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Least Disturbed Condition for European Mediterranean rivers

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HIGHLIGHTS

- Mediterranean Least Disturbed Streams (LDS) show various types of hydromorphological alterations.
- Common LDS thresholds were found for all river types in water quality and land use.
- But a lower threshold value for DO (60%) was retained for temporary streams.
- Invertebrate, diatom and macrophyte data were used to settle biological Least Disturbed Condition.

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ABSTRACT

The present report describes a three-step approach that was used to characterize and define thresholds for the Least Disturbed Condition in Mediterranean streams of four different types, regarding organic pollution and nutrients, hydrological and morphological alterations, and land use. For this purpose, a common database composed of national reference sites (929 records) from seven countries, sampled for invertebrates, diatoms and macrophytes was used. The analyses of reference sites showed that small (catchment <100 km^2) siliceous and non-siliceous streams were mainly affected by channelization, bank alteration and hydropeaking. Medium-sized siliceous rivers were the most affected by stressors: 25–43% of the samples showed at least slight alterations regarding channelization, connectivity, upstream dam influence, hydropeaking and degradation of riparian vegetation. Temporary streams were the least affected by hydromorphological changes, but they were nevertheless affected by alterations in riparian vegetation. There were no major differences between all permanent stream types regarding water quality, but temporary streams showed lower values for oxygenation (DO) and wider ranges for other variables, such as nitrates. A lower threshold value for DO (60%) was determined for this stream type and can be attributed to the streams' natural characteristics. For all other river types, common limits were found for the remaining variables (ammonium, nitrate, phosphate, total P, % of artificial areas, % of intensive and extensive agriculture, % of semi-natural areas in the catchment). These

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values were then used to select the list of reference sites. The biological communities were characterized, revealing the existence of nine groups of Mediterranean invertebrate communities, six for diatoms and five for macrophytes: each group was characterized by specific indicator taxa that highlighted the differences between groups.

1. Introduction

The Mediterranean climate (sensu KÖPPEN, 1923) is a variety of subtropical climate occurring not only in the Mediterranean basin but also in other areas of the world, such as California, western and southern Australia, southwestern Africa, central Asia and central coastal Chile. This climate is characterized by marked seasonal differences, with dry summers and mild winters. Rivers under the influence of a Mediterranean climate (hereby called Mediterranean rivers) show characteristic sequences of floods in autumn–winter and droughts that develop continuously and gradually over the summer (Resh et al., 1990; Gasith and Resh, 1999). The freshwater communities are thus adapted to the natural variability through shorter life spans, mechanisms to resist or avoid desiccation, and higher colonization rates (Lytle and Poff, 2004; Bonada et al., 2007; Stromberg et al., 2008; Santos, 2010). These Mediterranean communities are, therefore, different from those of temperate rivers, showing inter-annual fluctuations in richness and composition and in trophic structure (Ferreira et al., 2002a, 2002b; Bonada et al., 2007; Sabater et al., 2008; Feio et al., 2010). Certain functional processes are also characteristic of these systems. Leaf litter decomposition is slower than in temperate areas, and is performed by different shredders and fungi (Gonçalves et al., 2006). Riparian inputs to these streams occur over longer periods of time, rather than being concentrated in autumn (Gasith and Resh, 1999), less allochthonous organic matter is retained (Sabater et al., 2008) than in temperate streams.

Mediterranean river ecosystems have a highly endangered biodiversity, which cannot be dissociated from the long history of human disturbances (Zeder, 2008). Human competition for water enhances the natural deficit in water resources, due to a mean annual precipitation lower than the mean potential evapotranspiration (Gasith and Resh, 1999). Additionally, water diversion, flow regulation, increased salinity, pollution and introduced species have impacted the Mediterranean ecosystems over time (Moyle, 1995; Gasith and Resh, 1999; Aguiar and Ferreira, 2005; Hooke, 2006).

The need to prevent further deterioration and protect and improve the status of aquatic ecosystems is one of the main aims of the Water Framework Directive (WFD; European Commission, 2000). The directive establishes a framework for the determination of the ecological status of all water bodies and their regular bioassessment, which should be measured through the deviation from reference conditions. According to the WFD, the reference conditions for each type of water body correspond to the high status, where physical–chemical, hydromorphological and biological elements show no or only very little influence of anthropogenic activities. However, the exact meaning of "very little influence" is not given in the WFD (Moss, 2008) and other potential synonyms such as the terms "minor changes" or "minimally impaired" or "near natural", frequently used in the literature, are also very difficult to define in a rigorous and consensual way. Additionally, the WFD establishes the existence of an Intercalibration Exercise, which aims to assure that class-boundaries are defined according to the normative definitions and are comparable among the members of the European Commission. This implies that reference conditions should also be comparable among these countries (Birk et al., 2012), as they are the basis for the establishment of classification systems and class boundaries.

In practice, most of the currently used methods to define reference conditions are based on the information collected now or in the recent past from reference sites. These are usually selected based on knowledge of the changes caused by anthropogenic activities. Many authors have discussed the existing constraints of reference conditions for bioassessment and have attempted to establish criteria for selecting reference sites (e.g., Reynoldson et al., 1997; Landres et al., 1999; Ferreira et al., 2002b; Hering et al., 2003; Nijboer et al., 2004; Stoddard et al., 2006; Sanchéz-Montoya et al., 2009; Hawkins et al., 2010; Birk et al., 2012; Pardo et al., 2012; Smith and Tran, 2012). Stoddard et al. (2006) defined four types of reference conditions: 1) the condition of ecosystems at some time in the past (historical condition), 2) the best of today's existing conditions (Least Disturbed Condition); 3) the condition of systems in the absence of significant human disturbance (Minimally Disturbed Condition); and 4) the condition to be achieved with improved management (Best Attainable Condition). Here, we use the second concept, Least Disturbed Condition (LDC) to describe the present best available situation in European Mediterranean rivers, which is a practical concept to which all countries can be anchored at the same point on the impact gradient. Considering the long history of human presence, the intensive water demand in the Mediterranean Basin and the difficulty in knowing the condition before human influence, it is not possible for this region to set the high ecological status (pristine state), as a key starting point as defended by Moss (2008), even though this would be the ideal approach.

In the context of the Intercalibration Exercise, it is equally important to have enough representativeness of sites for all river types. In view of this, we propose here a selection method of "benchmarks" for IC purposes, which have a comparable and known level of anthropogenic degradation corresponding to no or only slight alterations. This study aims, therefore, to characterize the present abiotic and biological (for invertebrates, diatoms and macrophytes) LDC in Mediterranean rivers. We intend also to list the main impacts affecting the various stream types, while proposing a methodology for the selection of reference sites, based on common criteria. For this purpose, we followed a sequential approach using the available information on water chemistry and physics, hydromorphology and land use. Data were provided by seven Mediterranean countries (Portugal, Spain, France, Italy, Slovenia, Greece and Cyprus) participating in the 2nd phase of the Intercalibration Exercise (2008 to 2011) within the Mediterranean Geographic Intercalibration Group (MedGIG).

