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Headwater streams: neglected ecosystems in the EU Water Framework Directive. Implications for nitrogen pollution control

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ABSTRACT

The European Union Water Framework Directive (WFD) aims to achieve the ''good status'' of waters by 2015, through monitoring and control of human impacts on ''bodies of surface water'' (BSWs), discrete elements for quality diagnosis and management. Headwater streams, however, are frequently neglected as they are not usually recognised as BSW. This poses limitations for the management of river catchments, because anthropogenic impacts on headwaters can constrain the quality of downstream rivers. To illustrate this problem, we compared nitrate levels and land use pressures in a small agricultural catchment with those recorded in the catchment in which it is embedded (Ega), and in the Ebro River Basin (NE Spain) comprising both. Agriculture greatly influenced water nitrate concentration, regardless of the size of the catchments: $R^2 = 0.91$ for headwater catchments (0.1–7.3 km^2), and $R^2 = 0.82$ for Ebro tributary catchments (223–3113 km^2). Moreover, nitrate concentration in the outlet of a non-BSW small river catchment was similar to that of the greater downstream BSW rivers. These results are of interest since, despite representing 76% of the length of the Ega catchment hydrographical network, only 3.1% of the length of the headwater streams has been identified as BSWs. Human activities affecting headwater streams should therefore be considered if the 2015 objective of the WFD is to be achieved.

1. Introduction

The global biogeochemical cycle of nitrogen (N) has been deeply altered, and the boundary within which humankind can operate safely has long been crossed (Rockstrom et al., 2009). N point and diffuse sources, such as human sewage, atmospheric deposition and agriculture, increase N loads in streams, resulting in the cultural eutrophication of aquatic ecosystems (Camargo and Alonso, 2006). Agriculture is currently recognised as the main driver of N pressures in many of the major catchments of the European Union (EU) (EEA, 2005; Grizzetti et al., 2008).

River drainage networks are hierarchically organized systems in which 1st and 2nd order streams, commonly referred as headwater streams, make up at least 70% of total stream length (Leopold et al., 1964; Meyer, 2007). Most of the N flowing through the whole hydrological network is estimated to come from the headwater catchments (Alexander et al., 2007). By contrast, significant in-stream retention of this transported N can occur in the headwater streams due their intense water–streambed interaction (Alexander et al., 2000; Peterson et al., 2001; Wollheim et al., 2001; Martí et al., 2006). Furthermore, there is a high degree of permanent loss of this N to the atmosphere through denitrification (Mulholland et al.,

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2009). In addition to the important role played by headwater streams in N cycling, they provide other important ecosystem services (Lowe and Likens, 2005). Headwater streams, however, particularly those in agricultural areas, are subjected to different pressures such as channelling, impoundment or burial (Veliz and Richards, 2005; Freeman et al., 2007).

Since 1972, legislation in the United States of America protects the nation's navigable waters under the Clean Water Act (CWA). At first, any tributary of a navigable-in-fact river was protected, but this consideration changed in 2001. After a 5-year debate, it was considered that the CWA must protect all waters with a "significant nexus" with the hydrological system (Nadeau and Rains, 2007; Leibowitz et al., 2008). In the European Union (EU), the Water Framework Directive (WFD; 2000/60/EC) aims to achieve the "good status" of EU waters by 2015 through the implementation of measures at basin scale by means of River Basin Management Plans (RBMPs). The WFD requires the identification of "bodies of surface water" (BSWs) as discrete and significant elements to be used for quality diagnosis and management. Due to their small size, headwater streams are not usually identified as BSWs and are therefore excluded from the measures implemented by RBMPs, regardless of their vulnerability to anthropogenic activities and their significant influence on downstream water quality.

Herein we present a case study focusing on a small catchment of the Ebro River affected by N pollution caused by agriculture. Study of such specific cases is necessary with regard to highlighting the importance of headwater streams in the management of water quality at large catchment level, in order for them to be considered in future RBMPs. The Ebro River Basin (NE, Spain) is the largest Spanish catchment. Similarly to other basins in EU countries, control of point source effluents has efficiently reduced P levels in surface waters, whereas less success has been had in abating the concentration of N compounds (Ibáñez et al., 2008), particularly nitrate, which is often the major constituent of the N pool in rivers. A study considering the overall Ebro River Basin (Lassaletta et al., 2009) recently reported that nitrate pollution has increased in many of the monitored sites of the Ebro River Basin over the last few decades. Moreover, agricultural land cover was closely related to nitrate concentrations recorded at different sites across the basin. These results could lead to non-compliance with WFD aims in 2015. From the management perspective it is important to ascertain the impact of headwaters upon downstream water quality. For this reason, we have selected a small agricultural catchment to compare N levels and pressures in surface waters with those observed at a higher scale.

The following hypotheses were contrasted: (1) stream nitrate response to agriculture varies with the spatial scale considered; (2) stream nitrate levels at the mouth of this small catchment (non-BSW) are similar to those observed downstream at sites (BSW) also influenced by agricultural activities. The hydrographical network of the Ega River Catchment was classified according to stream hierarchies and to considerations of the WFD. The aim of this study is to assess whether current EU legislation adequately considers the significance of headwater streams in the achievement of specific water quality targets.

