© Asociación Ibérica de Limnología, Madrid. Spain. ISSN: 0213-8409

The performance of biological indicators in assessing the ecological state of streams with varying catchment urbanisation levels in Coimbra, Portugal

Ana Isabel Del Arco[1,2,*], Verónica Ferreira[2] and Manuel A. S. Graça[2]

[1] Current address: IMAR-CMA, Department of Life Sciences, University of Coimbra, P.O. Box 3046, 3001-401 Coimbra, Portugal.
[2] Department of Animal Biology, Plant Biology and Ecology, University of Jaén, Campus Las Lagunillas, Ed. Zabaleta (B3), C.P. 23071 Jaén, Spain.

[*] Corresponding author: aarco@ujaen.es

Received: 27/12/2010 Accepted: 14/6/2011

ABSTRACT

The performance of biological indicators in assessing the ecological state of streams with varying catchment urbanisation levels in Coimbra, Portugal

The growth of human populations has resulted in the expansion of metropolitan areas and changes in land use, both of which affect watersheds and streams. The ecological integrity of streams is likely to be negatively affected by urbanisation, compromising freshwater ecosystem services. The aim of this study was to assess how efficient structural and functional indicators are in evaluating the ecological conditions of water in urban stream ecosystems. Two urban streams crossing the city and one stream crossing a suburban area of Coimbra, Portugal were selected. Total impervious area (TIA) was used as an indicator of urbanisation. Physical and chemical parameters of water were measured and analysed within the Water Framework Directive (WFD/2000/60/EC). Benthic macroinvertebrates were used as structural indicators, and the IBMWP biotic index (modified) and the Portuguese $IPtI_S$ index were calculated. The decomposition rates of oak (*Quercus robur*) and alder (*Alnus glutinosa*) leaves were used as indicators of functional quality. Biotic indices and litter decomposition rates indicated poor ecological conditions in the urban streams compared to the suburban stream, consistent with the degree of urbanisation. The decrease in ecological quality in urban streams most likely reflected decreases in dissolved oxygen and increases in water temperature and conductivity. We emphasise (a) the need to combine physical and chemical data with biological data and (b) the high performance of a novel functional indicator based on litter breakdown rate as an accurate, efficient and integrative measure of ecological integrity in urban streams.

Key words: Urban streams, ecological integrity, macroinvertebrates, litter decomposition.

RESUMEN

Utilización de indicadores biológicos en la evaluación del estado ecológico de ríos urbanos de varias cuencas urbanas en Coímbra, Portugal

El crecimiento de la población humana en las últimas décadas ha provocado el aumento de las áreas urbanas y cambios en los usos del suelo que afectan a las cuencas hidrográficas y a sus ríos. Es probable que la integridad ecológica de los ríos urbanos se vea afectada negativamente, comprometiendo los servicios ecosistémicos proporcionados por las aguas dulces. El objetivo de este estudio fue comparar el rendimiento entre diferentes metodologías en la evaluación química y ecológica del agua en ríos urbanos. Se han seleccionado dos ríos urbanos que cruzan la ciudad de Coimbra, Portugal, y un río que cruza un área suburbana. Como indicador de urbanización se usó el área total impermeable (TIA). Se han utilizado parámetros físico-químicos que se han analizado siguiendo la Directiva Marco del Agua (2000/60/EC). Como indicadores estructurales se han utilizado los macroinvertebrados bentónicos, calculándose el índice biológico IBMWP (modificado) y el índice $IPtI_S$ *portugués. Como indicadores funcionales de la calidad ecológica se han usado las tasas de descomposición de hojarasca de roble (*Quercus robur *L.) y aliso (*Alnus glutinosa *(L.) Gaertner). Los parámetros físicos y químicos de todos los ríos indicaron "buena calidad" según los parámetros de calidad nacionales y la Directiva Marco del Agua. Sin embargo,* *los indicadores biológicos y las tasas de descomposición de hojarasca indicaron malas condiciones ecológicas en los ríos urbanos comparados con el río en el área suburbana coincidiendo también con el grado de urbanización. Esto sugiere que los indicadores biológicos (estructurales y funcionales) proporcionan información más temprana, precisa e integrada del sistema que las medidas de la química del agua que no reflejan el valor ecológico de las aguas, su calidad o los impactos ecológicos potenciales. En el estudio se hace especial énfasis en (a) la necesidad de combinar datos físico-químicos con medidas biológicas y (b) la elevada eficiencia de la descomposición como indicador preciso de la integridad ecológica y calidad ambiental en un número creciente de ríos urbanos.*

Palabras clave: *Ríos urbanos, integridad ecológica, macroinvertebrados, descomposición de hojarasca.*

INTRODUCTION

Approximately 50 % of the world's population lives in urban areas, and this proportion is expected to increase to 60 %-80 % in developed regions by 2030 (UN Population Division 2005). In line with this trend, approximately 60 % of the Portuguese population presently lives in urban areas, with an annual increase of 1.4 % (http://data.un.org/CountryProfile.aspx?crname=PORTUGAL). This global increase in urban populations will inevitably lead to changes in land use as more space for housing, commercial and industrial settings is needed, and this process will affect increasing numbers of streams.

The stressors imposed on the growing number of streams draining urban areas (hereafter called 'urban streams') and their biological responses have been encapsulated in the concept of the 'urban streams syndrome' (Walsh *et al.,* 2005). This syndrome consists of changes to the hydrological, morphological, geological, chemical and biological attributes of streams, which arise from four primary consistent stressors on urban streams: (a) increase in impervious area (e.g., roads, parking lots, rooftops); (b) catchment drainage and channelisation, usually as a flood-control measure; (c) changes in the riparian vegetation, including its removal as well as invasion by exotic species; and (d) sewage discharges (treated or untreated) (reviewed by Paul & Meyer, 2001; Walsh *et al.,* 2005; Wenger *et al.,* 2009). These stressors usually lead to poor water quality, typified by increases in water temperature, dissolved nutrients, pollutants and conductivity (Morgan *et al.*, 2007; Imberger *et al.,* 2008). Increased incidences of flash floods and reductions in riparian vegetation also decrease the amount of benthic organic matter in urban streams (Meyer *et al.,* 2005; Walsh *et al.,* 2005). These changes have the potential to negatively affect aquatic communities and ecosystem processes.

Benthic macroinvertebrates have frequently been used in monitoring studies of urban streams, mainly due to their value as indicators of ecological integrity (Metcalf, 1989; Wenger *et al.,* 2009), with the abundances of sensitive (e.g., Ephemeroptera, Plecoptera and Trichoptera) and tolerant (e.g., many Chironomidae and Oligochaeta species) taxa changing in response to changes in environmental quality. Macroinvertebrates are also used in biotic indices (Booth *et al.*, 2004; Walsh *et al.*, 2007; Miserendino *et al.,* 2008; Purcell *et al.,* 2009). Although changes in benthic communities may result from multiple stressors, a strong negative relationship has been found between benthic variables and impervious area (Paul & Meyer, 2001; Booth *et al.*, 2004; Walsh *et al.,* 2007).