2. Material and methods

2.1. Dataset

For the composition of an initial dataset, the seven countries provided data from their national reference sites, that were selected based on their national criteria, and that could be included in one of four Intercalibration river types (previously defined by the Mediterranean GIG), as follows:

1. Type 1: small rivers (catchment area <100 km^2), siliceous geology (e.g., schist, granite), highly seasonal hydrological regime
2. Type 2: rivers with medium sized catchments (100–1000 km^2), siliceous geology, highly seasonal hydrological regime
3. Type 3: rivers with small and medium-sized catchments (<1000 km^2), non-siliceous (e.g. calcareous, ophiolite), highly seasonal regime
4. Type 4: rivers with small and medium-sized catchments (<1000 km^2), temporary hydrological regime.

Samples of invertebrates (455 samples: 157 of type 1; 30 of type 2; 208 of type 3; and 60 of type 4), diatoms (311 samples: 115 of type 1; 46 of type 2; 121 of type 3; and 29 of type 4) and macrophytes (139 samples: 34 of type 1; 26 of type 2; 61 of type 3; and 18 of type 4) were collected over several years (1998–2010) and seasons. The abiotic data gathered during the field campaigns were also used here, as well as the information that could translate the effect of human stressors and the condition of streams (reach, segment and catchment scales) regarding morphology, hydrology, land use, and organic and nutrient pollution. Variables that could be provided by most of the MS with a minimum loss of information were retained for further analyses (Table 1). The information related to stream hydromorphology was provided in the form of categorical data, in order to better harmonize the data available for each MS. This proved to be necessary, as an a priori comparative study showed that the sources of information for each variable varied from country to country even though there were many parallels. For example, while Portugal and Slovenia used the River Habitat Survey (EA, 2003) to assess channelization, Italy and Cyprus used the Caravaggio method (see Buffagni et al., 2010), which is an adaptation of the former and France used the QRB index (Munné et al., 2003). Therefore, for those variables, sites were classified by each country based on the available information, in relation to their degree of alteration from the natural state, as: 1 (no alterations), 2 (slight alterations), 3 (moderate alterations) or 4 (strong alterations). A common understanding of the meaning of each class for each variable was initially established among countries and is described in Table 2. Along with their classifications all countries also provided the sources of the information. Even though all variables fall in the above-mentioned four categories, the actual variables translating each type of stressor could in the end be different, depending on whether they were collected at a sampling site for diatoms, invertebrates or macrophytes (see Table 1). The information available on diatom sampling sites was less detailed, as, traditionally, hydromorphological changes were not considered relevant for this algal group and therefore this information was often not collected along with the biological samples. For invertebrates this information was available for most sites; and as macrophytes were collected later on, many during the Intercalibration Exercise, it was also possible to gather this information for most of the sites. The variables for water chemistry and physical properties, organic and land use were numerical and based on spot measurements for all parameters. All variables used in this study are listed in Table 1.

Table 1
List of stressor variables used in the data analyses, with respective scales of measurement.

Category of stressor	Variables	Scale of measurement
Morphology	Channelization (1–4)[a,c]	Reach
	Bank alteration (1–4)[a,c]	Reach
	Local habitat alteration (1–4)[a,c]	Site
	Riparian vegetation (1–4)[a]	Site
	General morphology[b]	Reach
Hydrology	Connectivity (1–4)[a]	Reach
	Stream flow (1–4)[a,c]	Site
	Upstream dam influence (1–4)[a,c]	Reach
	Hydropeaking (1–4)[a,c]	Reach
	General hydrology[b]	Reach
Water physics and chemistry	DO (mg/l)[c]	Site (spot measurements)
	DO (%)[a,b]	
	N-NH4$^+$ (mg/l)[a,b,c]	Site (spot measurements)
	N-NO$_3^-$ (mg/l)[a,b,c]	Site (spot measurements)
	P-Total (mg/l)[a,b,c]	Site (spot measurements)
	P-PO$_4^{3-}$ (mg/l)[a,c]	Site (spot measurements)
Land use	% artificial areas[a,b,c]	Catchment
	% intensive agriculture[a,b,c]	Catchment
	% extensive agriculture[a,b,c]	Catchment
	% semi-natural areas[a,b,c]	Catchment
	% urbanization (reach)[b]	Reach
	% non-natural land use[b]	Reach
	% agriculture[b,c]	Reach
	% urbanization[a,c]	Reach

[a] From invertebrate database.
[b] From diatom database.
[c] From macrophyte database.

The biological databases were composed of data from the selected benchmarks (see below). Data were derived from samples collected in spring for invertebrates, spring–summer season for macrophytes, and all year-round for diatoms. Macroinvertebrates were sampled (Surber or handnet; 500 μm mesh) in all countries with a multi-habitat procedure and were identified to family level. All individuals in a sample were counted. Epilithic diatom assemblages were sampled, treated in the laboratory and studied according to European Standards (EN, 13946, 2003; EN, 14407, 2004; EN, 14996, 2006) and as described by Kelly et al. (1998). Diatoms were identified to the lowest taxonomic rank possible (usually to species level) and 400 valves were counted, so the data consists of relative abundance. Diatom taxonomy was also harmonized at the European level during this exercise, and the current classifications were used in this study (Kahlert et al., 2012). Macrophytes (vascular plants, bryophytes, algae) were sampled according to European standards (EN, 14184, 2003; EN, 14996, 2006) by zig-zagging across the channel or by walking along banks and identified to species level, except for some macroalgae which were identified only to genus level. The percentage cover of each species was recorded and used for data analyses.

2.2. Data analyses

As described above, the concept behind our data analysis is that: a) pristine sites no longer exist in the Mediterranean region and therefore, the best sites that we can possibly find correspond to the least-disturbed conditions; b) it is necessary to find a common interpretation among all Mediterranean countries of the meaning of “Least Disturbed”; and c) sites with no changes in channel and bank morphology, riparian vegetation or flow, with no impoundments and no alterations in habitats will guarantee overall quality, up to a certain point, as the absence of these changes indicates no influence of human presence on the site and reach. In spite of this assumption, the use of the 90th or 10th percentiles in Step II (see below) aimed to further refine the selection and prevent for unexpected chemical disturbance at the site or significant changes in land use in the catchment.

In view of this, the abiotic data were accomplished through a three-step procedure, where sample records were treated independently (flow chart in Fig. 1):

Step I Initial selection of reference sites. Samples from the entire database of national reference sites were first selected if all the categorical variables were classified in class 1, no impact. This selection corresponded, therefore, to sites that are not affected by hydrological or morphological alterations, including in the channel, banks, habitats, connectivity, and riparian corridors.

