x	x	x	x	x	predator
Temnocephalla sp.	x	x	x	x	x	x	parasites
Nematoda	x	x	x	x	x	x	gathering-collector
Naididae Naidinae	x	x	x	x	x	x	gathering-collector
Naididae Pristininae	x	x	x	x	x	x	gathering-collector
Naididae Tubificinae	x	x	x	x	x	x	gathering-collector
Naididae Rhyacodrilinae	x	x	x	x	x	x	gathering-collector
Opistocystidae	x	x				x	gathering-collector
Enchytraeidae	x	x	x	x	x	x	gathering-collector
Aelosomatidae				x		x	gathering-collector
Hirudinea	x	x	x	x	x	x	predator
Pomacea canaliculata	x	x		x	x	x	scrapers
Heleobia parchappei	x	x	x	x	x	x	scrapers
Drepanotrema kermatoides	x	x	x	x	x	x	scrapers
Biomphalaria peregrina	x	x	x	x		x	scrapers
Musculium sp.		x		x	x	x	scrapers
Hebetancylus moricandi	x	x	x	x	x	x	scrapers
Eupera sp.		x			x	x	scrapers
Chilina sp.			x			x	scrapers
Pisidium vile	x		x	x	x	x	filtering-collectors
Pisidium sterkianum	x	x	x	x	x	x	filtering-collectors
Corbicula fluminea	x	x	x	x	x	x	filtering-collectors
Diplodon delodontus	x	x			x	x	filtering-collectors
Tardigrada	x	x	x	x	x	x	gathering-collector
Sminthuridae	x	x	x		x	x	gathering-collector
Caenis sp.	x	x	x	x	x	x	gathering-collector
Americabaetis sp.	x	x	x	x	x	x	gathering-collector
Callibaetis sp.		x				x	gathering-collector
Campsurus sp.	x				x		gathering-collector
Perithemis sp.	x	x				x	predator
Aeshna sp.	x					x	predator
Phyllogomphoides joaquini	x	x	x	x	x	x	predator
Coenagrionidae	x	x	x	x	x	x	predator
Hydrophilidae larvae	x	x	x	x	x	x	predator
Hydrophilidae adults	x	x	x	x	x	x	gathering-collector
Elmidae larvae	x	x	x	x	x	x	gathering-collector
Elmidae adults	x	x	x	x	x	x	gathering-collector
Curculionidae larvae	x					x	gathering-collector
Dytiscidae		x	x	x		x	predator
Noteridae		x				x	predator
Staphylinidae		x				x	predator
Corixidae	x			x		x	predator
Belostoma elegans		x	x	x	x	x	predator
Veliidae		x				x	predator
Chironomidae	x	x	x	x	x	x	gathering-collector
Ceratopogonidae	x	x	x	x	x	x	gathering-collector
Tipulidae	x					x	gathering-collector
Ephydridae	x	x				x	gathering-collector
Empididae			x	x	x	x	gathering-collector
Culicidae		x				x	filtering-collectors
Tabanidae				x		x	predator
Leptoceridae		x			x		gathering-collector
Hidroptilidae			x			x	gathering-collector
Hyallela curvispina	x	x	x	x	x	x	gathering-collector
Palaeomonetes argentinus	x	x	x		x	x	gathering-collector
Macrobrachium borelii	x	x			x	x	gathering-collector
Aegla bonariensis		x	x	x	x	x	gathering-collector
Ostracoda	x	x	x	x	x	x	filtering-collectors
Daphnidae	x	x	x	x	x	x	filtering-collectors
Macrothricidae	x	x	x	x	x	x	filtering-collectors
Chidoridae	x	x	x	x	x	x	filtering-collectors
Leydigia sp.	x					x	filtering-collectors
Cyclopoida	x	x	x	x	x	x	predator
Harpacticoida	x	x	x	x	x	x	gathering-collector
Acari	x	x	x	x	x	x	predator

In the samples collected immediately after fertilization (December 2007 through February 2008), a marked increase in the proportion of filtering collectors was noted, though this abrupt change did not persist in subsequent samples. In May and December 2008 the predators became the dominant group, whereas in July the prevalence of gathering collectors increased, and by September the filtering collectors once again held sway (Fig. 2b). According to the BACI analysis these results were not statistically significant for predators ($F = 2.73$, $p > 0.05$) and scrapers ($F = 2.69$, $p > 0.05$),

however, filterers ($F = 7.29$, $p = 0.01$) and gathering collectors ($F = 11.79$, $p = 0.003$) showed significant differences.