In addition to changes in the structure of aquatic communities, recent studies have reported that anthropogenic disturbances affect ecosystem functions. Nutrient uptake seems to decrease as urbanisation increases, most likely as a result of increased loads of toxic substances and decreases in benthic organic matter and associated microbes, whereas stream metabolism (primary production and respiration) is reported to be less sensitive to urbanisation (Meyer *et al.,* 2005). The rate of litter breakdown, another important ecosystem function, was reported to increase with urbanisation, presumably because of increased physical abrasion related to flash floods and the stimulation of microbial activity by increases in dissolved nutrients and temperature (Meyer *et al.,* 2005; Chadwick *et al.,* 2006; Imberger *et al.,* 2008). However, at very high levels of urbanisation, breakdown rates may decrease as a result of high concentrations of toxic compounds (Chadwick *et al.,* 2006).

As a result of the stressors imposed on urban streams and their negative effects on water quality, aquatic communities and processes, many of these streams have decreased in social and economic value (Booth *et al.,* 2004). To rehabilitate urban streams, their physical, chemical, morphological and biological (structural and functional) components should first be assessed. However, urban stream assessment and management represent novel challenges for both aquatic ecologists and city planners (Wenger *et al.,* 2009).

The aim of this study was to determine the effectiveness of biological (structural and functional) indicators, which are usually used in more natural stream ecosystems in little-impacted or non-urbanised areas for detecting and identifying impairment in urban streams. This was addressed in three streams in the city of Coimbra in central Portugal. Streams were selected based on variation in the proportion of impervious area within their watersheds, which was assumed to be correlated with urbanisation intensity and related impacts on the streams (Paul & Meyer, 2001). The assessment was based on the benthic macroinvertebrate communities (a measure of structural integrity) and on the decomposition rates of leaves from two tree species, one labile and the other recalcitrant (a measure of functional integrity; Gulis *et al*., 2006; Castela *et al.,* 2008).

MATERIALS AND METHODS

Study sites

For our study we selected one suburban stream (São Paulo de Frades, S1) and two urban streams (Coselhas, S2, and an unnamed stream in Vale das Flores, S3) in Coimbra. All of the streams are tributaries of the Mondego River in central Portugal. The substrate of all streams was dominated by small-sized particles: approximately 90 % gravel in S1, 90 % sand and silt in S2, and 90 % silt and clay in S3. Only stream S1 had aquatic macrophytes (approximately 10 %). Riparian vegetation and canopy cover were present along all three streams, although it was not abundant: at S1, canopy cover was approx. 5 % (*Salix atrocinerea* and *Ficus carita*), although it was notably denser in the upstream section; S2 had approx. 50 % cover of *Salix* spp. and 10–25 % cover of exotic bushes; and S3 had approx. 10 % cover of native (*S. atrocinerea* and *Populus* spp.) and non-native (*Eucalyptus globulus*) tree species and 10 % cover by exotic bushes.

The three streams were channelised to different degrees. Whereas streams S1 and S2 were channelised without any artificial walls, the upstream section of stream S3 was modified by a cement wall along both banks. The drainage system in the catchment of stream S2 was mostly of the combined type (29 km), but in some areas storm water and sanitary sewers were drained separately (1.2 km). We can assume that the combined system is the most common drainage system throughout the city of Coimbra; however, no drainage discharges are known in stream site S1, and a discharge of unknown origin existed upstream from stream site S3. Stream S1 was considered to represent the best possible circumstances for an urban stream based on its potential for ecological integrity, given its peripheral location outside the most densely urbanised zone of Coimbra.

Urbanisation variables

Total impervious area (TIA), which represents the area of non-permeable surfaces (e.g., parking lots, paved roads, rooftops), increases with urbanisation (Yang *et al.,* 2003) and is an important indicator of urban land use and water quality (Arnold & Gibbons, 1999; Morgan *et al.,* 2007; Imberger *et al.,* 2008). Therefore, TIA can be used as an indicator of watershed impairment and pressure on urban streams (Paul & Meyer, 2001;

Walsh *et al.,* 2001). The percentages of the watersheds of the three streams in this study that were covered by total impervious area (% TIA) were calculated using ArcGIS 9.3.1 software as the percentage of all built-up areas with more than 0 % imperviousness, based on the Urban Atlas developed by the Global Monitoring for Environment and Security (GEMS) of the European Environmental Agency (EEA).

Physical and chemical variables

Each stream was surveyed four times between 2 February and 18 March 2010. During each survey, total dissolved solids (TDS), conductivity, temperature (WTW LF 330, Wissenschaftlich-Technische Werkstätten GmbH, Germany), dissolved oxygen (WTW OXI 92, Wissenschaftlich-Technische Werkstätten GmbH, Germany), pH (JENWAY 3310, Bibby Scientific Limited, UK), wet channel width, depth, and current velocity (VALEPORT 15277, Valeport Limited, UK) were determined using field probes. Discharge was computed as width × depth × current velocity. On the same occasion, water samples were filtered (Millipore APFF04700, Millipore, MA, USA) and transported cold to the laboratory. Water was analysed for nutrients and other ions using an ion chromatograph (Dionex DX-120, Sunnyvale, CA, USA). Soluble reactive phosphorus (SRP) concentration was determined using the ascorbic acid method, and alkalinity was determined by titration to an endpoint of pH 4.5 (APHA, 1995).

Biological variables

Structural variables: benthic macroinvertebrates

Benthic macroinvertebrates were sampled from all streams on two occasions in winter 2010 (3 February and 18 March), following standard procedures (INAG, 2009; WFD 60/EC/2000). Each sample was composed of six kicks taken with a hand net (0.25 × 0.25 m opening, 500 μm mesh), with a duration of 30 s each, along 1 m in the upstream direction. Samples were taken within a 50-m long reach of each stream, and located based on the relative proportions of all major habitats. Samples were either processed fresh within 48 h of collection or fixed with formalin (4 % final concentration). In either case, samples were washed with tap water through a series of sieves (0.5, 1, and 2 mm), and the sieved invertebrates were sorted and preserved in 80 % ethanol for later identification. Identification was carried out to the lowest possible taxonomic level, usually genus or species except for Oligochaeta (family) and Diptera (subfamily or tribe), following Tachet *et al*. (2002).