Step II Least Disturbed Condition. Based on the above selection, water chemical and physical conditions and land use in the catchment area were characterized by IC type using histograms and boxplots. Special attention was given to the potential differences between permanent and temporary streams, especially during summer, where low water levels can naturally lead to higher concentrations of nutrients, higher temperatures and lower oxygenation levels. Thresholds (maximum or minimum admissible values for Mediterranean reference sites) were then established by calculating the 90th percentile for most numerical variables (maximum admissible value), based on the sites selected in Step I, with the exception of % semi-natural areas (10th percentile), % dissolved oxygen and dissolved oxygen (mg/l) (an interval is accepted based on the 5th and 95th percentiles). The use of these percentiles allowed

Table 2
List of categorical stressor variables used and meaning of each quality class.

Variables	Pressure intensity			
	1. No/unaltered	2. Low/slightly altered	3. Medium/altered	4. High/highly altered
Channelization	No channelization, no alteration of the "natural" cross section (no "hard work" affecting the entire river). No flow velocity increase.	Slight alteration (less than 10% of the segment affected by "hard work"). No flow velocity increase.	Significant alteration (a main part of the segment is affected by "hard work"). Flow velocity increase.	Strong alteration (straightened river, technical-uprofile section). Flow velocity increase.
Bank alteration	No alteration; natural vegetation, no artificial erosion due to vegetation removal or bank mowing.	Small alterations.	Significant alterations.	Clear bank alteration through livestock and human use.
Local habitat alteration	No alteration of instream habitats, no "soft work" (bank protection), no significant sedimentation, no important degradation of the river bed (incision, deepening).	Slight alterations. <20% of the site is affected by "soft works".	Significant alterations.	Strong alterations.
Riparian vegetation	No alteration of the riparian vegetation (i.e. adjacent riparian woods appropriate to the type and geographical location of the river).	Slight alteration of the riparian vegetation. Good riparian forest cover with only a few and isolated alien species.	Strong alteration of the riparian vegetation. Non-continuous riparian corridor.	Riparian vegetation completely altered due to human activities, including replacement by alien invasive species.
General morphology	No alterations. Should summarize all the above aspects in channelization, bank alteration, local habitat alteration, and riparian vegetation.	Small alterations. Should summarize all the above aspects in channelization, bank alteration, local habitat alteration, and riparian vegetation.	Significant alterations. Should summarize all the above aspects in channelization, bank alteration, local habitat alteration, and riparian vegetation.	Strong alterations. Should summarize all the above aspects in channelization, bank alteration, local habitat alteration, and riparian vegetation.
Connectivity	No alterations in longitudinal connectivity related to the presence of artificial barriers (dams, weirs or other).	Small alterations in longitudinal connectivity (presence of a minor dam or weir made of natural material, e.g. stones) and allowing for water flow over it.	Significant alterations.	Strong alterations.
Stream flow	Natural hydrological features.	Slow flow regime alteration due to a small water diversion or some small water diversions.	Significant alterations.	Strong alterations.
Upstream dam influence	No influence of dam located upstream the site itself (flow regulation, temperature, sedimentation, reservoir flushing).	Slight influence of dam located upstream from the segment itself (flow regulation, temperature, sedimentation, reservoir flushing). No clear potential effect on the fish fauna at the site.	Significant influence of dams.	Strong influence of dam located upstream from the reach itself (flow regulation, temperature, sedimentation, reservoir flushing).
Hydropeaking	No hydropeaking, no alteration of the hydrograph.	Hydropeaking, slight alteration of the hydrograph.	Significant alteration.	Hydropeaking, alteration of the hydrograph.
General hydrology	No alterations. Should summarize all the above aspects in connectivity, stream flow, upstream dam influence and hydropeaking.	Small alterations. Should summarize all the above aspects in connectivity, stream flow, upstream dam influence and hydropeaking.	Significant alterations. Should summarize all the above aspects in connectivity, stream flow, upstream dam influence and hydropeaking.	Strong alterations. Should summarize all the above aspects in connectivity, stream flow, upstream dam influence and hydropeaking.

the exclusion of possible outliers, but also the definition of a threshold close to the maximum (or minimum) values that presently characterize the best conditions for Mediterranean rivers. A single value was attempted for all stream types, but exceptions were considered for water chemistry, assuming that the highly variable flow conditions could naturally affect the concentration of chemical substances and oxygen in the water.

Step III Selection of benchmarks. Sites excluded in Step 1 but with categorical variables classified as up to 2 (i.e., slight alterations) and simultaneously with all numerical values below the thresholds defined in Step II, were re-included in the final list of benchmarks. This allowed a better representation of reference sites by type while maintaining a high level of quality, since only slight hydrological modifications were accepted at this level, and values for water chemistry and non-natural land use were kept low.

The Least Disturbed communities were characterized through multivariate analyses for each biological element (invertebrates, diatoms and macrophytes). Patterns in taxa distribution (groups) were first defined by classification analyses (UPGMA; Bray–Curtis similarity; Primer 6). The select groups should be clearly distinct in the cluster and include at least 5 sites. Isolated sites or too small groups (<5 sites) were excluded from this analysis because they could not be used to define biological patterns. The groups were complementarily checked for consistency with Multidimensional Scaling analyses. Those groups must also have a significant degree of segregation ($R > 0.3$ and $p < 0.05$) in ANOSIM tests (Primer 6). This is a non-parametric test, applied to the rank similarity matrix and analogous to the ANOVA, analysis of variance (Clarke and Warwick, 2001). IC types were not considered a priori, since they were expected to be too broad to be meaningful for the three biological elements individually. Nevertheless, this consistency was also checked by plotting IC type symbols to sites in the Multidimensional Scaling ordination. Each group was also characterized by calculating the mean ± SD for their major abiotic characteristics (altitude, geology, hydrological regime and catchment area and, additionally, conductivity for diatoms and macrophytes). For each biological element, significant differences in the abiotic features for the groups were also tested through the permutational multivariate analysis of variance, PERMANOVA (Primer 6 + PERMANOVA).

Indicator taxa were determined for each of the groups (PcOrd vs 6.0). The Indicator Species Analysis (Dufrene and Legendre, 1997) selects the species/taxa that distinguish the groups, i.e., those that are frequent and abundant at the majority of the sites of a given group but not in the others. The indicator value for each taxon (IV) was determined based on its relative frequency and relative average abundance in clusters. The highest indicator value for a given taxon (IVmax) across groups is the overall indicator value of that taxon. Its statistical significance (p-value) was evaluated through the Monte Carlo method.

For all the above analyses, data for invertebrates and diatoms were previously transformed by fourth root and data for macrophytes by square root.