Analyses of gut contents

Detritus was an abundant component within the gut contents of most of the taxa studied (Fig. 3). With the Coenagrionidae, the second most abundant item was animal remains, principally composed of the remains of Chironomidae and Copepoda. Animal remains were
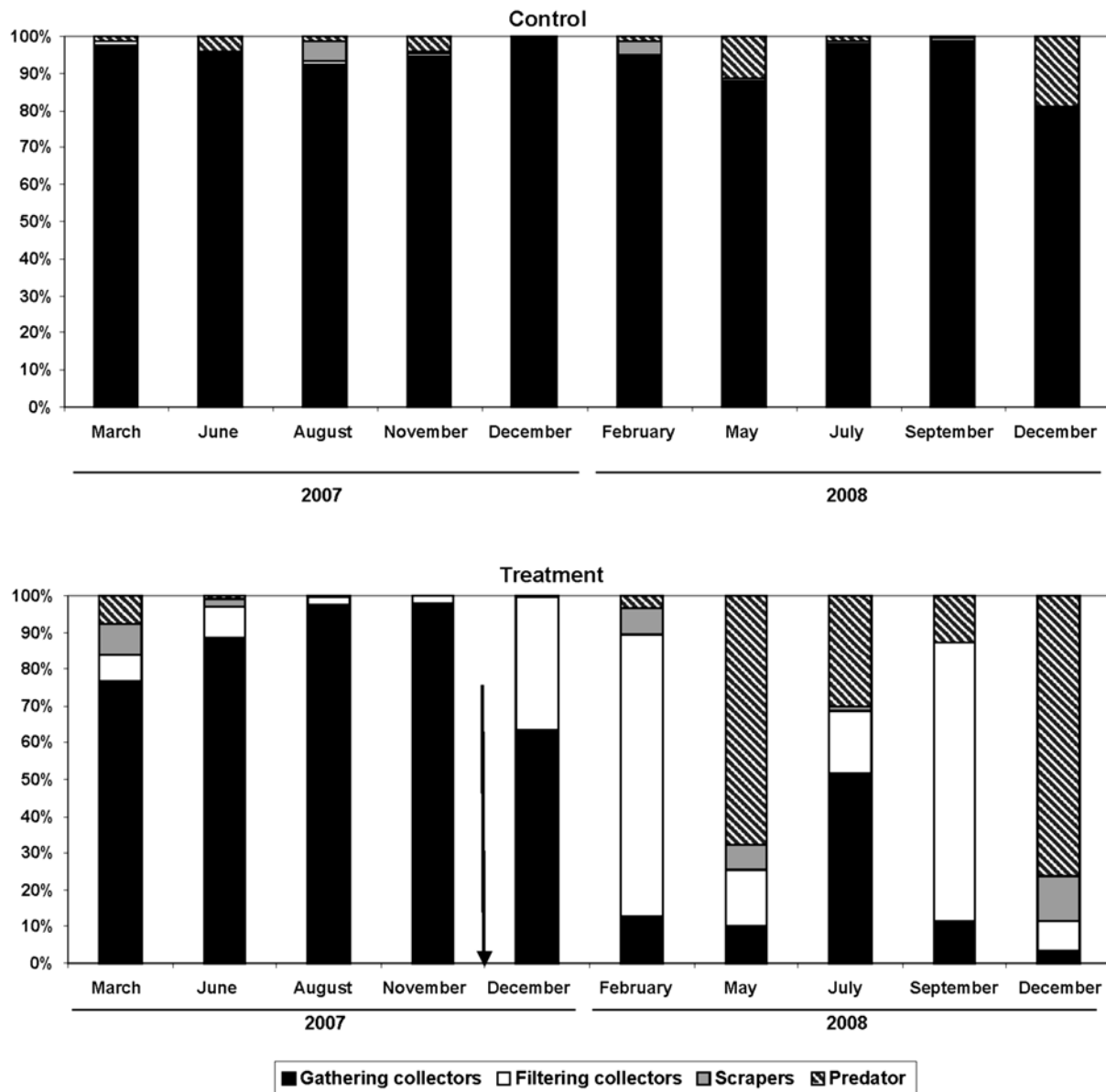
Fig. 2. Percentage of functional feeding groups in the control and the treatment sites in the La Choza Stream. **a)** control, **b)** treatment. Arrow shows fertilization date.

observed in the gut contents of *P. joaquini* at the control site but not in the treatment site. The Student t test, however, showed that these differences between sites were not significant (t = −0.01, p > 0.05).

In the gut of *D. delodontus* at the control site diatoms were the second most abundant item (mean value, 25 %); the same was found for *C. fluminea* at the treatment site although here the diatoms were at times accompanied by the presence of animal remains (at 3 %). At the treatment site a significant decrease in the prevalence of diatoms in guts was noted following fertilization along with an increase in the level of detritus (Table 2).