2. Headwater streams in the EU legislation

The "good status" condition defined by the WFD is reached by a water body when both its "ecological status" and "chemical status" are considered to be at least "good". The "ecological status", a great improvement of the Directive, is an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters. Evaluation of this quality involves consideration of biological elements and other quality elements supporting these, such as physical–chemical and hydromorphological elements. Nutrient conditions are considered as an evaluative element within the classification of "ecological status".

To achieve its environmental objectives, the WFD enforces the implementation of integrated management plans (RBMPs) at the whole basin level (River Basin District). The first steps are the characterization of the River Basin District and the identification of the BSWs. Another step, previous to developing and implementing the RBMP, involves a risk assessment process. As part of this, the Competent Authorities shall collect and maintain information on the type and magnitude of the significant anthropogenic pressures to which the BSWs are liable to be subjected. Additionally, the WFD requires the identification of their susceptibility to the recognised pressures. The information on pressures and susceptibility is then used to assess the risk of failing to achieve "good status" of waters. Specific pressures highlighted in the WFD include diffuse pollution of substances contributing to eutrophication of inland and coastal waters. In particular, nitrate is one of the main pollutants considered in the identification of environmental pressures and for achieving the "good ecological status".

Since the BSWs are the basic units of assessment, identification thereof constitutes a key step for the future development of the RBMPs. Although the purpose of the WFD is to establish a framework for the protection of all water bodies, hydrographical networks include a large number of very small streams; the administrative burden of managing these waters could therefore be enormous. The smallest area range of a catchment considered by the Common Implementation Strategy of the WFD (EC, 2003) is 10–100 km^2. However, it is recognised that in some regions with many small water bodies, this general approach will need to be adapted. Thus, Member States have flexibility to decide whether the aims of the WFD can be achieved without the identification as BSWs of every minor but discrete and significant element of surface water. In any case, identification of a small element as a BSW is recommended when it is significant in the context of the WFD's purposes and objectives (e.g. if it causes significant adverse impacts on other surface waters) (EC, 2003). A total of 700 BSW has been identified for the Ebro River Basin, but no consideration is given to many small streams such as the ones draining the catchment included in this study (Galbarra Stream Catchment, with an area of 23 km^2: CHE, 2005).

In addition to the WFD, the Urban Waste Water Directive (91/271/EEC), the Drinking Water Directive (80/778/EEC) and particularly the Nitrates Directive (91/767/EEC) oblige the Competent Authorities to monitor the water N levels. The Nitrates Directive requires Member States to designate "Nitrate Vulnerable Zones" (NVZs; areas draining into nitrate-polluted groundwater), to develop action programmes within these and to establish codes of good agricultural practices (to be implemented voluntarily by farmers). Furthermore, farmers affected by the implementation of the WFD can receive subsidies and are also conditioned to the implementation of good agricultural practices if they receive subsidies from the Common Agricultural Policy. Good agricultural practices codes include fertilizer management measures.

3. Materials and methods

Herein we compare nitrate data in stream waters obtained in nested hydrological systems (Fig. 1) in order to establish whether or not stream nitrate concentration responds similarly at different scales. Our case of study centres on the Galbarra Stream Catchment (Fig. 1c), a small watershed devoted to cereal cultivation, intensively sampled by our team during 2002 and 2003. Available public data on higher spatial scales of Ega River (Fig. 1b) and Ebro tributaries and the Ebro River mouth (Fig. 1a) were obtained from the Water Quality Control Network of the Ebro Basin Confederation (Confederación Hidrográfica del Ebro: CHE), which mainly places sampling sites in river sections of great hydrographical order. Due to the extensive nature of these data, we considered a 5-year period (2001–2005) to conduct the inter-scales comparison.

3.1. *Study area and sampling sites*

The Ebro River (NE Spain), with a fluvial network of 13 049 km, discharges 9930 hm^3/y (2001–2005 average) into the Western Mediterranean Sea as a 7th order stream, after 910 km of main channel (Fig. 1a). It is the largest Spanish fluvial catchment, with a drainage area of 85 566 km^2, covering 17% of Spanish Iberian territory. The catchment is heterogeneous in terms of geology, topography, and climatology. In general, silicic materials dominate at high altitudes, while calcareous materials are found at lower elevations. The topography modulates Mediterranean climatic patterns throughout the catchment, with a distinct transition from a semiarid environment in its centre to humid conditions at its northern ranges, influenced by the Pyrenees. Mean annual precipitation ranges from 342 mm to 2188 mm and mean annual temperature ranges from 7.1 °C to 17.4 °C (1975–2000 period; http://www.chebro.es/). Agricultural and natural areas represent 48% and 50% of the total catchment area, respectively (year 2000 data from the CORINE Land Cover (CLC) Project; EEA, 2007), with a human population of circa 3 million inhabitants. Fig. 1a shows the 30 hydrologically independent sampling sites considered herein (ET sites) and their corresponding drainage areas ranging from 223 km^2 to 3113 km^2 (Table 1), with a median value of 1096 km^2. The Ebro River mouth sampling site (ER site) is also shown.