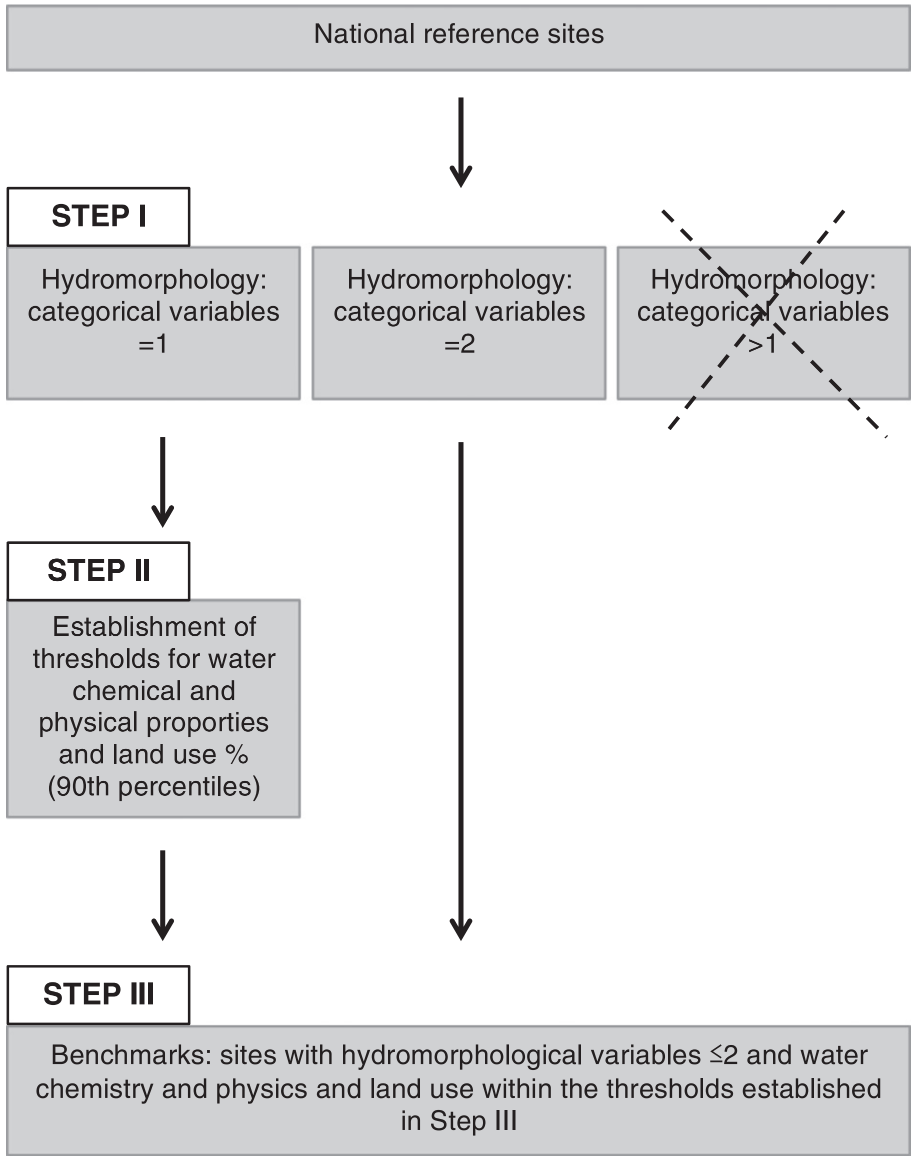

Fig. 1. Flow chart outlining the three main steps followed in the data analysis aiming the selection benchmarks from national reference sites.

3. Results

3.1. Abiotic thresholds and LDS selection

In spite of the difference in categorical variables used, a priori test showed that national reference sites common to diatoms and invertebrates or macrophytes ($n = 63$) were classified similarly in 96% of the cases for morphology and 86% for hydrology. This comparison was made between the general morphology classification of diatom sites against a global evaluation of morphology, based on the worst classification among the 4 morphological variables and the 3 hydrological variables. In any case the difference was greater than one class. Therefore the categorical classification systems (diatoms vs macrophytes and invertebrates) were considered equivalent.

After Step I, 140 samples from invertebrate sites, 110 from diatom sites and 60 from macrophyte sites were selected, corresponding respectively to 30%, 35% and 44% of the initial number. The main hydromorphological stressors affecting the streams of each IC type are shown in Table 3. In small siliceous (type 1) and non-siliceous streams (type 3) channelization, bank alteration and hydropeaking were the variables responsible for more exclusions after Step I. Medium-sized siliceous rivers were affected by a larger number of stressors, with 25–43% of the samples with at least slight alterations regarding channelization, connectivity, upstream dam influence, hydropeaking and degradation of riparian vegetation. Riparian vegetation alteration was the major problem affecting temporary streams (type 4), although this river type was the least affected overall.

Boxplots showed that there were no major differences (Fig. 2a) in mean values between stream types 1, 2 and 3, even though there were often many outliers in water quality variables, especially for nitrates in types 1 and 2 (Fig. 2a). On the other hand, temporary streams showed lower oxygenation levels (Fig. 2b); while the median level of dissolved oxygen was ≈100% in permanent streams, it was closer to 90% but with a wider dispersion and lower 5th percentile in temporary streams (Fig. 2b).

Differences between stream types regarding land use in the catchment were mainly in the percentages of intensive agriculture and natural areas (Fig. 3). Artificial areas occupied a low percentage of the catchment areas (<1% in most cases) but with a slightly broader distribution of values and two outliers for the siliceous rivers. Catchments of temporary rivers were not occupied by a measurable amount of artificial areas. Major differences appeared for the percentage of intensive

Table 3
Percentage of sites classified in class 1 or above (>1) or class 2 (>2) or above by IC stream types (type 1, type 2, type 3 and type 4).

	TYPE 1		TYPE 2		TYPE 3		TYPE 4	
	>1	>2	>1	>2	>1	>2	>1	>2
Channelization	55	3	30	29	68	3	7	4
Bank alteration	57	3	14	0	66	2	12	3
Connectivity	15	4	41	29	5	0	1	0
Local habitat alteration	15	0	14	0	7	0	6	1
Stream flow	6	1	21	5	2	0	0	0
Upstream dam influence	1	1	26	5	0	0	0	0
Hydropeaking	34	1	26	5	59	0	0	0
Riparian vegetation	31	3	43	0	12	3	36	5

agriculture: while non-siliceous streams had only up to 2%, temporary streams accounted for ca. 23%.

The comparison of 90th and 10th percentiles (Table 4) showed that global values were nevertheless similar, with or without the inclusion of temporary streams. The P-PO_4^{3-} level was high in summer (0.10 compared with the overall 0.06 global value), but not when averaged over all seasons. The exception was O_2 for which the 10th percentile was lower than for other rivers (60.3% all year-round, and 22.5% in summer). Therefore, the global thresholds obtained for all rivers and seasons were adopted, with the exception of the lower oxygenation level for temporary rivers (where type 4 all-season values were adopted).