Benthic macroinvertebrate composition was used to compute two biotic indices to represent water quality classes and ecological integrity: 1) a modification of the Iberian Biological Monitoring Working Party Index (IBMWP; Alba-Tercedor *et al.,* 2002), in which benthic macroinvertebrates were not checked *in situ* for new families and therefore all samples correspond to the same sampling effort, and 2) the Portuguese $IPtI_S$ index (INAG, 2009). The IBMWP was calculated by summing the scores attributed to each family represented by more than 1 individual, based on the family's tolerance to organic contamination (score 1, most tolerant; score 10, least tolerant). The final IBMWP scores fall into five water quality classes: I (IBMWP > 101), very good; II (100 > IBMWP > 61), good; III (60 > IBMWP > 36), moderate; IV (35 > IBMWP > 16), poor; and, V (IBMWP < 15), very poor (Alba-Tercedor *et al.*, 2002). The $IPtI_S$ was calculated by applying the following formula: $IPtI_S$ = no. families × 0.2 + no. EPT families × 0.2 + (IASPT − 2) × 0.4 + $\log_{10}$ (Sel. EPTCD + 1) × 0.2, where no. EPT families is the number of Ephemeroptera, Plecoptera, and Trichoptera families, IASPT is the Iberian Average Score Per Taxon given by (IBMWP/number of IBMWP families), and Sel. EPTCD is the number of individuals from selected Ephemeroptera, Plecoptera, Tricoptera, Coleoptera and Diptera families (Chloroperlidae, Nemouridae, Leutricidae, Leptophlebiidae, Ephemerellidae, Philopotamidae, Limnephilidae, Psychomyiidae, Sericostomatidae, Elmidae, Dryopidae and Athericidae). Final $IPtI_S$ scores fall into four water quality classes: I (0.70 < $IPtI_S$ < 0.95), excellent; II (0.47

< $IPtI_S$ < 0.70), good; III (0.23 < $IPtI_S$ < 0.47), moderate; and IV ($IPtI_S$ < 0.23), poor (INAG, 2009). Benthic metrics were also calculated (e.g., taxa richness, number of EPT taxa, percentage of shredder individuals; USEPA, 1998).

Functional variables: litter breakdown rates

The breakdown rates of alder (*Alnus glutinosa* (L.) Gaertner) and oak (*Quercus robur* L.) leaves were determined in all of the streams because litter breakdown rate has been proposed for use as an indicator of stream functional integrity (Gessner & Chauvet, 2002) and its usefulness has been demonstrated (Pascoal *et al.,* 2003; Gulis *et al.,* 2006; Castela *et al.,* 2008; but see Hagen *et al*., 2006; Bergfur, 2007, Pérez et al., 2011). Alder and oak leaves were chosen due to their contrasting physical and chemical characteristics and the distinctive sensitivity of their breakdown rates to two of the major pressures in urban streams: physical abrasion and nutrient enrichment. Alder leaves are thin and fragile, and therefore their breakdown rate is potentially sensitive to increased physical abrasion caused by flash floods (Ferreira *et al.*, 2006a). Oak leaves are nutrient-poor (high carbon/nitrogen), and therefore, their breakdown rate is potentially sensitive to increases in dissolved nutrient concentrations. In oak leaves the microbial community will most likely be nutrient-limited and stimulated by an increase in the availability of external nutrients, as microbes have the ability to retrieve nutrients from both the organic substrate and the water (Ferreira *et al.,* 2006b; Gulis *et al.*, 2006). Leaves were collected just after abscission, air dried, and stored in the dark until used. Batches of 3±0.15 g of leaves were weighed, rehydrated, and enclosed in coarse mesh bags (10 × 15 cm, 10 mm mesh opening). Seventy-two litter bags were deployed in the streams on 2 February 2010 (d0) and incubated for up to 34 days. After 7, 15, and 34 days, 4 bags of each litter species were retrieved and transported to the laboratory on ice. The litter was rinsed with tap water, oven-dried (105 °C for 24 h), weighed (± 0.1 mg), incinerated (550 °C for 4 h), and reweighed (± 0.1 mg) to determine the ash-free dry mass (AFDM) remaining. Four extra bags of each leaf species were taken to the streams on d0, immersed for ~ 30 min, and immediately transported to the laboratory to calculate the initial air-dry to initial AFDM conversion factor, taking into account handling losses.

Data treatment

Physical and chemical variables were compared between streams using 1-way ANOVA, with Tukey's test for multiple comparisons. Principal Components Analysis (PCA; CANOCO for Windows 4.5, Microcomputer Power, New York, USA; ter Braak & Smilauer, 1998) was performed to determine whether environmental variables could be used to discriminate among streams. We also used PCA (CANOCO) to investigate whether sites differed significantly in terms of macroinvertebrate composition (invertebrate abundances were $\log(x + 1)$ transformed). The relationships between PCA axis coordinates from both ordinations and physical-chemical variables were assessed using Pearson correlations.

Litter breakdown rates (k) were estimated as the slope (k) of a linear regression of the fraction of remaining mass (ln transformed) over time. This assumes an exponential decay, which is expressed by the negative exponential model $M_t = M_i\, e^{-k \cdot t}$, where M_t is the AFDM remaining at time t (days), M_i is the initial AFDM, and k is the breakdown rate coefficient. Breakdown rates of each litter species were compared between streams by ANCOVA, with Tukey's test for multiple comparisons. Comparisons among streams were also made using the ratio $k_{\text{impacted}}/k_{\text{best stream}}$ (based on Gessner & Chauvet, 2002), considering stream S1 as the best stream. All analyses were performed with STATISTICA 7 software (StatSoft, OK, USA) unless otherwise indicated.

RESULTS

Urbanisation, physical and chemical variables

The watersheds of the selected streams had total impervious areas of 3, 19 and 61 % for streams S1, S2 and S3, respectively. Therefore, the se-

Table 1. Physical and chemical variables (mean ± SE) of the three streams along an urbanisation gradient in Coimbra. Comparisons among sites were made using ANOVA (*F* and *p* values are given); different letters indicate significant differences among streams (Tukey's test, $p < 0.05$). *Medidas físicas y químicas (media ± SE) de los ríos urbanos en Coimbra. Las comparaciones entre sitios se hicieron usando ANOVA (se presentan los valores* F *y* p*); differentes letras indican diferencias entre ríos (Tukey's test,* $p < 0.05$*).*