The Ega River (Fig. 1b), with a length of 112 km, is a 5th order stream and a tributary of the Ebro River with a fluvial network of 1313 km. Its catchment covers an area of 1461 km^2 with calcareous materials in its high-middle section and clays, marls and gypsums in the lowest one. Mean annual precipitation ranges from 374 mm to 1355 mm and mean annual temperature ranges from 9.7 °C to 13.9 °C (1987–2000 period; http://www.chebro.es/). Agricultural and natural areas represent 43% and 57% of the total catchment area, respectively, with a human population of circa 33 600 inhabitants. Fig. 1b shows the 3 sampling sites considered in our study (Ega-1, Ega-2 and Ega-3); their respective drainage areas are 898 km^2, 1049 km^2 and 1383 km^2, respectively (Table 1).

The Galbarra Stream (Fig. 1c and Table 1) is a 3rd order tributary of the Ega River, with a main channel of 8.6 km and a fluvial network of 26.1 km. The catchment covers an area of 23 km^2 on a calcareous shelf. It presents a transitional Mediterranean climate with an annual precipitation of 850 mm and a mean annual temperature of 12.1 °C. Of a total agricultural cover of 37% of the catchment, 90% is devoted to winter cereal cultivation in rotation (wheat, barley, forage or fallow), and natural areas represent almost all of the remaining 63%. There are 5 small villages (not identified by the CLC Project) with 213 inhabitants. Sub-catchment areas of the Galbarra Stream Catchment ranged from 0.2 km^2 to 7.3 km^2, with 0.8 km^2 of median value. They are drained by 1st and 2nd order tributaries, and therefore considered as headwater streams. Neither the Galbarra Stream nor its tributaries are identified as BSWs. One sampling site (the GS site) was set up at the mouth of the Galbarra Stream (Fig. 1c) and 9 sites were located at the mouth of Galbarra Stream tributaries (GT sites). Land uses of the tributary sub-catchments ranged from 100% natural areas to 100% agricultural cover (Table 1).

3.2. *Surface water monitoring*

We collected surface water samples in the Galbarra Stream Catchment (Fig. 1c) at the 9 GT sites and at the GS site, from January 2002 to October 2003, throughout 16 campaigns, covering the range of precipitations, periods of soil fertilization and sowing, growth and harvest times. Each sampling site consisted of a 50 m-long section of stream, along which 5 L samples of running water were collected in polyethylene containers. We measured water flow by using cross-sectional areas divided into subsections of known width and depth, and by recording water velocity at the centre of each subsection with a current meter (OTT C2 10.150, Germany). Two 1 L aliquots of sample were kept refrigerated (4 °C) in darkness until it was analyzed in the laboratory. Nitrate concentration data corresponding to the 3 Ega sites and to the 30 ET sites were provided to us by the Water Quality Department of the CHE. These corresponded to stream water samples taken periodically, between the years 2001 and 2005, at fixed sites of the Water Quality Control Network of the CHE. Sample collection, preservation and transportation were performed in accordance with APHA (1992).

3.3. *Analytical methods*

We performed analytical determinations of waters, always following good laboratory practices such as standard protocols, blank measurements, spiked and duplicated filtered samples (APHA, 1992; Hach, 1992). Nitrate (NO_3^-) and ammonium (NH_4^+) concentrations in water samples of GT and GS sites were determined in our laboratory by means of