Application of these thresholds (Table 5) to all samples from sites with no or only slight alterations (classes 1 or 2 for categorical variables) resulted in the selection of 587 benchmark samples from all three databases, which corresponded to ca. 64% of the initial number of national reference sites provided. In a few cases (4 sites), a site was accepted as a benchmark if it had an occasional higher value (above the limit) for a given sample, but simultaneously the mean value of all samples remained within the limits, and/or all other samples collected for several seasons/years were consistently within the limits.

After all samples were analyzed, 222 sites were retained as benchmarks (Fig. 4).

3.2. Biological LDC

From the classification analyses of reference sites it was possible to select nine main biological groups for invertebrates, with significant differences and good segregation between them (Fig. 5a; ANOSIM Global R: 0.695; $p < 0.001$), six groups for diatoms (Fig. 6a; ANOSIM Global R: 0.454; $p < 0.001$) and five for macrophytes (Fig. 7a; ANOSIM Global R: 0.668; $p < 0.001$). As predicted, these groups were not coincident with the IC types (Figs. 5, 6, 7b). More than one biological group was included in one IC type for all elements, and the biological groups were spread through different types. For example, for invertebrates, Group a included 17 sites from type 3 and 7 from type 4, while Group c included 7 types from type 4 and 3 from type 3. For diatoms, for example, Group e included sites from three types (11 from type 1, 9 from type 2 and three from type 3) and the same for Group f sites (6 sites from type 4 and 4 from type 3). In the case of macrophytes, some of the natural groups also contained three IC types, such as Group a (8 sites from type 1, 4 from type 2, 3 from type 4) and Group e (7 sites from type 2, 3 sites from type 3 and 1 site from type 4). There was also a certain segregation of the samples by country, especially in the case of Cyprus for diatoms and invertebrates; and some groups were composed of sites from only one country.

The indicator species highlighted the major differences between the invertebrate groups (Supp. Mat. Table 1) which were also significantly different in their major abiotic characteristics (PERMANOVA: Pseudo-F = 23.026; p = 0.001; 998 permutations). Invertebrate Group a was composed of the eastern- and southernmost streams, with a medium altitude (mean altitude ± SD = 320 ± 94.5 m), with sedimentary ophiolitic geology with both permanent and temporary regimes, and all located in Cyprus. The leeches of the family Erpobdellidae, which are usually found attached to stones, the decapod family Potamidae, the Odonata families Euphaeidae and Coenagrionidae and the Trichoptera Lepidostomatidae characteristic of slow-flowing waters distinguished this group (Supp. Material Table 1). Group b consisted of temporary streams with siliceous geology and medium altitudes (altitude = 351 ± 115 m) located in central Mediterranean latitudes. The only indicator taxon found for this group was the Trichoptera Beraeidae, with preference for slow-flowing waters, water-saturated muck, and stony substrates (Supp. Material Table 1). Group c was composed by very small temporary streams (mean catchment area of 2.8 ± 0.6 km^2), located at medium altitudes (325 ± 176 m), in the Iberian Peninsula, and characterized by the dominance of several families from the orders Heteroptera (Notonectidae, Hydrometridae, Nepidae), and Odonata (Corduliidae, Libellulidae) and class Gastropoda (Physidae, Planorbidae, Lymnaeidae) associated with lentic systems and with vegetation and detritus. Streams of Group d had mainly siliceous geology, and included both temporary and highly seasonal hydrological regimes with small to medium-sized streams (mean catchment area of 43.5 ± 55.5 km^2) located in the Iberian Peninsula. The gastropod family Thiaridae was the indicator taxon of these streams. Group e included only permanent streams with a highly seasonal regime, from small to larger streams (mean: 102.8 ± 179.6 km^2) with siliceous geology and medium-high

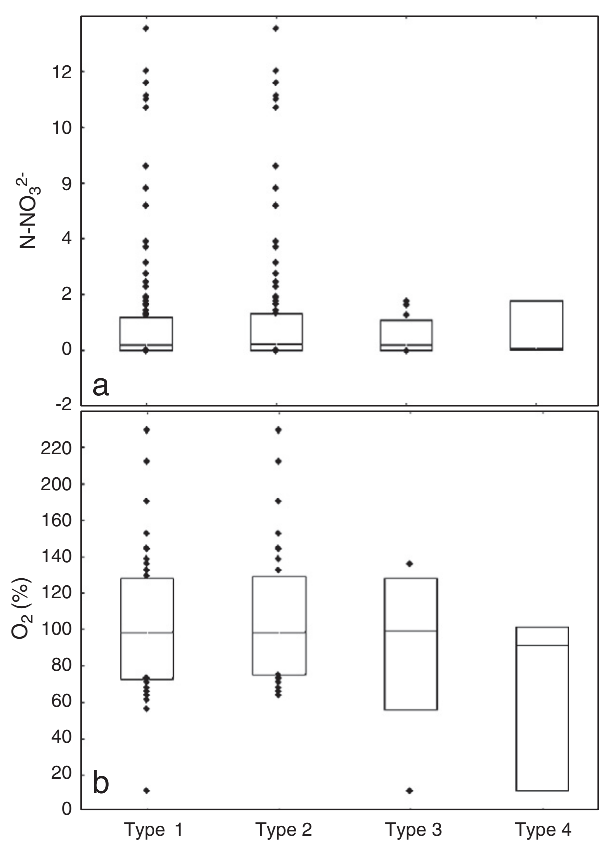

Fig. 2. Boxplots showing the distribution of values for (a) O_2% and (b) N-NO_3^{2-} found at reference sites after Step 1 (only class 1— no modification for all categorical variables). (◆) indicates the values above or below the box limits, which were defined as the 5–95th percentiles for O_2 (%) and 10–90th percentiles for N-$NO^{2-}{}_3$ and all other chemical parameters; (—) indicates the median.