Variables	Sites			*F*-value	*p*-value	Legal limits*
	S1	S2	S3			
Temperature (°C)	$10.5{\pm}0.9^{a}$	$11.8{\pm}0.9^{ab}$	$12.9{\pm}1.2^{b}$	5.417	0.025	
DO (mg/L)*	$10.9{\pm}0.3^{a}$	$10.6{\pm}1.1^{a}$	$8.7{\pm}1.5^{a}$	4.117	0.059	> 5 mg O_2/L
DO (%)*	$99.2{\pm}0.9^{a}$	$98.8{\pm}10.1^{a}$	$79.8{\pm}15.2^{a}$	3.610	0.076	60-120 %
Conductivity (μS/cm)	$137{\pm}82^{a}$	$423{\pm}58^{b}$	$386{\pm}91^{b}$	17.584	0.001	
TDS (mg/L)	$150{\pm}90^{a}$	$463{\pm}64^{b}$	$414{\pm}11^{b}$	15.521	0.001	
pH*	$7.2{\pm}0.1^{a}$	$7.9{\pm}0.8^{ab}$	$7.4{\pm}0.5^{b}$	6.610	0.015	6-9
Alkalinity (mg $CaCO_3$/L)	$28.8{\pm}5.8^{a}$	$147.4{\pm}36.0^{b}$	$117.2{\pm}9.2^{b}$	24.582	0.0001	
Discharge (m^3/s)	$0.45{\pm}0.72^{a}$	$0.14{\pm}0.04^{a}$	$0.05{\pm}0.01^{a}$	2.976	0.108	
SRP (μg/L)*	$17.37{\pm}3.30^{a}$	$68.01{\pm}32.44^{ab}$	$49.69{\pm}21.03^{b}$	5.121	0.027	≤ 130
NO_2^- (mg/L)	< 0.01	$0.05{\pm}0.04^{a}$	$0.32{\pm}0.08^{b}$	47.871	0.0001	
NO_3^- (mg/L)*	$3.80{\pm}0.52^{a}$	$4.74{\pm}1.34^{a}$	$3.77{\pm}0.88^{a}$	1.426	0.281	≤ 25
NH_4^+ (mg/L)*	$0.17{\pm}0.15^{a}$	$0.24{\pm}0.11^{a}$	$0.18{\pm}0.06^{a}$	0.484	0.631	≤ 1
Total N (mg/L)	$0.99{\pm}0.21^{a}$	$1.32{\pm}0.35^{a}$	$1.09{\pm}0.24^{a}$	1.732	0.222	
CH_3CO_2 (mg/L)	$0.46{\pm}0.40^{a}$	$1.90{\pm}\ 2.46^{a}$	$0.62{\pm}0.33^{a}$	0.835	0.465	
F^- (mg/L)	$0.06{\pm}0.00^{a}$	$0.11{\pm}0.02^{b}$	$0.07{\pm}\ 0.01^{a}$	11.646	0.0001	
Cl^- (mg/L)	$15.49{\pm}0.70^{a}$	$4.15{\pm}0.05^{b}$	$8.23{\pm}2.84^{b}$	10.480	0.003	
Na^+ (mg/L)	$13.15 \pm 0.55\ ^{a}$	$18.28 \pm 3.12\ ^{b}$	$8.08 \pm 2.11\ ^{c}$	22.101	0.0001	
SO_4^{2-} (mg/L)	$21.34{\pm}1.48^{a}$	$26.91{\pm}6.25^{a}$	$12.13{\pm}3.76^{b}$	7.231	0.009	
K^+ (mg/L)	$1.65{\pm}0.20^{a}$	$3.61{\pm}0.67^{b}$	$2.91{\pm}0.84^{b}$	11.406	0.002	
Mg^+ (mg/L)	$9.13{\pm}0.75^{a}$	$21.56{\pm}3.59^{b}$	$7.55{\pm}3.59^{a}$	31.647	0.0001	
Ca^{2+} (mg/L)	$11.60{\pm}2.11^{a}$	$44.60{\pm}5.69^{b}$	$25.05{\pm}6.37^{c}$	50.345	0.0001	

* Physical and chemical measurements regulated by the WFD.

* Medidas físicas y químicas reguladas por la DMA.

lected streams fell along an urbanisation gradient (Stepenuck *et al.*, 2002), with stream S1 likely being the least-impaired stream and streams S2 and S3 being the most-impaired.

Although stream S1 had significantly lower conductivity, TDS and alkalinity than streams S2 and S3, as well as a lower SRP concentration and pH than S3 (Table 1), the water variables regulated by the Water Framework Directive (WFD 2000/60/EC) fell within acceptable legal values in all the streams, indicating good water quality. Temperature, fluoride (F^-), chloride (Cl^-), nitrite (NO_2^-), sodium (Na^+), sulphate (SO_4^{-2}), potassium (K^+), magnesium (Mg^+) and calcium (Ca^{2+}) levels differed significantly among streams, with a tendency for the values to be lowest in stream S1 (Table 1).

The ordination of physical and chemical variables by PCA discriminated between sites (Fig. 1). The sites were distributed along axis 1, which explained 49.6 % of the variability and was positively related to total N, SRP and several ions; and along axis 2, which explained 32.4 % of the variability and was positively related to temperature and alkalinity and negatively related to dissolved oxygen and discharge (Table 2).

Benthic macroinvertebrates

Over the first sampling period, a total of 338, 169 and 3620 individuals distributed in 23, 8 and 2 families were collected in streams S1, S2 and S3, respectively (Table 3). Shredders were

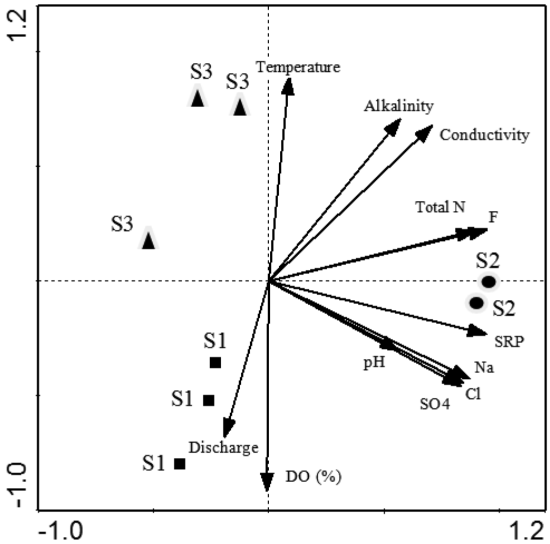

Figure 1. Principal components analysis (PCA) of environmental variables of the suburban (S1) and urban (S2 and S3) streams in Coimbra. *Análisis de componentes principales (ACP) usando variables ambientales de los ríos suburbanos (S1) y urbanos (S2 y S3) en Coimbra.*

present only in stream S1, representing approximately 4 % of the total number of individuals. On the second sampling period, the samples were composed of 305, 213 and 214 individuals,

Table 2. Axis eigenvalues and correlation between axes and environmental variables used in the principal components analysis of environmental variables from three streams along an urbanisation gradient in Coimbra, Portugal. Pearson correlation: * denotes r values with $p < 0.05$. *Valores propios de los ejes y correlación entre los ejes y variables ambientales usados en el análisis de componentes principales de las variables ambientales de los tres ríos a lo largo de un gradiente de urbanización en Coimbra, Portugal. Correlacion de Pearson: * indica valores de* r *con* p *< 0.05.*