In the guts of the Chironomidae at the control site, a greater abundance of diatoms, vascular plants, and animal matter was observed, whereas in the guts of this same group at the treatment site the proportion of detritus increased at the expense of diatoms, and neither vascular plants nor animal matter were present.

In the guts of *P. canaliculata* at the control site, detritus (44 %), diatoms (32 %), and vascular plants (in lesser amounts at 21 %) were principally observed, whereas in the guts of this species analyzed at the treatment site the vascular plants were dominant over the diatoms, whose proportion had become significantly lower (F = 3.738, p = 0.03; Table 2).

The analysis of interaction between sites (control vs. impact data) indicated that in some instances differences between the two reaches existed before the experimental addition of the nutrients. Nevertheless,
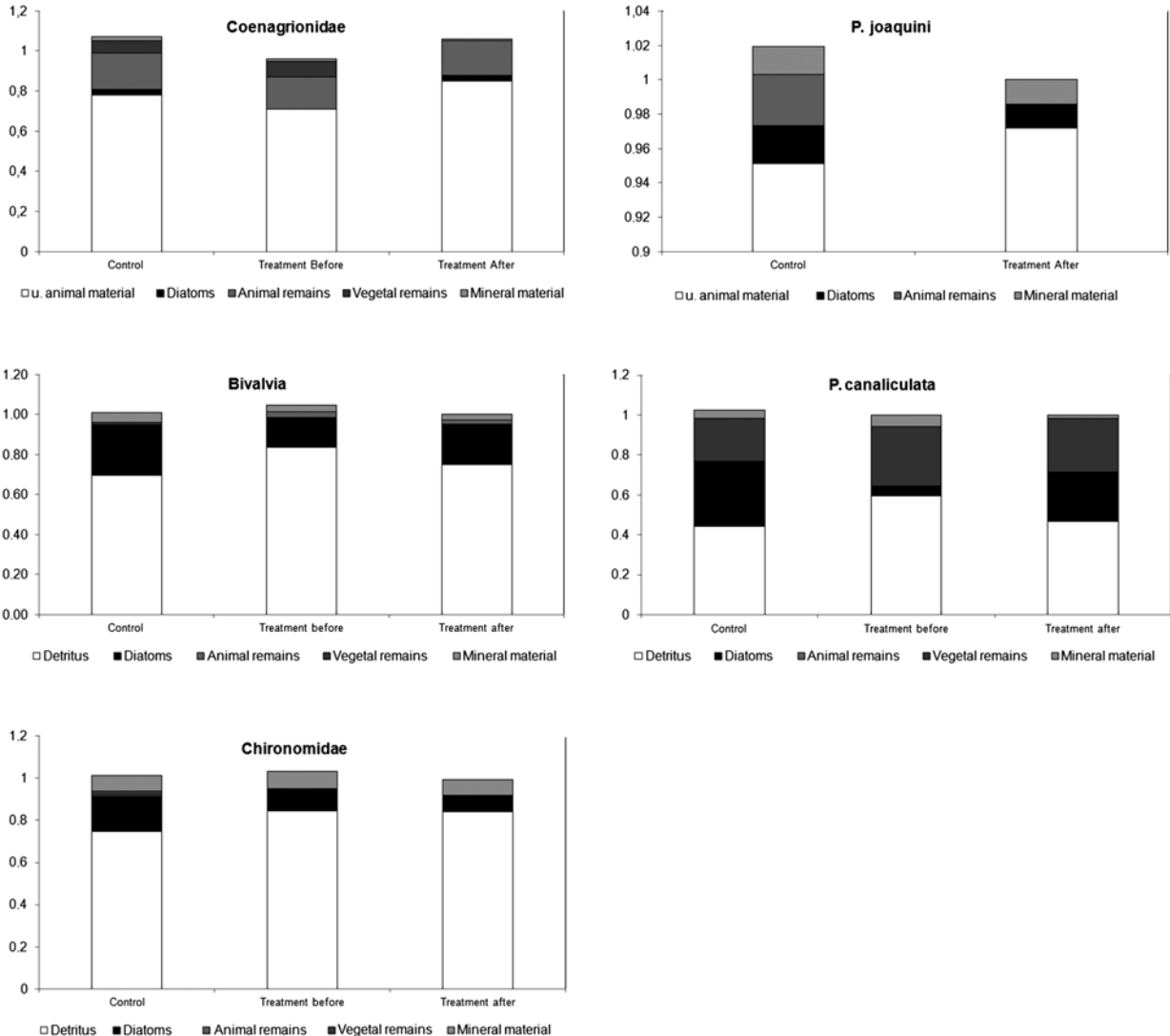
Fig. 3. Mean relative abundances of food items in gut contents for each of the taxa studied in the La Choza Stream.