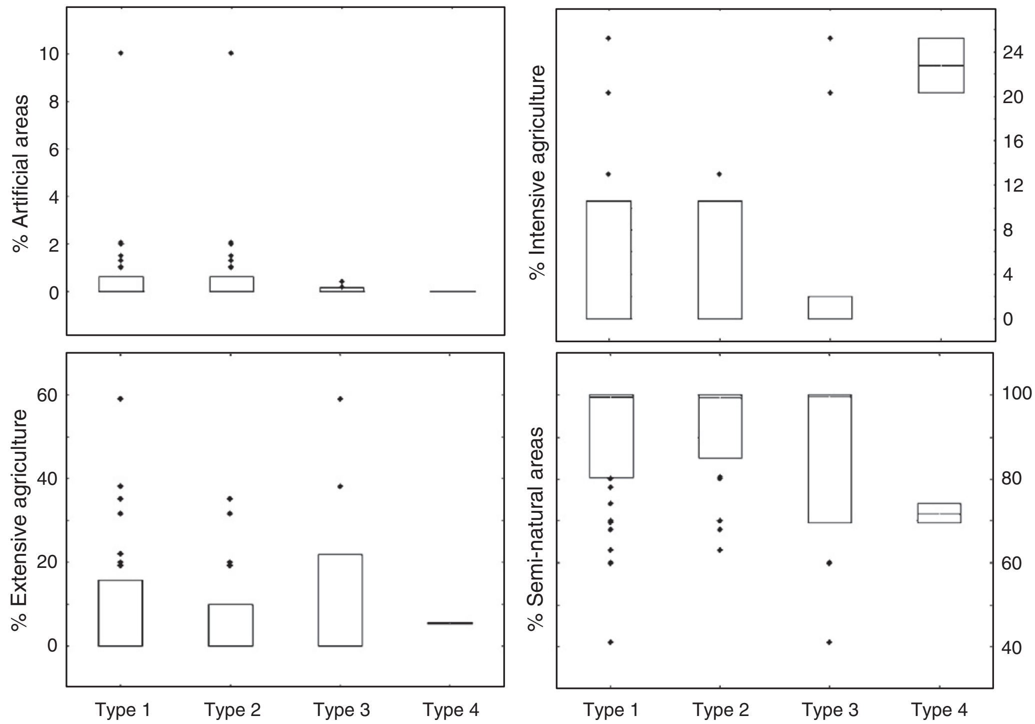

Fig. 3. Boxplots showing the distribution of values for land use in the catchment for reference sites selected after Step 1 (only class 1— no modification for all categorical variables). (◆) indicates the values above or below the box limits, which were defined as the 10th–90th percentiles for all land use %; (—) indicates the median.

altitudes (473.1 ± 212.2 m mean altitude). This was the most geographically widespread group, with sites from all countries except Cyprus. The indicator taxa Aphelocheiridae (Hemiptera) and the Trichoptera Calamoceratidae distinguished this group, and can be found in several types of aquatic habitats, including fast-flowing riffle zones and in backwaters. Group f included western-European streams (Iberian Peninsula), located at high altitudes (mean altitude: 1034 ± 209.6 m) with a highly seasonal hydrological regime (permanent) and small to medium catchments (mean: 28.9 ± 49.3 km^2). Their invertebrate communities are characterized by a diversity of insect families, which include the Plecoptera Capniidae, Ephemeroptera Ephemeridae, the Odonata Aeshnidae and Cordulegasteridae characteristic of well-aerated zones and clean waters; and the Stratiomyidae, found in decomposing organic matter. Group g was composed of medium-sized streams (mean catchment area 87.7 ± 101.4 km^2) at medium altitudes (394.7 ± 269.6 m), with only calcareous geology and highly seasonal hydrological regimes. It includes sites from Spain and France. Intermediately sensitive taxa such as the Dryopidae (Coleoptera), Gomphidae (Odonata), Oligoneuriidae (Ephemeroptera) and Tabanidae (Diptera) characterize this group. The most northernmost streams were in Group h, all located in France, at high altitudes (mean altitude: 832 ± 244.4 m) and with siliceous geology and relatively small catchments (36.9 ± 37.7 km^2). This is the most diverse group of Mediterranean streams, with higher numbers of Trichoptera (8 families), Plecoptera (5 families) and Ephemeroptera (3 families) among the indicator taxa (see Supp. Material Table 1), most of them typical of oligotrophic, cold and well-aerated waters (e.g., Brachycentridae, Sericostomatidae, Limnephilidae, Perlodidae, Leuctridae). Finally, Group

Table 4
Comparison of 90th percentiles (and 10th lower limit of O_2) obtained for all rivers and all seasons for all river types, with the exception of temporary rivers, and for temporary rivers in all seasons and in summer only.

Variables	All seasons, all IC types	All seasons except temporary rivers	All seasons temporary rivers	Summer temporary rivers
DO (mg/l)	6.39–13.70	6.90–12.81	6.78–13.76	–
O_2 (%)	73.7–127.9	76.2–128.4	60.3–127.8	22.5–100.8
N-NH4$^+$ (mg/l)	≤0.09	≤0.09	≤0.06	≤0.04
N-NO_3^- (mg/l)	≤1.15	≤1.24	≤0.78	≤1.09
P-Total (mg/l)	≤0.07	≤0.08	≤0.06	≤0.05
P-PO_4^{3-} (mg/l)	≤0.06	≤0.07	≤0.04	≤0.10

Table 5
Thresholds established for the final selection of benchmarks in Step III.

Variables	Benchmarks are accepted if	
	Types 1, 2, 3	Type 4
Channelization (1–4)[a]	≤2	
Bank alteration (1–4)[a]		
Local habitat alteration (1–4)[a]		
Riparian vegetation (1–4)		
General morphology[b]		
Connectivity (1–4)		
Stream flow (1–4)[a]		
Upstream dam influence (1–4)[a]		
Hydropeaking (1–4)[a]		
General hydrology[b]		
DO (mg/l)[c]	6.39–13.70	
O_2 (%)	73.72–127.92	60.34–127.92
N-NH4$^+$ (mg/l)	≤0.09	
N-NO_3^- (mg/l)	≤1.15	
P-Total (mg/l)	≤0.07	
P-PO_4^{3-} (mg/l)	≤0.06	
% artificial areas (catchm)	≤1	
% intensive agriculture (catchm)	≤11	
% extensive agriculture (catchm)	≤32	
% semi-natural areas (catchm)	≥68	
% urbanization (reach)[b]	≤1	
% land use (reach)[b]	≤20	
% agriculture (reach)[b]	≤20	

[a] Variables used for invertebrates and macrophytes.
[b] For diatoms only, instead of land use in the catchment.
[c] For macrophytes only, instead of O_2 (%).

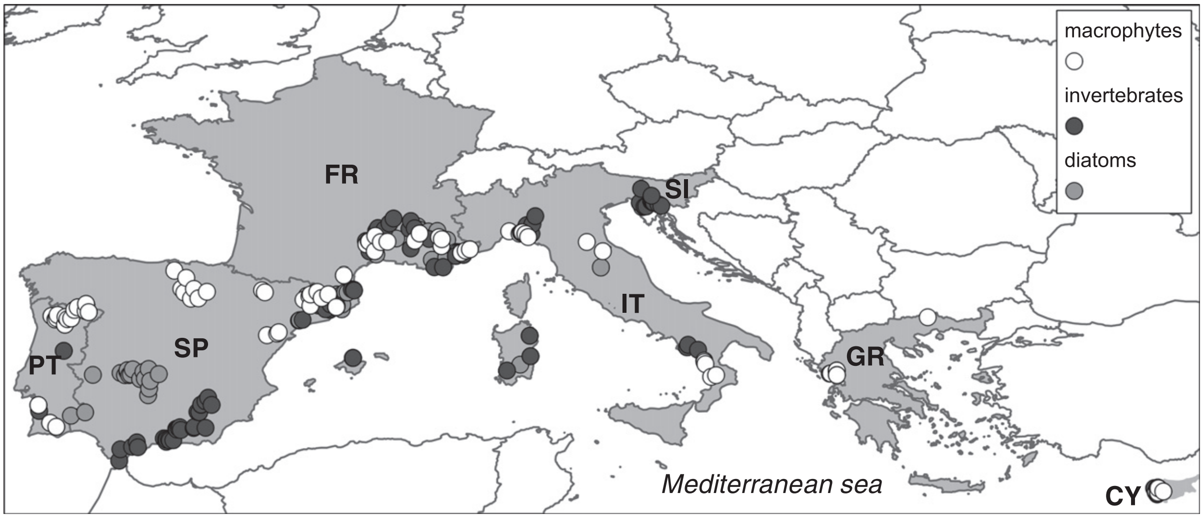

Fig. 4. Location of site of final benchmarks selected after Step III in Mediterranean countries, according to the original database for biological element. PT, Portugal; SP, Spain; FR, France; IT, Italy; SI, Slovenia, GR, Greece; and CY, Cyprus.