	Axis 1	Axis 2
Eigenvalues	49.6	32.4
Temperature	0.09	0.89*
DO	–0.01	–0.92*
Conductivity	0.71*	0.68
pH	0.56	–0.30
Alkalinity	0.57	0.71*
Log Discharge	0.07	–0.95*
Log SRP	0.75*	–0.63
Total N	0.90*	0.22
F^-	0.95*	0.23
Cl^-	0.84*	–0.44
Na^+	0.87*	–0.42
SO_4^-	0.83*	–0.46

distributed in 15, 3 and 2 families in streams S1, S2 and S3, respectively. In this period, shredders were absent from all three streams (Table 3). On both sampling occasions, stream S3 contained the fewest taxa. The total number of taxa, number of intolerant taxa, number of clinger taxa, and percentage of EPT taxa were highest in stream S1 (Table 3); all of these metrics are related to good ecological integrity. In contrast, stream S3 had higher scores for metrics associated with low water quality, such as percentage of Diptera individuals, percentage of Oligochaeta individuals (Table 3). Stream S2 also had high percentages of Diptera individuals and high numbers of tolerant taxa (Table 3). The IBMWP and the $IPtI_S$ indices indicated that stream S1 was in a 'good' ecological state, while streams S2 and S3 were 'impacted' or 'highly impacted'. The ordination of benthic macroinvertebrate assemblages using abundance data discriminated among the streams (Fig. 2). The two urban streams were

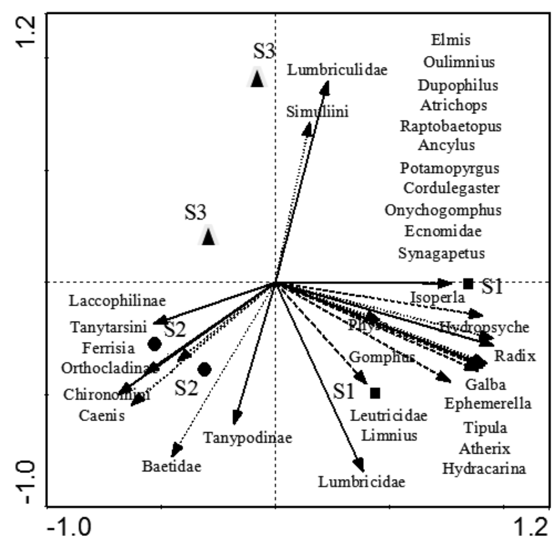

Figure 2. Principal components analysis (PCA) of benthic macroinvertebrate communities of the three streams along an urbanisation gradient in Coimbra. Solid and dashed arrows represent tolerant and intolerant taxa, respectively. Tolerant taxa have a score of 1, 2 or 3 while intolerant taxa have a score of 8, 9 or 10 in the IBMWP index. *Análisis de componentes principales (ACP) usando las comunidades de macroinvertebrados bentónicos de los tres ríos a lo largo de un gradiente de urbanización en Coimbra. Las flechas continuas y discontinuas representan taxones tolerantes e intolerantes respectivamente. Los taxones tolerantes tienen un valor de 1, 2 y 3; los intolerantes tienen un valor de 8, 9 y 10 en el índice IBMWP.*

Table 3. Selected biotic indices and metrics for benthic macroinvertebrates in three streams along an urbanisation gradient in Coimbra. *Selección de índices biológicos y métricas usando macroinvertebrados bentónicos de los ríos urbanos en Coimbra.*

Metrics	1st sampling			2nd sampling		
	S1	S2	S3	S1	S2	S3
Biotic indices						
IBMWP score (quality class)	121	31	6	85	9	3
	(I)	(IV)	(V)	(II)	(V)	(V)
$IPtI_S$ score (quality class)	0.93	0.28	0.09	0.77	0.20	0.00
	(II)	(IV)	(IV)	(II)	(III)	(IV)
Abundance						
Total no. of individuals	338	169	3620	305	213	214
Richness measures						
Total no. taxa	34	10	2	24	10	4
No. EPT taxa[1]	7	2	0	8	2	0
Composition measures						
% EPT individuals	22	55	0	27	62	0
% Diptera individuals	13	41	6	8	28	43
% Chironomidae individuals	22	55	0	27	62	0
% Oligochaeta individuals	17	1	95	9	9	57
Tolerance						
No. intolerant taxa[2]	11	0	0	8	0	0
No. tolerant taxa[3]	12	2	1	8	7	1
% Dominant taxon individuals	20	47	95	29	39	57
Trophic groups						
% Shedders individuals	4	0	0	0	0	0
% Grazers individuals	39	58	0	48	62	0
Habitat measures						
No. clinger taxa (insects)	24	8	1	18	7	3

[1] EPT = Ephemeroptera + Plecoptera + Tricoptera.

[2] Intolerant families have scores of 8, 9 and 10 in the IBMWP index.

[3] Tolerant families have scores of 1, 2 and 3 in the IBMWP index.

distributed along axis 1, which explained 44 % of the variability and was negatively related to SRP and alkalinity ($r = -0.82$ and -0.87, respectively, $p < 0.05$), and along axis 2, which explained 31 % of the variability but was not correlated with any water variable. Abundance of tolerant taxa such as Lumbriculidae (Oligochaeta) best explained the discrimination of stream S3. Tolerant dipteran taxa, such as tribe Chironomini, tribe Tanytarsini or subfamily Orthocladinae, discriminated stream S2 from the other two. In contrast to these taxa, the most sensitive groups, such as *Atherix* spp., *Isoperla* spp. or *Gomphus* spp., were associated with stream S1 (Fig. 2).

Functional variables: litter breakdown rates

Alder breakdown rates varied between 0.0089/d and 0.0265/d across streams. Oak breakdown rates were slower, and varied between 0.0062/d and 0.0138/d. The alder leaf breakdown rate was significantly faster in stream S1 than in streams S2 and S3 (ANCOVA, $p = 0.003$), while the oak leaf breakdown rate was significantly faster in streams S1 and S2 than in stream S3 (ANCOVA, $p < 0.001$) (Fig. 3). Using the Gessner & Chauvet (2002) functional approach and assuming that stream S1 represents the best available comparable stream, the ratio of breakdown