Table 2. Comparisons made by BACI method for each taxon studied and for each food item. Only statistically significant values are shown. n.s.: not significant.

Comparisons	Detritus	Diatoms	Vascular plants	Animal matter	Mineral material
Chironomidae					
Before-After	n.s.	n.s.	n.s.	n.s.	n.s.
Control-Impact	$F = 302.3$, $p = 0.000$	n.s.	n.s.	n.s.	$F = 22.0$ $p = 0.019$
Before-After * Control-Impact	n.s.	n.s.	n.s.	n.s.	n.s.
Times (Before-After)	n.s.	n.s.	n.s.	n.s.	n.s.
Control-Impact * Times (Before-After)	$F = 9.7$, $p = 0.000$	$F = 16.9$, $p = 0.000$	n.s.	n.s.	$F = 7.4$ $p = 0.000$
Bivalvia					
Before-After	n.s.	n.s.	n.s.	n.s.	n.s.
Control-Impact	n.s.	n.s.	n.s.	n.s.	n.s.
Before-After * Control-Impact	n.s.	n.s.	n.s.	n.s.	n.s.
Times (Before-After)	n.s.	n.s.	n.s.	n.s.	n.s.
Control-Impact * Times (Before-After)	$F = 47.8$, $p = 0.000$	$F = 62.7$, $p = 0.000$	–	–	n.s.
Odonata					
Before-After	n.s.	n.s.	n.s.	n.s.	n.s.
Control-Impact	n.s.	n.s.	n.s.	n.s.	n.s.
Before-After * Control-Impact	n.s.	n.s.	n.s.	n.s.	n.s.
Times (Before-After)	n.s.	n.s.	n.s.	n.s.	n.s.
Control-Impact * Times (Before-After)	n.s.	n.s.	n.s.	n.s.	n.s.
Pomacea					
Before-After	n.s.	n.s.	n.s.	–	$F = 17.9$, $p = 0.014$
Control-Impact	$F = 8.4$, $p = 0.006$	n.s.	n.s.	–	$F = 9.3$, $p = 0.009$
Before-After * Control-Impact	$F = 4.5$, $p = 0.037$	n.s.	n.s.	–	n.s.
Times (Before-After)	$F = 105.9$, $p = 0.009$	n.s.	n.s.	–	n.s.
Control-Impact * Times (Before-After)	n.s.	$F = 3.7$, $p = 0.03$	n.s.	–	n.s.
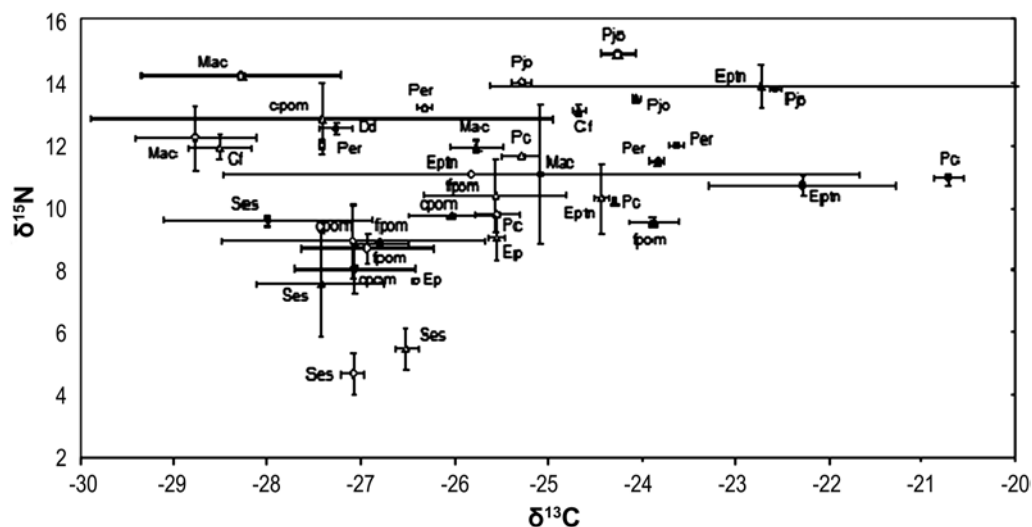
Fig. 4. Stable-isotope (δ^{15}N and δ^{13}C) values for basal resources and for each species analyzed. Symbols: Circles: control, Triangles: treatment, white symbols: before, black symbols: after, Per – *Perithemis* sp., Pjo – *P. joaquini*, Pc – *P. canaliculata*, Cf – *C. fluminea*, Dd – *D. delodontus*, Ses – seston, COPM – coarse organic particulate matter, FOPM – fine organic particulate matter, Ep – epipelon, Eptn – epiphyton, Mac – macrophytes.