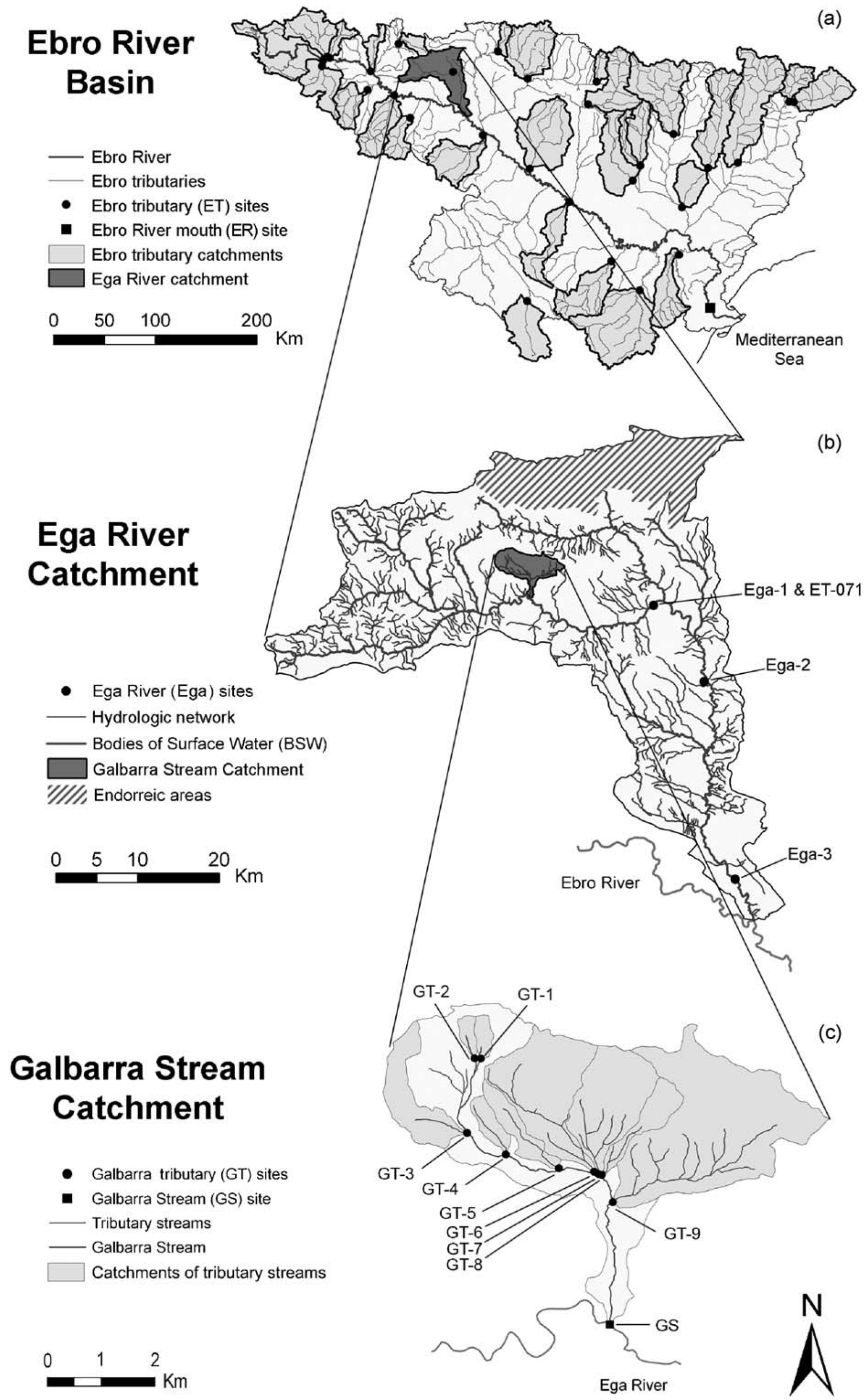

Fig. 1 – Study area and location of the sampling sites. (a) Ebro River Catchment, the 30 hydrologically independent sub-catchments considered and their respective sampling sites (ET sites) and the Ebro River mouth (ER site); (b) Ega River Catchment showing the 3 sampling sites of the Ega River considered (Ega sites); (c) Galbarra Stream Catchment, showing the sampling site at the mouth of the stream (GS site) along with the 9 sites in its tributaries (GT sites), and their respective drainage areas.

spectrophotometry and cadmium reduction and Nessler methods, respectively (Hach, 1992). Waters from Ega, ET and ER sites were determined by the Water Quality Laboratory of the CHE with ion chromatography (for further information, see Lassaletta et al., 2009). Nitrate and ammonium values are expressed as mg/L of NO_3^- and mg/L of NH_4^+, respectively.

This study focuses on nitrogen pollution and water quality policy. This implies the use of nitrate concentrations and not

Table 1 – Hydrographical characteristics, land cover information, human population and average water flows of the 9 headwater-stream tributaries of the Galbarra Stream (GT sites), the mouth of the Galbarra Stream (GS site), 3 sampling sites in the Ega River downstream GS confluence (Ega sites), sampling sites in 30 Ebro River tributaries (ET sites) and the mouth of the Ebro River (ER site).

Site code	Stream		Drainage area (km^2)	Stream order	Length of drainage network (km)	Land cover (CLC 1st level)[a]			Population		Water Flow (L/s)[b]	BSW[c]
						Agricultural areas (%)	Natural areas[d] (%)	Artificial surface (%)	Total (inh)	Density (inh/km^2)		
GT-1	Galbarra Stream tributary		0.2	1	0.1	1.2	98.8	0	0	0	5 ± 3.1	No
GT-2	Galbarra Stream tributary		0.2	1	0.5	0.1	99.9	0	0	0	8 ± 3.4	No
GT-3	Galbarra Stream tributary		1.4	1	1.1	35.6	64.4	0[e]	63	44	20 ± 9.7	No
GT-4	Galbarra Stream tributary		0.4	1	0.8	100	0	0	0	0	4 ± 2.4	No
GT-5	Galbarra Stream tributary		0.7	1	0.9	100	0	0	0	0	9 ± 4.5	No
GT-6	Galbarra Stream tributary		3.1	1	3.2	42.5	57.5	0[e]	31	10	34 ± 20	No
GT-7	Galbarra Stream tributary		2.5	1	0.7	52.9	47.1	0[e]	26	10	34 ± 15	No
GT-8	Galbarra Stream tributary		0.8	1	0.2	45.6	54.4	0	0	0	8 ± 4.7	No
GT-9	Galbarra Stream tributary		7.3	2	9.1	25.2	74.8	0[e]	45	6	48 ± 26	No
GS	Galbarra Stream		23	3	26.1	37.1	62.9	0[e]	213	9	272 ± 136	No
Ega-1	Ega River (071)[f]		898	5	704.8	34.6	65	0.5	14 335	16	8878 ± 1963	Yes
Ega-2	Ega River (239)[f]		1049	5	954.9	30.3	69.1	0.6	25 350	24	na	Yes
Ega-3	Ega River (003)[f]		1383	5	1302.9	42.9	56.5	0.6	33 560	24	12 412 ± 1642	Yes
ET	Ebro tributaries ($n = 30$)	Min	223	nc	nc	2.2	7.7	0.1	628	2	600 ± 100	Yes
		Max	3113	nc	nc	91.2	97.1	2.7	68 366	126	27 001 ± 5193	Yes
ER	Ebro River		83 281[g]	7	>10 000[g]	48.0	50.2	1.1	3×10^6 [g]	34[g]	272 702 ± 60 285	Yes