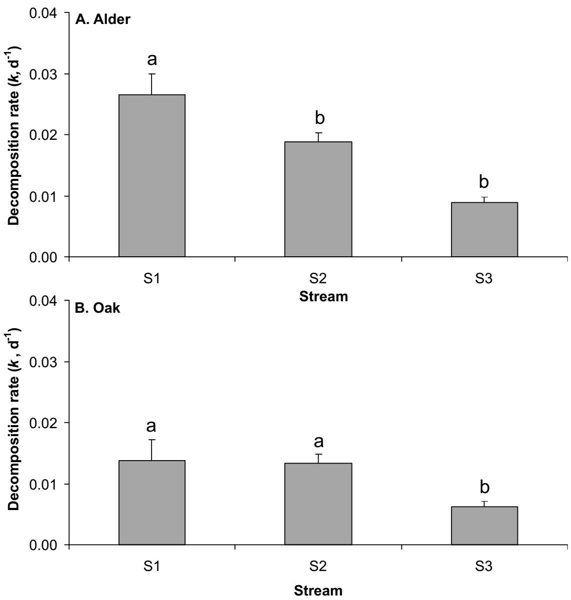

Figure 3. Litter breakdown rates (mean ± SE) for alder and oak leaves incubated in coarse-mesh bags in three streams along an urban gradient in Coimbra. Comparisons between streams were made for each species using ANCOVA; different letters indicate significant differences among streams (Tukey's test, $p < 0.05$). *Tasas de descomposición de hojarasca (mean ± SE) de aliso y roble incubados en bolsas de malla gruesa en los ríos urbanos de Coimbra a lo largo de un gradiente de urbanización. Las comparaciones entre ríos para cada especie se realizaron usando ANCOVA; diferentes letras indican diferencias significativas entre ríos (Tukey's test,* p $<$ *0.05).*

rates ($k_{\text{impacted}}/k_{\text{best stream}}$) indicated that streams S2 and S3 were likely to have impaired ecosystem functions, with the worst conditions in stream S3 (Table 4).

DISCUSSION

Total impervious area, which reflects urban land use in a catchment, suggested an increasing degree of impairment from stream S1 (suburban stream) to stream S3 (located in a highly populated area within the city of Coimbra). However, based on WFD guidelines, the water chemistry was in good condition for all three streams, although the values of the specific parameters differed (for example, the temperature or SRP concentration). We will first discuss the significance of the chemical and physical data, then the structural biological parameters and finally

Table 4. The ratio of breakdown rates for alder and oak leaves between the likely impacted streams (S2 and S3) and the 'best' stream (S1) ($k_{\text{impacted}}/k_{\text{best stream}}$). Scores are based on Gessner & Chauvet (2002). *Relaciones entre las tasas de descomposición de los ríos potencialmente impactados (S2 y S3) y el río en mejor condición ecológica (S1)* ($k_{impactados}/k_{mejor\ río}$) *encontradas en las incubaciones con hojarasca de roble y aliso. Los valores de referencia están basados en Gessner & Chauvet (2002).*

Streams	Litter species			
	Alder		Oak	
	Ratio	Score*	Ratio	Score*
S2	0.71	1	0.97	2
S3	0.33	1	0.26	1

* Score 1: compromised ecosystem functioning; Score 2: no evidence of an impact on ecosystem functioning.

the impairment of litter decomposition and its significance for environmental management. The three sites differed in temperature, which was consistent with their shading in the sampling sites themselves and several hundred meters upstream. Given the importance of temperature in the metabolism of invertebrates and microorganisms, a temperature difference has the potential to affect the biota.

Total N concentration did not differ significantly between streams, but was an important discriminating factor in the PCA analysis. Nutrient concentrations were high for all three streams, especially SRP, compared with concentrations found in other streams in the same area but not crossing the city (6.40 µg SRP/L; Gama *et al.,* 2007; Castela *et al.,* 2008). These differences might be explained by spot wastewater inputs, increased runoff and decreased riparian vegetation in the urban streams. Both N (Morgan *et al.*, 2007) and P (Brett *et al.*, 2005; Imberger *et al.*, 2008) have been reported to increase with denser urbanisation (reviewed by Paul & Meyer, 2001). However, according to the WFD chemical criteria, the SRP concentrations were within the limits of "good ecological status" (INAG, 2009). Therefore, we have three streams with similarly "acceptable" water qualities. However, water quality could change under high discharge events, which was not addressed in this study.

Benthic invertebrate metrics, biotic indices and functional feeding groups differed between streams, and the variation across streams was correlated with urbanisation (TIA), which indicates an increasing gradient of impairment from stream S1 to stream S3. Therefore, although the chemical data did not indicate strong impacts on water quality in the three streams, biotic data showed that they were heavily ecologically impaired. For example, the number of taxa at the suburban site (S1 with 3 % TIA) was higher than in the two urban streams (S2 and S3 with 19 and 61 % TIA, respectively). This is consistent with observations by Stepenuck *et al.* (2002), who reported a decrease in the number of taxa in more urbanised areas (along a gradient of 8 to 12 % TIA). The number of EPT taxa also decreased as the TIA increased, which agrees with previous reports by Roy *et al.* (2003), Freeman & Schorr (2004) and Walsh *et al.* (2007). The same applies to the biotic indices, which decreased from stream S1 to stream S3. This relationship between proportion of imperviousness and biotic scores has also been reported by Booth *et al.* (2004), Miserendino *et al.* (2008) and Purcell *et al.* (2009).

Why were the biological and chemical data inconsistent? The differences observed between sites may be the result of prior site conditions which prevented the establishment of sensitive taxa in the urban streams. Conditions such as chemical fluctuations or storm events can impair the establishment of sensitive taxa. Past unfavourable conditions can also be reflected in the availability of resources for invertebrates. Streams S1 and S2 contained shredders and grazers, while in stream S3 they were replaced by consumers of fine particles of organic matter. Aside from a lower litter input into urbanised streams (negatively affecting shredders) and a high rate of sedimentation, which covers stable substrates where algae may grow (negatively affecting grazers), perturbed streams receive large amounts of fine particles and dissolved organic matter, which accumulates in the stream bed and is consumed by microorganisms and collectors. In addition to a history of unfavourable conditions and the availability of food resources, a third factor that could explain the differences in the biotic community between streams is the physical habitat condition. We expect a high level of heterogeneity to accommodate more species and a higher degree of ecosystem function, as well as protection/resistance against runoff. Stream S1 had the most diverse habitat, in which submerged stones, sand, aquatic plants, riffles and pools were present, and the complex river banks potentially protected in-stream biota and habitat from runoff. The stream with the least diverse habitat and more exposure to storm waters due to a high degree of channelization, clear river banks and direct discharges from pipes was S3. Here the substrate was dominated by sand, with a high degree of embeddedness. Such habitats have frequently been referred to as poor (e.g., Quinn & Hickey, 1990). In addition, direct discharges and the absence of riverine vegetation drastically affect habitat heterogeneity and stream biota.