the changes recorded with the Chironomidae and the Bivalvia for the gut items detritus and diatoms and with *P. canaliculata* for diatoms after the fertilization can be attributed only to that intervention (e.g., in the BACI analysis: the interaction between the control and impact data compared for the before and after time points). With the Odonata, however, significant differences were never observed between the two sites (Table 2).

According to the quantitative analysis of the stable isotopes (Fig. 4), $\delta^{15}N$ indicated that the Odonata were always predators although the insects of that order also had a tendency to decrease in $\delta^{15}N$ signatures in the treatment site after fertilization perhaps the consumption of less enriched preys. The Student t test showed that with respect to this shift a significant difference had occurred with *Perithemis* sp. (t = −2.21, p = 0.03) at that site after nutrient input, though not with *P. joaquini* (t = −1.16, k > 0.05). For bivalves, which tend to be detritivores, we found evidence of the consumption of animal matter (*C. fluminea*, t = 4, p = 0.02). The mixing model results showed the importance of detritus (CPOM mainly) for both species of bivalves (Fig. 5). *P. canaliculata*, however, exhibited a more herbivorous behavior, albeit with some tendency to consume detritus. These differences, however, were not significant (t = 0.96, p > 0.05). The mixing model results for this species showed that before fertilization the more abundant item was epipelon in the control site and detritus in the treatment site. After nutrient addition in both sites the more abundant item was macrophytes (Fig. 6). With respect to $\delta^{13}C$ the majority of the organisms studied were related with detritus (coarse and fine particulate organic matter) as basal resource, whereas the filterers *D. delodontus* and *C. fluminea* (before fertilization) and the predator *Perithemis* sp (control site, before fertilization) were related with suspended ultrafine particulate organic matter (seston). In a similar way *P. canaliculata* (control, after fertilization) was related with epiphyton. For stoichiometric ratios of primary producers who responded to treatment through an increase in their P content and a decline of the stoichiometric ratios were epiphyton (< C:N), epipelon (< C:N, C:P and N:P), CPOM (< C:P and N:P) and FPOM (< C:N) (Table 3).

Discussion

At both the experimental and control sites a similar pattern with respect to the abundance of the FFGs was maintained before fertilization, as characterized by a predominance of gathering collectors. This pattern continued at the control site for the duration of the study period, whereas the treatment site exhibited
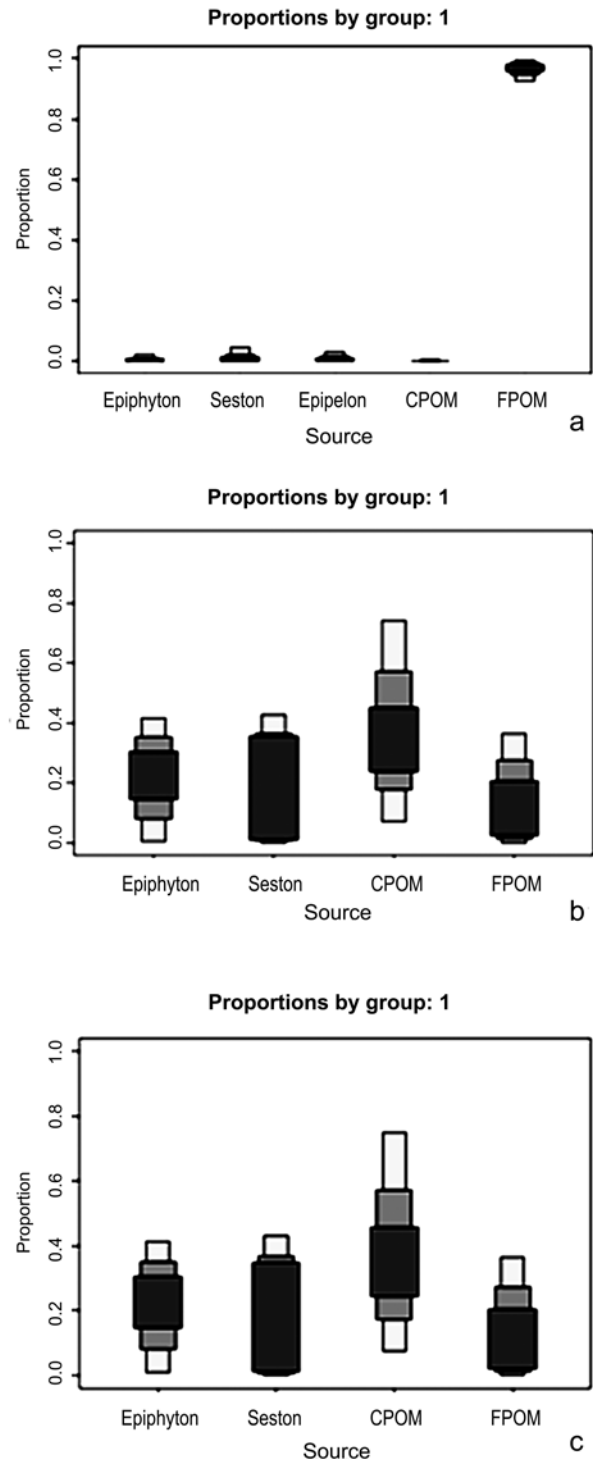
Fig. 5. Boxplot of the proportions of different sources for Bivalvia species. **a)** *C. fluminea* in treatment site, before fertilization, **b)** *C. fluminea* in treatment site, after fertilization, **c)** *D. delodontus* in control site, after fertilization.