i was composed of larger calcareous streams located in France (mean catchment area 171.9 ± 137.6 km^2) with a highly seasonal hydrological regime. The taxon with the highest indicator value was the Gammaridae (Crustacea), which is typical of alkaline waters. However, this is a very diversified group that also includes Trichoptera (Goeridae, Psychomyiidae, Hydroptilidae), Ephemeroptera (Ephemerellidae, Baetidae), Diptera, Coleoptera (Elmidae), Bivalvia (Sphaeriidae), Platyhelminthes (Dugesiidae) and also Decapoda (Cambaridae) among the indicator taxa.

For diatoms (Supp. Material Table 2), the biological groups established were also significantly different in terms of major abiotic characteristics (PERMANOVA: Pseudo-F = 8.696; p = 0.001; 997 permutations). Group a was composed of sites located in the central part of the Mediterranean basin (France) from medium-sized calcareous streams (catchment area = 182 ± 172 km^2), at medium-high altitudes (503.4 ± 247.4 m), with a highly seasonal hydrological regime and high conductivity (508.4 ± 295.2 μS cm^{-1}). Accordingly, this group was dominated by taxa that are alkaliphilic, sensitive to nutrients, and associated with high oxygen concentration (i.e.

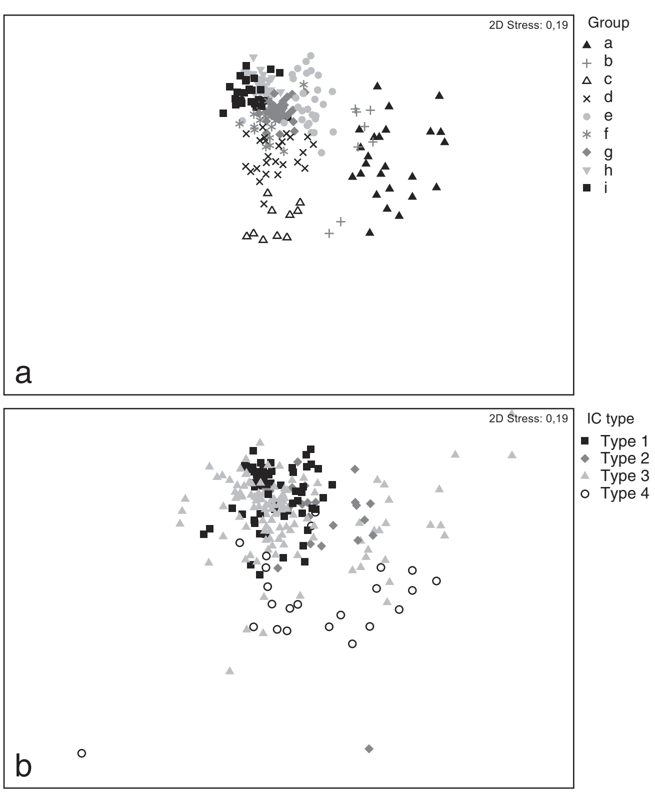

Fig. 5. Ordination of final benchmarks based on their invertebrate assemblages (fourth root transformation). Symbols represent: a) natural groups (obtained in the cluster); and b) types.

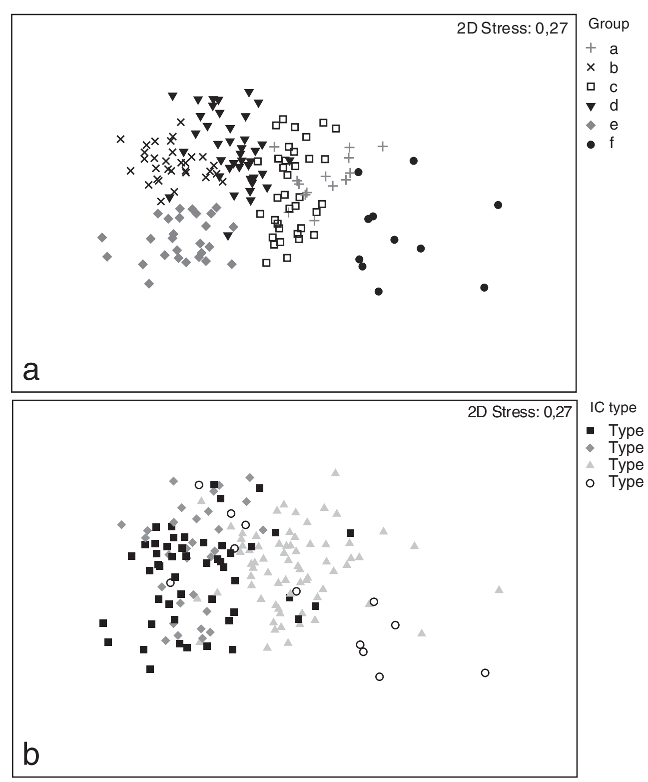

Fig. 6. Ordination of final benchmarks based on their diatom assemblages (fourth root transformation). Symbols represent: a) natural groups (obtained in the cluster); and b) IC types.