A final measurement of environmental quality was a functional one: litter breakdown. The breakdown rates of the alder leaves incubated in our streams were lower than those reported for another local stream not affected by urbanisation (0.0413/day; Abelho, 2009). This result was unexpected, given that our streams had high nutrient concentrations, which are known to stimulate decomposers and consequently litter processing (Ferreira *et al.*, 2006b; Gulis *et al.*, 2006). However, this stimulation could have been minor in the case of the nutrient-rich alder leaves, where decomposers are most likely not nutrient-limited and therefore not sensitive to the concentration of dissolved nutrients. The breakdown of the thin and soft alder leaves was, however, expected to be sensitive to the projected increase in physical abrasion resulting from high current velocity and sediment displacement during floods in our streams, which also stimulates litter breakdown (Ferreira *et al.*, 2006a).

The breakdown rates of oak leaves in our streams were within the range found in non-impacted local streams (0.0058–0.0138/d; Castela *et al.*, 2008; Abelho, 2009). They were therefore below our expectations because we anticipated that the decomposers on the nutrient-poor oak leaves would be nutrient-limited and would therefore be more responsive to the high nutrient concentration in the water of urbanised streams, leading to accelerated breakdown rates when compared with those found for streams with lower nutrient availability (Ferreira *et al.*, 2006b; Gulis *et al.*, 2006). The generally lower breakdown rates in our streams when compared with those in non-urbanised streams may be related to the presence of compounds inhibiting the microbial and macroinvertebrate communities in the sampled streams.

Consistent with our results, in a large-scale survey, Feio *et al.* (2010) also found that the decomposition rates of alder and oak were negatively correlated with ammonium and nitrite concentrations and with urban area in the catchment, and positively correlated with dissolved oxygen. Our results contrast with those reported by Meyer *et al.* (2005) and Imberger *et al.* (2008), who found an increase in litter breakdown rate with an increase in urbanisation, which the authors attributed to increased physical abrasion related to flash floods and stimulation of microbial activity caused by increases in dissolved nutrients and temperature. However, at very high levels of urbanisation, breakdown rates were reported to decrease as a result of high concentrations of toxic compounds affecting the invertebrate community, as reported by Chadwick *et al.* (2006).

In summary, the urbanisation gradient, translated into a gradient of total impervious area from stream S1 to stream S3, was reflected in the changes in the benthic macroinvertebrate communities and in the litter breakdown rates, which indicated ecological impairment at structural and functional levels. However, the effects of urbanisation were not detected in water physical-chemical variables. This suggests that biotic (structural and functional) indices integrate changes over longer periods, providing more accurate information on the system state than a measure of water quality at a specific time, which may not reflect previous quality states or related ecological risks and impacts (Metcalf, 1989). Therefore, it is important to combine physical-chemical measurements with ecological integrity bioindicators to obtain an accurate bioassessment. Particularly important in this context is the litter decomposition rate as a functional indicator of environmental quality because it integrates the presence and activity of decomposers and invertebrate consumers, which may be affected by urbanisation in several ways.

ACKNOWLEDGEMENTS

We thank Alan Covich, Ian Dodkins, Nathan P. Nibbelink and Maria João Feio for technical help, field support and several discussions. Elsa Rodrigues and Cristina Docal operated the ion chromatograph. We also acknowledge the helpful comments provided by Chris Walsh and an anonymous referee on an earlier version of the manuscript. Financial support was provided by Talentia (Junta de Andalucía, fellowship to AO, Talentia/2008), EMAE, IMAR-CMA, and the QREN-POPH, the European Social Fund and National Funds through the FCT-Portuguese Science Foundation (fellowship to VF, SFRH/BPD/34368/2006).

REFERENCES

ABELHO, M. 2009. Leaf-litter mixtures affect breakdown and macroinvertebrate colonization rates in a stream ecosystem. *Internat. Rev. Hydrobiol.*, 94: 436–451.

ALBA-TERCEDOR, J., P. JÁIMEZ-CUÉLLAR, M. ÁLVAREZ, J. AVILÉS, N. BONADA, J. CASAS, A. MELLADO, M. ORTEGA, I. PARDO, N. PRAT, M. RIERADEVALL, S. ROBLES, C. E. SÁINZ-CANTERO, A. SÁNCHEZ-ORTEGA, M. L. SUÁREZ, M. TORO, M. R. VIDAL-ABARCA, S. VIVAS & C. ZAMORA-MUÑOZ. 2002. Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes 'BMWP'). *Limnetica*, 2: 175–185.

APHA. 1995. *Standard methods for the examination of water and watershed.* 19th edition. American public health association. Washington DC, USA. 1050 pp.

ARNOLD, C. L. & C. J. GIBBONS. 1999. Impervious surface coverage: the emergence of a key environmental indicator. *J. Am. Planning.,* 62: 243–258.

BERGFUR, J. 2007. Seasonal variation in leaf-litter breakdown in nine boreal streams: implications for assessing functional integrity. *Fundamental and Applied Limnology*, 169: 319–329.

BOOTH, D. B., J. R. KARR, S. SCHAUMAN, C. P. KONRAD, S. A. MAOLEY, M. G. LARSON & S. J. BURGES. 2004. Reviving urban streams: land use, hydrology, biology, and human behaviour. *J. Am. Water Res. Ass.*, 40: 1351–1364.

BRETT, M. T., G. B. ARHONDITSIS, S. E. MUELLER, D. M. HARTLEY, J. D. FRODGE & D. E. FUNKE. 2005. Non-point source impacts on stream nutrient concentrations along a forest to urban gradient. *Environ. Manag.*, 35: 330–342.

CASTELA, J., V. FERREIRA & M. A. S. GRAÇA. 2008. Evaluation of stream ecological integrity using litter decomposition and benthic invertebrates. *Environ. Poll.*, 153: 440–449.

CHADWICK, M. A., DOBBERFUHL, D. R., BENKE, A. C., HURYN, A. D., SUBERKROPP, K. & J. E. THIELE. 2006. Urbanization affects stream ecosystem function by altering hydrology, chemistry, and biotic richness. *Ecol. Appl.*, 16: 1796–1807.

FEIO, M. J., T. ALVES, M. BOAVIDA, A. MEDEIROS & M. A. S. GRAÇA. 2010. Functional indicators of stream health: a river-basin approach. *Freshwat. Biol.*, 55: 1050–1065.

FERREIRA, V., M. A. S. GRAÇA, J. L. M. P. DE LIMA & R. GOMES. 2006a. Role of physical fragmentation and invertebrate activity in the breakdown rate of leaves. *Archiv Hydrobiol.*, 165: 493–513.

FERREIRA, V., V. GULIS & M. A. S. GRAÇA. 2006b. Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. *Oecologia*, 149: 718–729.

FREEMAN, P. L. & M. S. SCHORR. 2004 Influence of watershed urbanization on fine sediment and macroinvertebrate assemblage characteristics in Tennessee Ridge and Valley Streams. *J. Freshwat. Ecol.,* 19: 353–362.