Table 3. Mean and standard deviation of stoichiometric ratios for basal resources in the La Choza stream. Decreases of the stoichiometric ratios related with treatment are shown in bold.

	Site	C:N Mean	C:N SD	C:P Mean	C:P SD	N:P Mean	N:P SD
Macrophyte							
Before	Control	17.588	4.875	557.683	184.267	32.879	11.692
Before	Treatment	–	–	–	–	–	–
After	Control	14.324	2.591	243.681	79.736	16.890	4.484
After	Treatment	14.238	1.037	267.021	22.871	18.768	1.235
Epiphyton							
Before	Control	11.644	–	456.316	–	39.190	–
Before	Treatment	**13.349**	–	402.849	–	30.178	–
After	Control	12.473	1.423	132.607	51.448	10.878	4.648
After	Treatment	**9.200**	0.898	93.726	10.368	10.182	0.353
Epipelon							
Before	Control	14.742	14.742	489.256	0.000	33.187	0.000
Before	Treatment	**14.538**	1.961	**714.120**	385.709	**50.076**	27.483
After	Control	50.346	–	3044.407	–	60.470	–
After	Treatment	**12.041**	1.111	**433.142**	321.772	**36.841**	29.733
Seston							
Before	Control	7.912	0.609	215.185	20.533	27.175	0.708
Before	Treatment	8.094	0.486	232.932	24.253	28.741	1.270
After	Control	8.294	0.344	124.462	20.714	14.958	1.854
After	Treatment	11.217	1.330	155.482	56.764	14.206	6.087
CPOM							
Before	Control	16.220	4.883	2.544	1.484	0.150	0.074
Before	Treatment	9.751	3.855	**4.070**	0.694	**0.459**	0.171
After	Control	23.586	0.568	5.872	0.219	0.249	0.008
After	Treatment	17.772	0.210	**2.374**	0.101	**0.134**	0.004
FPOM							
Before	Control	12.282	0.428	2.467	1.043	0.199	0.079
Before	Treatment	**11.769**	1.531	**1.579**	0.075	**0.135**	0.011
After	Control	11.104	1.809	1.125	0.129	0.102	0.008
After	Treatment	**8.266**	0.870	**1.966**	0.422	**0.238**	0.044

an increase in the proportions of other groups, principally the filtering collectors; with these organisms feeding mainly on fine or ultrafine organic matter and on diatoms that had been enriched by the addition of the nutrients.

The experimental addition of nutrients to this pampean stream caused slight changes in the diet of some invertebrates. Detritus was an abundant component of the gut contents in most of the taxa studied. Several reports have established that detritus is a key source of nutrition for lotic food webs (e.g., Cummins et al. 1966, Hildrew et al. 1985, Closs & Lake 1994, Acuña et al. 2005) in agreement with our results in the La Choza Stream. Motta & Uieda (2004) concluded that the stability of the trophic structure of the insect community in Ribeirão do Atalho (Brazil) may be related to the great prevalence of organic matter as a food re-

source for insects. Thus, organic matter may be a significant source of nutrients – and one of limitless availability – for the various communities of tropical and subtropical streams. Shieh et al. (2002) asserted that, with the exception of predators, all the invertebrate taxa consume a higher amount of amorphous detritus than any other type of food. This food resource would be a dietary supplement – and one that would be very advantageous in nutritionally unstable environments. The detritus from the treatment site had a higher nutritional quality after the nutrient addition (Feijóo et al. 2012) as had been previously observed by Sabater et al. (2011). In the studied stream the detritus is a mixture of epipelon, epiphyton and macrophytes, with the largest contribution from algae.