na: not available data; nc: not considered data.
[a] Data of classes at 1st level of CORINE Land Cover Project (CLC) in year 2000.
[b] Average flow for the study period ± standard error: 2002–2003 period for GT and GS sites, and 2001–2005 period for Ega, ET and ER sites.
[c] BSW is body of surface water (WFD).
[d] Natural areas: forest and semi-natural areas in CLC nomenclature.
[e] Five small villages are not recognised in the CLC data.
[f] Original code of the site in the Water Quality Control Network of the Ebro Basin Confederation (CHE).
[g] Data from WFD document (CHE, 2005).

fluxes. In order to clarify the source of the nitrate, however, its instant yield (mg s^{-1} km^{-2}) at GT sites was calculated by multiplying measured concentration and instant water flow and by dividing by the corresponding site drainage area.

3.4. Geographic Information System

All the geographic data were processed using the GIS software ArcGIS. Drainage areas upstream from the sampling sites were delimited by means of topographic data layers at a scale of 1:50 000 for the macro-level of the Ebro River Basin and the Ega River Catchment (http://oph.chebro.es) and a scale of 1:25 000 for the Galbarra Stream Catchment (CNIG, 2009). The hydrographical network, stream orders (Strahler, 1957) and lengths were determined for the entire Ega catchment, with the use of the topographic data layers 1:25 000, the closest scale to the 1:24 000 scale recommended by Leopold et al. (1964). Land use maps for the year 2000 from the CLC Project (EEA, 2007) were used to calculate the cover (expressed as %) of every land use class in each catchment at the first level of CLC classification. This level includes five categories: (1) artificial surface; (2) agricultural areas; (3) forest and semi-natural areas; (4) wetlands; and (5) water bodies.

3.5. Statistical analysis

To test the effect of agricultural land cover on stream nitrate concentrations, we constructed two linear regression models considering nitrate as the response variable and percentage of agricultural cover in the drainage area as the predictor variable. The first model was developed with nitrate average data on each GT site at meso-scale level, and the second model with nitrate average data of each ET site corresponding to macro-scale level. Next, the two models were compared to test the homogeneity of their slopes by means of a parallelism test. In this test, which is a part of the analysis of covariance designs, the null hypothesis implies that the slopes of both regression models are similar (Wildt and Ahtola, 1987). Another model was performed to confirm the agricultural origin of nitrate pollution in Galbarra streams using nitrate yield in GT sites as the response variable and the same predictor variable as the previous models. In order to evaluate whether differences in nitrate concentration existed between the Galbarra Stream mouth (GS) and the Ega River sites (Ega-1, -2 and -3), downstream GS, a one-way ANOVA was performed (Zar, 1998).

Additionally, the available data recorded from 1981 to 2005 at the 3 Ega sites were used to detect significant historical trends in nitrate concentrations ($n = 50$ for Ega-1, $n = 38$ for Ega-2 and $n = 49$ for Ega-3). The non-parametric Seasonal Kendall test (SK test) (Hirsch et al., 1982) was performed following Lassaletta et al. (2009). The MsExcel® tool developed by Libiseller (2004) was used to perform the SK test; a similar tool was developed to estimate the corresponding slopes. With the exception of trend analysis, we performed all the statistical analyses using the computer programme STATISTICA 6.0 (StatSoft Inc., 2001), and establishing 0.05 as the critical significance level.

4. Results

Nitrate was the dominant form of Dissolved Inorganic Nitrogen (DIN). None of the sites presented an averaged nitrate/ammonium molar ratio lower than 1 (Table 2). With the exception of GT-1 and GT-2, with no agriculture or urban presence in their catchments, nitrate was 1 or 2 orders of magnitude higher than ammonium. GT-3 was the only site with an ammonium mean concentration higher than 0.15 mg/L, which was due to the wastewater effluent from a small

Table 2 – Nitrate and ammonium concentrations (expressed as mg/L) and molar ratios of nitrate/ammonium in waters of the 9 headwater-stream tributaries of the Galbarra Stream (GT sites), the mouth of the Galbarra Stream (GS site), 3 sampling sites in the Ega River downstream GS confluence (Ega sites), sampling sites in 30 Ebro River tributaries (ET sites) and the mouth of the Ebro River (ER site).