GAMA, M., A. L. CONÇALVES, V. FERREIRA, M. A. S. GRAÇA & C. CANHOTO. 2007. Decomposition of fire exposed eucalyptus leaves in a Portuguese lowland stream. *Internat. Rev. Hydrobiol.*, 92: 229–241.

GESSNER, M. O. & E. CHAUVET. 2002. A case for using litter breakdown to assess functional stream integrity. *Ecol. Appl.*, 12: 498–510.

GULIS, V., V. FERREIRA & M. A. S. GRAÇA. 2006. Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. *Freshwat. Biol.*, 51: 1655–1669.

HAGEN, E. M., J. R. WEBSTER & E. F. BENFIELD. 2006. Are leaf breakdown rates a useful measure of stream integrity along an agriculture landuse gradient? *J. N. Am. Benthol. Soc.*, 25: 330–343.

IMBERGER, S. J., C. J. WALSH & M. R. GRACE. 2008. More microbial activity, not abrasive flow or shredder abundance, accelerates breakdown of labile leaf litter in urban streams. *J. N. Am. Benthol. Soc.*, 27: 549–561.

INAG. 2009. *Critérios para a classificação do estado das massas de água superficiais-Rios e albufeiras*. Ministério do Ambiente, do Ordenamento e do Desenvolvimento Regional. 71 pp.

METCALF, J. 1989. Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status in Europe. *Environ. Poll.*, 60: 101–139.

MEYER, J. L., M. J. PAUL & W. K. TAULBEE. 2005. Stream ecosystem function in urbanizing landscapes. *J. N. Am. Benthol. Soc.*, 24: 602–612.

MISERENDINO, M. L., C. BRAND & C. Y. DI PRINZIO. 2008. Assessing urban impacts on water quality, benthic communities and fish in streams of the Andes Mountains, Patagonia (Argentina). *Water Air Soil Pollut.*, 194: 91–110.

MORGAN, II R. P., K. M. KLINE & S. F. CUSHMAN. 2007. Relationships among nutrients, chloride and biological indices in urban Maryland streams. *Urban Ecosyst.*, 10: 153–166.

PASCOAL, C., M. PINHO, F. CÁSSIO & P. GOMES, 2003: Assessing structural and functional ecosystem condition using leaf breakdown: studies on a polluted river. *Freshwat. Biol.,* 48: 2033–2044.

PASCOAL, C. & F. CASSIO. 2004. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. *Appl. and Environ. Microbiol.*, 70: 5266–5273.

PAUL, M. J. & J. L. MEYER. 2001. Streams in the urban landscape. *Ann. Rev. Ecol. Syst.*, 32: 333–365.

PÉREZ, J., M. MENÉNDEZ, S. LARRAÑAGA & J. POZO. 2011. Inter- and intra-regional variability of leaf litter breakdown in reference headwater streams of Northern Spain: Atlantic versus Mediterranean streams. *Internat.Rev. Hydrobiol.*, 96: 105–117.

PURCELL, A. H., D. W. BRESSLER, M. J. PAUL, M. T. BARBOUR, E. T. RANKIN, J. L. CARTER

& V. H. RESH. 2009. Assessment tools for urban catchments: developing biological indicators based on benthic macroinvertebrates. *J. Am. Water Res. Ass.*, 45: 306–319.

QUINN, J. M. & C. W. HICKEY. 1990. Characterisation and classification of benthic invertebrate communities in 88 New Zealand rivers in relation to environmental factors. *New Zeal. J. Mar. Fresh. Res.,* 24: 387–409.

ROY, A. H., A. D. ROSEMOND, M. J. PAUL, D. S. LEIGH & J. B. WALLACE. 2003. Stream macroinvertebrate response to catchment urbanization (Georgia, U.S.A.) *Freshwat. Biol.,* 48: 329–346.

STEPENUCK, K. F., R. L. CRUNKILTON & L. Z. WAN. 2002. Impacts of urban landuse on macroinvertebrate communities in southeastern Wisconsin streams. *J. Am. Water Res. As.*, 38: 1041–1051.

TACHET, H., P. RICHOUX, M. BOURNAUD & P. USSEGLIO-POLATERRA. 2002. *Invertébrés d'eau douce. Systématique, biologie, écologie,* CNRS Editions, Paris. 588 pp.

TER BRAAK, C. J. F. & P. SMILAUER. 1998. *CANOCO reference manual and user's guide to CANOCO for Windows: software for canonical community ordination, version 4*. Microcomputer Power. Ithaca, NY. 351 pp.

UN Population Division. 2005. *Urban and rural areas 2005*. United Nations. New York, USA. 210 pp.

USEPA. 1998. *Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish*. US Environmental Protection Agency. 339 pp.

WALSH, C. J., A. K. SHARPE, P. F. BREEN & J. A. SONNEMAN. 2001. Effects of urbanization on streams of the Melbourne region. Victoria, Australia, I. Benthic macroinvertebrates communities. *Freshwat. Biol*., 46: 535–551.

WALSH, C. J., A. H. ROY, J. W. FEMINELLA, P. D. COTTINGHAM, P. M. GROFFMAN, II & R. P. MORGAN. 2005. The urban stream syndrome: current knowledge and the search for a cure. *J. N. Am. Benthol. Soc.*, 24: 706–723.

WALSH, C. J., K. A. WALLER, J. GEHLING & R. M. NALLY. 2007. Riverine invertebrate assemblages are degraded more by catchment urbanization than by riparian deforestation. *Freshwat. Biol.*, 52: 574–587.

WATER FRAMEWORK DIRECTIVE 2000/60/EC. 2000. *Water Framework Directive of the European Parliament and the Council, of 23 October 2000, establishing a framework for Community action in the field of water policy*. Official Journal of the European Communities., L327: 1–72.

WENGER, S. J., A. H. ROY, C. R. JACKSON, E. S. BERNHARDT, T. L. CARTER, S. FILOSO, C. A. GIBSON, W. C. HESSION, S. S. KAUSHAL, E. MARTÍ, J. L. MEYER, M. A. PALMER, M. J. PAUL, A. H. PURCELL, A. RAMÍREZ, A. D. ROSEMOND, K. A. SCHOFIELD, E. E. SUDDUTH & C. J. WALSH. 2009. Twenty-six key research questions in urban stream ecology: an assessment of the state of science. *J. N. Am. Benthol. Soc.*, 28: 1080–1098.

YANG, L., C. HUANG, G. COLLIN, HOMER, C.G., WYLIE, B. K. & COAN, M. J. 2003. An approach for mapping large-scale impervious surfaces: synergistic use of Landsat-7 ETM+ and high spatial resolution imagery. *Can. J. Remote Sensing*, 29: 230–240.