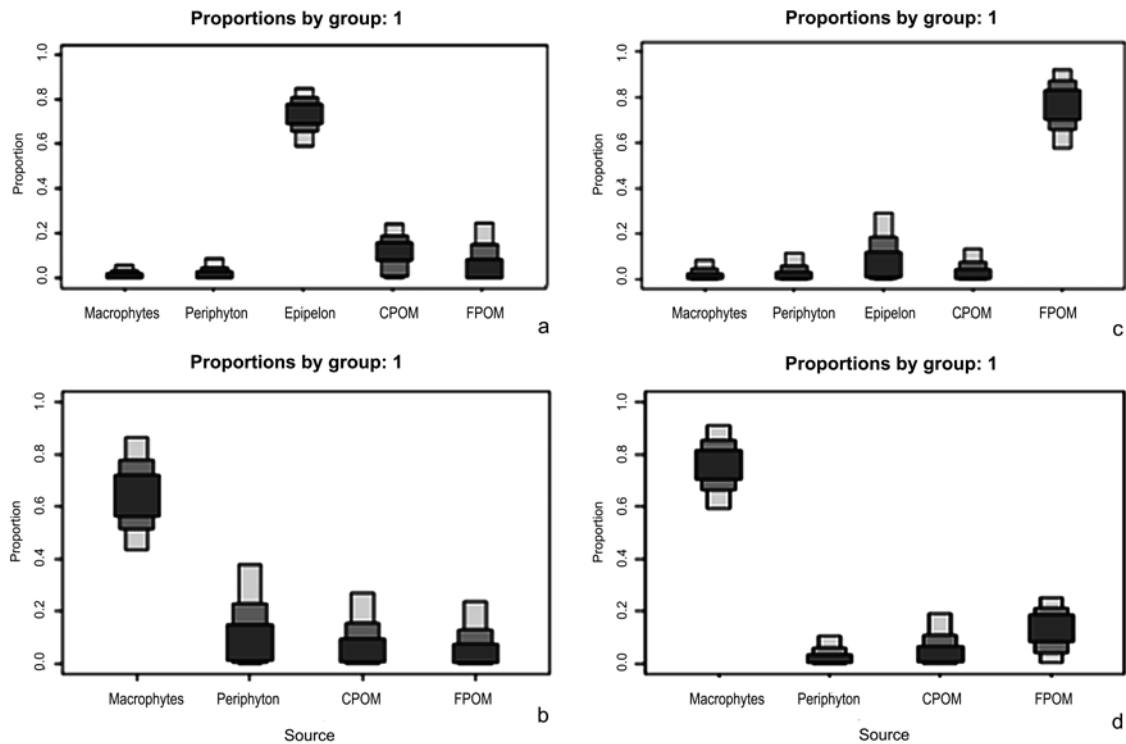Shieh et al. (2003) demonstrated that the relative prevalence of animal prey in the diet of macroinverte-

Fig. 6. Boxplot of the proportions of different sources for *P. canaliculata*. **a)** control site, before fertilization, **b)** control site, after fertilization, **c)** treatment site, before fertilization, **d)** treatment site, after fertilization.

brates decreased with increasing environmental stress (increases of nutrients concentration), whereas under the same circumstances the occurrence of diatoms increased. In our study, there were no significant differences in predator gut contents with respect to animal matter between the two experimental situations (BACI analyses). Measurement of $\delta^{15}N$, however, indicated differences in the general diet of some predatory species. Furthermore, our results indicated that – after the enrichment – detritus, rather than diatoms, became the most abundant basal food-web resource of all the FFGs. Nevertheless, the gut diatom content did exhibit differences, but only for *P. canaliculata* (a scraper) and *C. fluminea* (a filterer), after the fertilization. Flexibility in the mode of feeding no doubt facilitates the adaptation of aquatic insects to changes in food availability within a given environment (Motta & Uieda 2004). Accordingly, Mihuc & Mihuc (1995) stressed that observed food habits do not necessarily reflect an accurate assessment of fundamental functional trophic relationships in benthic invertebrates since most taxa may function as generalists, if necessary, even when reported as specialists. Consequently, Albariño & Díaz Villanueva (2006) indicated that a generalist strategy would be better adapted during unpredictable changes

in environmental conditions when specific resources may become unavailable. Because of this, the stable isotopes analyses are a useful tool to determine that the organisms are really assimilating.