Site code	n	NO_3^- (mg/L)						NH_4^+ (mg/L) mean ± SE[a]	NO_3^-/NH_4^+ mean ± SE[a]
		Mean ± SE[a]	Minimum	1st quartile	Median	3rd quartile	Maximum		
GT-1	10	0.5 ± 0.1	0.2	0.3	0.4	0.4	1.3	0.15 ± 0.06	1 ± 0
GT-2	10	0.4 ± 0.0	0.1	0.4	0.4	0.4	0.4	0.08 ± 0.02	2 ± 1
GT-3	10	11.7 ± 1.9	1.8	8.5	10.5	15.7	20.8	2.67 ± 0.96	14 ± 6
GT-4	10	35.9 ± 2.2	29.3	31.9	34.2	39.1	45.6	0.05 ± 0.01	255 ± 60
GT-5	10	33.3 ± 2.7	19.4	25.3	36.9	39.1	44.8	0.06 ± 0.01	217 ± 51
GT-6	10	13.6 ± 3.4	0.9	7.8	9.4	21.8	30.7	0.10 ± 0.02	57 ± 17
GT-7	10	14.0 ± 1.9	6.7	10.0	11.8	16.4	24.2	0.07 ± 0.01	93 ± 30
GT-8	10	13.1 ± 2.2	4.8	7.8	11.8	18.1	26.5	0.05 ± 0.01	67 ± 15
GT-9	10	18.0 ± 3.0	7.9	12.8	18.0	21.7	31.3	0.08 ± 0.03	98 ± 26
GS	13	15.4 ± 1.7	7.0	10.7	11.8	19.4	28.0	0.11 ± 0.02	171 ± 35
Ega-1	11	18.9 ± 2.6	8.6	13.2	17.2	22.5	40.1	0.07 ± 0.01	83 ± 10
Ega-2	6	14.6 ± 1.5	10.3	11.3	14.8	17.3	19.2	0.33 ± 0.16	20 ± 11
Ega-3	18	16.9 ± 1.2	7.1	14.4	15.9	20.0	24.7	0.07 ± 0.00	86 ± 9
ET[b]	30	11.6 ± 1.8	1.4	3.4	9.7	16.0	42.4	0.27 ± 0.12	38 ± 5
ER	59	10.5 ± 0.3	6.2	11.8	10.1	11.8	17.2	0.12 ± 0.02	40 ± 2

[a] SE: standard error of the mean.
[b] ET data correspond to the 30 nitrate means of the ET sites, which were calculated for the whole data population.

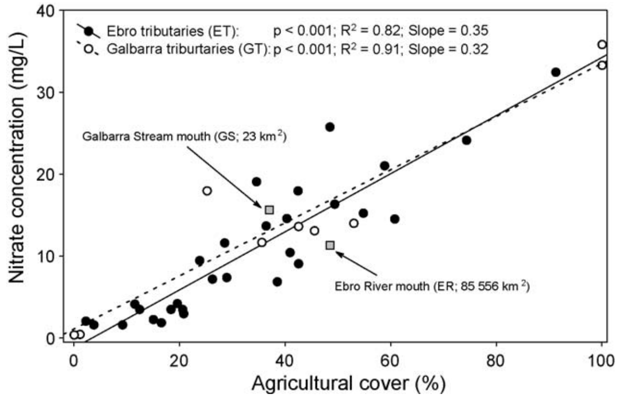

Fig. 2 – Comparison of the two relationships established between agricultural cover of the drainage area and nitrate concentration in stream waters: the first at meso-scale level of headwater sub-catchments (Galbarra Stream tributary streams; GT sites; *n* = 9) and the second one at macro-scale level of Ebro sub-catchments (Ebro River tributary streams; ET sites; *n* = 30). Data on Galbarra Stream (GS site) and Ebro River mouths (ER site) have been included in the representation but not in the two regression analyses.

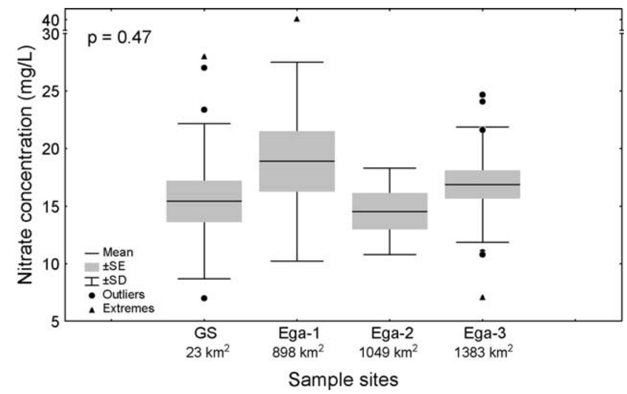

Fig. 3 – Mean, standard error (SE) and standard deviation (SD) of nitrate concentrations in surface waters of Galbarra Stream mouth (GS site) and the 3 sites of Ega River (Ega sites); outlier and extreme values are shown. *p* value corresponds to the one-way ANOVA performed to compare nitrate concentrations.

village upstream from the sampling site. However, nitrate was the predominant form in most cases. Only GT-1 and GT-2 presented a nitrate mean concentration close to the background concentration of 0.44 mg/L proposed by Meybeck (1982) for major unpolluted rivers. Moreover, with the cited exceptions, the rest of the sites exceeded 8.8 mg/L, which is the threshold proposed by Camargo et al. (2005) for protecting the most sensitive freshwater species.