C. fluminea is capable of both filter-feeding and pedal-feeding – i.e., drawing from both the water column and the bottom sediments, respectively, for nutrition (Wittman et al. 2008). In our study, the dominant item was detritus, with a particle size ranging from 10 to 350 µm and corresponding to fine-to-ultrafine material, with various diatoms being well represented. In the example of *D. delodontus*, the gut contained principally ultrafine detritus and minerals at the control site. The statistical analyses (BACI) revealed significant differences in the proportions of detritus and diatoms within the gut contents of the Bivalvia, with this difference being attributable to the fertilization. Since, however, *C. fluminea* and *D. delodontus* are two different bivalve species; the results do not necessarily allow a definitive conclusion.

In the present study, the gut contents of the Chironomidae larvae contained detritus, minerals, and diatoms, and although fertilization resulted in a general preference for the ingestion of detritus in this group, the size of the ingested material was always between

0.5 µm and 1 mm – in agreement with the findings of Chessman (1986) and Henríquez de Oliveira et al. (2003). These organisms are usually classified as gathering collectors, as has been seen in our study, but the FFGs of scrapers, predators, and shredders are also represented within this family. This confirms that the FFG approach is not always a better metric for evaluating the feeding habit of macroinvertebrates.

P. canaliculata are extremely polyphagous snails, feeding on vegetal, detrital, and animal matter. This species is primarily macrophytophagous, preferring floating or submerged plants (Estebenet & Martín 2002) and our observations also show that their gut contents contained conspicuous vegetal matter and periphytic organisms. Nutrient addition furthermore changed the proportions of the food items in the diet of this species resulting in a significant decrease in diatom content at the treatment site.

Bottom-up effects of nutrient enrichment can stimulate consumer biomass as a result of increased primary production, but also through detrital-microbial pathways (Cross et al. 2005, Cross et al. 2007). Because bacteria are organisms with a high affinity for phosphorus (Coveney & Wetzel 1992), these microbes may react to nutrient enrichment. Accordingly, Sabater et al. (2011) found an increase in bacterial density and activity after nutrient addition in their comparable experimental study. This increase can mean a more balanced elemental content (e.g., C, N, and P) of the detritus that would alter the nutritional relationship between the producers and the consumers (Sterner & Elser 2001) in favor of the consumption of detritus by the latter. In our study the artificial fertilization caused increases in the quantity and quality (decreases in stoichiometric ratios) of some primary resources so as to produce a bottom-up effect in this manner. Our results are consistent with those of Gómez et al. (2009), who found that the microalgae associated with the sediment responded significantly to the addition of nutrient by doubling their biomass. Those authors also found changes in the structure of the diatom taxocenosis along with an increase in their density in response to the fertilization of the La Choza Stream.

The statistical analyses indicated significant differences between the status of the stream before and after fertilization with respect to some of the observed items in the gut contents of certain taxa. Although, contrary to expectations, the nitrogen levels in the water did not become elevated after fertilization during the experiment, the phosphorus loading of this lotic system doubled. The decreases in nitrogen concentrations were due to denitrification processes that occur in this water course principally at night (Acuña et al. 2011). This phosphorus enrichment affected the responses of the organism present in terms of both the feeding preferences and the proportions of the food items ingested. A general increase in detritus ingestion was observed in the scrapers and the collectors. The trophic structure also exhibited changes, in terms of the proportions of the different FFGs undergoing notable variations. Slavik et al. (2004) found that densities of invertebrate taxa such as certain Chironomidae and Ephemeroptera increased in a fertilized reach while filterer densities were higher in the reference reach. Alterations in resource supply have been shown to dramatically change trophic dynamics of streams in arctic tundra (Peterson et al. 1993) and those in forested streams (Wallace et al. 1999). According to Cross et al. (2007) a longer period of fertilization would be needed to confirm this conclusion statistically since effects on community composition require at least three years of continuous fertilization, as was previously observed. However, effects may be seen in the short term when it comes to sensitive taxa or assemblages of primary producers even in eutrophic environments (Gómez et al. 2009), but especially in environments with low nutrient concentration (Slavik et al. 2004, Sabater et al. 2011).We can therefore conclude that the premise on which the study was based – i.e., that the artificial fertilization would result in dietary changes in the scrapers and detritivores – proved valid since the effects on those biota were, in fact, observed, and can even be extended to the predators. These results are meaningful in constituting a first approximation of the functional responses of macroinvertebrates to the environmental alterations expected within the context of global land-use change in temperate plain streams of South America.

Acknowledgements

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