The nitrate mean concentrations of the GT sites and ET sites were closely related to the agricultural cover of their drainage areas ($R^2 = 0.91$ and $p < 0.001$ for GT sites, and $R^2 = 0.82$ and $p < 0.001$ for ET sites). Fig. 2 shows how the nitrate mean concentration of both catchment sizes (0.2–7.3 km^2 and 223–3113 km^2 for GT and ET sites, respectively) responded similarly to agricultural cover. Although the variance explained by the regression model for GT sites was slightly higher, the slopes of both models were similar (parallelism test, $p > 0.05$). Nitrate mean concentrations not included in the models, corresponding to the mouths of the Galbarra Stream and the Ebro River (GS site and ER site, respectively), were incorporated into Fig. 2, and exhibited similar responses.

Mean instant nitrate yield at the GT sites was also strongly related to agricultural cover ($R^2 = 0.98$; $p < 0.001$). Moreover, the calculated instant yields at the GT sites were highly variable, being highest in winter (mean value, 261.8 mg s^{-1} km^{-2} of nitrate) and lowest in summer (mean value, 0.8 mg s^{-1} km^{-2} of nitrate). As a result, export from the GS to the Ega River was also very variable (from 0.1 mg s^{-1} km^{-2} to 244 mg s^{-1} km^{-2} of nitrate).

The one-way ANOVA (Fig. 3) indicated no significant difference in nitrate concentration among the GS, Ega-1, Ega-2 and Ega-3 sites ($F_{3, 46} = 0.85$, $p = 0.47$). In none of these sites did the mean of nitrate concentration surpass 20 mg/L of nitrate, the threshold currently adopted by the CHE (2008) to achieve the "good ecological status" of its BSWs; nonetheless, this value was occasionally exceeded in some observations.

A significant increasing historical trend in nitrate concentration (1981–2005 period) was detected in Ega-1 and Ega-3 ($p < 0.01$; their slopes were 0.39 and 0.40 mg L^{-1} y^{-1} of nitrate, respectively), whereas no significant trend was found for Ega-2 ($p = 0.051$). If these two sites steadily increase their nitrate

Table 3 – Number of streams, total, mean and relative stream lengths by order for the Ega River Catchment and their percentage of inclusion as a BSW or situation in a NVZ.

Stream order	Total streams (*n*)	Total length (km)	Mean length (km)	Relative length (%)	Total length as BSW[a] (%)	Total length in NVZ[b] (%)
1	574	737	1.3	56	1.2	0
2	156	263	1.7	20	8.4	0
3	33	129	3.9	10	60.8	0
4	9	72	7.9	5	98.3	0
5	1	112	–	9	100	0
Hydrographical network	773	1313	1.7	100	22.3	0

[a] Percentage of the total length of each order considered as a body of surface water (BSW) by the WFD.
[b] Percentage of the total length of each order situated in a Nitrate Vulnerable Zone (NVZ).

concentrations according to the estimated slope of the trends, in 2015 they will reach 23.3 mg/L and 24.5 mg/L of nitrate, respectively, surpassing the threshold established by the CHE.

Table 3 summarizes information on the stream orders of branching that constitute the Ega River network. Within the Ega River Catchment (Fig. 1b), 773 river sections were identified, with a combined distance of 1313 km. 1st and 2nd order streams present a mean length of 1.3 km and 1.7 km, respectively; whereas the 5th order main channel measures 112 km. A total of 76% of stream network length corresponds to 1st and 2nd order streams, while the 1st order streams alone represent 56% of stream length. Only 3.1% of the whole length of headwater streams was identified as BSW according to the WFD. Finally, only 22.3% of the Ega River network length falls under the BSW category. No Nitrate Vulnerable Zones have been declared in the Ega River Catchment.

5. Discussion

The response of nitrate concentration in surface waters to the agricultural cover of the catchment was similar regardless of spatial scale. The strong relationship between nitrate levels and agricultural cover found in Galbarra headwater catchments has also been reported in several studies conducted at different scales (Liu et al., 2000; Kyllmar et al., 2006; Lassaletta, 2007; Broussard and Turner, 2009). Mean instant yield of nitrate was also closely related to agricultural cover in the Galbarra headwater streams, corroborating the agricultural origin of fluvial N. Moreover, the observed seasonal oscillations in nitrate yield could be explained by the variations in rainfall, temperature and vegetation phenology. These oscillations induce an asynchrony, characteristic in Mediterranean catchments, between the plant stages of maximum N demand and the wet periods with highest nitrate availability derived from N flushing out of soil (Meixner and Fenn, 2004). This pattern is enhanced by winter fertilization in some agricultural areas such as the Galbarra Stream Catchment, determining very high N exports from headwaters to downstream BSWs in this season (Lassaletta, 2007).

Nitrate concentrations in the mouth of a non-BSW were similar to those recorded in a large downstream river, considered as BSW, which have similar or even higher percentages of agricultural cover in their catchments and are influenced by large cities.

All the results suggest that downstream nitrate levels in receiving waters are closely connected to distant landscape sources and headwater streams, as addressed by Alexander et al. (2007) and Dodds and Oakes (2008). Control of nitrate levels in headwater streams might therefore be crucial in order to avoid nitrate pollution in large rivers and estuaries, most of these considered as BSWs.

Several programmes aimed at controlling N pollution in continental waters have been successful in some European countries (Iital et al., 2005; Kronvang et al., 2008) but not in others (Oenema et al., 2005; Bechmann et al., 2008). A delay in the response of the catchment to the new practices in place very much explains the absence of a clear response to these improvements (Jackson et al., 2008; Cherry et al., 2008). These programmes include reductions or control of fertilizer applications, improvements in farm management practices or regulations on point source discharges from wastewater treatment plants (i.e. acting at the sources). Shilling and Wolter (2009) show how fertilizer reduction can represent the most effective measure with regard to reducing N pollution. Similarly, Oenema et al. (2009) emphasize the effectiveness of "N fertilization balance" measures enhancing an increase in nitrogen use efficiency by crops and a reduction of N losses.

Improvement of N-retention and process capacities of riparian buffer strips and rivers, especially in headwater catchments (i.e. acting in relation to transport) can embody an important complementary action (Craig et al., 2008). Headwater riparian ecotones present a major contact surface between crops and streams, these ecosystems representing hotspots in the nutrient retention process. Dodds and Oakes (2008) suggest that protection of downstream riparian zones alone is insufficient with regard to protecting water quality if the influence of small upland streams is not considered. In addition, a recent study performed in a Mediterranean agricultural headwater catchment shows that permanent N removal via stream denitrification can be greater than in forest or urban catchments and can account for 68% of total nitrate uptake (von Schiller et al., 2009). In agricultural N rich catchments, however, this process is saturated and efficiency is reduced when high nitrate concentrations are reached (Bernot et al., 2006). Consequently, integrating simultaneous management strategies in relation to sources and transport could constitute the most effective approach.

Almost 80% of the length of the Ega River Catchment drainage network is not classified as a BSW, most of it corresponding to 1st and 2nd order streams. Unfortunately, the WFD does not usually consider these headwater streams, despite their great importance for the achievement of the "good ecological status" of downstream rivers by 2015. Although relatively high nitrate concentrations are frequently observed in alluvial zones associated with irrigated agriculture (Arauzo et al., 2008), in the Ega catchment no groundwater bodies were declared NVZ by the Competent Authorities (http://www.saecoop.com/zonas%20vulnerables.htm). This means that the regulations referring to good agricultural practice will only be applied voluntarily.

Consideration of all streams in the drainage network as BSWs would have been unfeasible from a logistic and economic perspective, and application of measures referring to fertilization also involves potentially high costs (Oenema et al., 2009). The results of this study, however, suggest that acting in small headwater agricultural catchments and in their streams, in relation both to sources and to transport, could be vital with regard to defining downstream water quality. That is, reaching relatively acceptable quality levels in the non-BSWs could be of great importance in order to attain the "good ecological status" of downstream BSWs, thus complying with the objectives of the WFD.

Efforts by the River Basin Management Plans to control point source and irrigation-related impacts, and to restore the banks and courses of large rivers, are undoubtedly crucial in relation to meeting WFD objectives. Nonetheless, we believe that managers should also keep in mind the important influence of headwater catchments, especially agricultural ones, as these can affect N export, not only because they are the elements in which agricultural pollution processes start, but also because of the great N-retention capacity exhibited by the well-conserved ones.

6. Conclusions

Nitrate concentration in the headwater streams (1st and 2nd order streams) studied herein may be higher than downstream river concentration, and clearly responds to percentage of agricultural land use in their catchment. The effect of agriculture on nitrate concentration was very high and independent from the size of the embedded catchments considered in this study (0.1–7.3 km^2 and 223–3113 km^2 catchment area, respectively), thus refuting our initial hypothesis. The nitrate concentration in the mouth of a small stream not identified as a ''body of surface water'' (BSW) was similar to concentration in the greater downstream BSW river. These results show that water quality in agricultural catchments can deteriorate in the first few kilometres of the river, and therefore suggest that downstream nitrate levels can be clearly influenced by upstream contamination processes.

Headwater streams comprise 76% of the length of the Ega River Catchment's hydrographical network, but only a small proportion (3.1% of their whole length) is identified as BSW according to the WFD. As result, only the 22.3% of the Ega hydrographical network's length is identified as a BSW. Although reaching the ''good ecological status'' of the remaining 77.7% does not constitute an aim of the WFD, this could have a clear influence on the BSWs. The competent Authorities should seriously take into account small agricultural catchments for the development and implementation of the River Basin Management Plans.

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