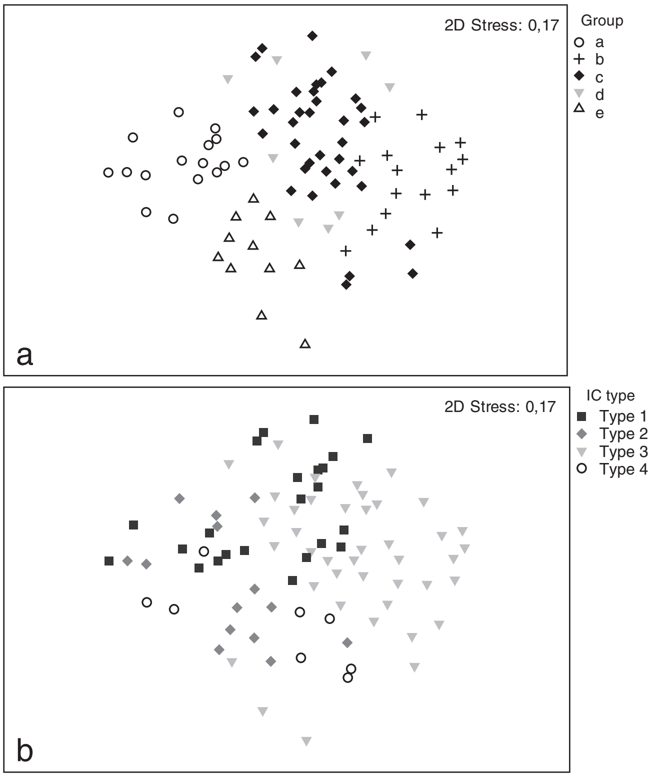

Fig. 7. Ordination of final benchmarks based on their macrophyte assemblages (square root transformation). Symbols represent: a) natural groups (obtained in the cluster); and b) types.

Cymbella parva, *Sellaphora stroemii*, *Brachysira neoexilis*). Group b was composed of sites from small and medium-sized rivers from Spain, with siliceous geology and low conductivity (124 ± 85.3 μS cm^{-1}). The most important diatoms were three species of the genus Fragilaria (*F. vaucheriae*, *F. ulna*, *F. rumpens*), two of the genus *Gomphonema* (*G. truncatum* and *G. gracile* and the *Amphora veneta*). Group c was composed of calcareous sites with high conductivity (411.5 ± 168.9 μS cm^{-1}), from medium-high altitudes (580 ± 286.8 m) in Spain and France and included in both streams in small and medium-sized river basins (<1000 km^2), all with a highly seasonal hydrological regime. Diatoms (*Delicata delicatula*, *Encyonopsis cesatii*, *Gomphonema lateripunctatum*) indicate alkaline, high oxygen concentrations. Group d was the largest, and the covered the widest longitudinal range, including 40 sites from several countries (Spain, France, Italy and Slovenia). Rivers included small to large catchment areas (122.1 ± 182.9 km^2), mixed geology, medium-high altitudes (424.2 ± 254.3 m) and with high conductivity (391.4 ± 160.0 μS cm^{-1}). This variability is reflected in the species that appear as indicators of this group, and in their lower indicator value, such as *Amphora inariensis*, *Navicula tripunctata*, *Rhoicosphenia abbreviata*, and *Cocconeis pediculus*, among others. These species are also mainly alkaliphic. Group e was composed of siliceous sites, with a wide range of sizes (catchment areas = 102.0 ± 164.2 km^2), at high altitude (mean 556.0 ± 278.0 m), but with the lowest pH (6.9 ± 0.7) and conductivity (90 ±116.0 μS cm^{-1}), located in the Iberian Peninsula and France. The most important indicators of this group are *Achnanthidium subatomoides*, *Hannaea arcus*, *Diatoma mesodon*, *Gomphonema rhombicum* and *Achnanthidium helveticum*, which are mostly acidophilic. Group f was composed only of sites from Cyprus, with relatively small catchment areas (63.0 ± 30.0 km^2), and low altitudes (204.8 ± 24.7 m), all with ophiolitic geology (pH = 8.2 ± 0.2) and high conductivity (520.1 ±76.8 μS cm^{-1}), which was also the case for macroinvertebrate Group a. This group includes diatom taxa that are mostly circumneutral (*Achnanthidium lineare*, *Encyonopsis subminuta*) or alkaliphilic (i.e. *Fragilaria biceps*, *Gomphonema tergestinum*, *Epithemia goeppertiana*), which means that they indicate neutral to alkaline waters. About half of the sites were temporary, and the others showed highly seasonal hydrological regimes.

The macrophyte biological groups (Supp. Material Table 3) also had different general abiotic characteristics (PERMANOVA: Pseudo-F = 6.908; p = 0.001; 997 permutations). Group a was composed of small and medium-sized streams (mean catchment area = 121.7 ± 193.2 km^2) at medium altitudes (382.0 ± 228.8 m) from the southwestern area, with mixed geology and relatively low conductivity (85.5 ± 119.1 μS cm^{-1}). The emergent species *Carex elata* ssp. *reuteriana* and *Galium broterianum* dominated on rocky substrata and in moderate to fast-flowing waters, along with the acidophillous rheophyte *Platyhypnidium lusitanicum*, especially in shallow depths but turbulent currents; whereas *Oenanthe crocata crocata* and *Juncus effusus* dominated in segments with smoother currents and finer substrates in both temporary and permanent rivers. Riverbanks are dominated by emergent species such as *Lotus pedunculatus*, *Prunella vulgaris*, *Polygonum hydropiper* or *Eupatorium cannabinum* and terricolous bryophyte indicators of shady and less disturbed substrates, such as *Kindbergia praelonga*, *Plagiomnium undulatum* and *Lunularia cruciata*. Strict hydrophyte communities were scarce in this group, and even mosses that are usually considered hydrophytes such as the neutrophilous *Fissidens crassipes* ssp. *warnstorffi* or the acidophillous *Platyhypnidium lusitanicum* can tolerate permanently or intermittently in submerged conditions. Group b was composed of relatively small montane calcareous streams (catchment area = 97.8 ± 102.9 km^2; altitude = 638.5 ± 220.0 m; conductivity = 472.4 ± 183.0 μS cm^{-1}) dominated by the cyanobacteria *Rivularia* sp. and by the macroalgae *Spirogyra*, *Nostoc* and *Bangia atropurpurea*. Rivers in this group were dominated by bryophytes that are characteristic of frequently submerged conditions and are usually associated to calcareous substrates, such as the mosses *Palustriella commutate* and *Pallustriella falcata* and the liverwort *Pellia endiviifolia*. These bryophyte species are indicators of undisturbed substrates, and are typical of Mediterranean montane riverbeds. *Chara vulgaris* frequently occurred in rivers with slow flows. Group c was composed of highly seasonal rivers with mixed geology, clear running-waters and coarse substrates (pebbles and boulders). This group includes sites at high altitudes (>1000 m; mean altitude = 632.5 ± 341.7 m), with small catchments (51.1 ± 79.9 km^2) and intermediate conductivity (333.5 ± 277.4 μS cm^{-1}). The sites were spread throughout the study area, from Spain to Cyprus. The moss *Platyhypnidium riparioides*, a neutrophilous and cosmopolitan species, occupied large portions of the substrates. This species can tolerate fast to slow currents, and along with the red algae *Lemanea* form low-diversity but highly abundant communities on rocky riverbeds with no sedimentation. The river segments with moderate to slow flows and more nutrients were usually colonized by populations of floating green filamentous algae, namely *Cladophora*, *Spyrogira*, and *Microspora*; and by small coverages of the yellow-green algae *Vaucheria*. Besides *Lemanea*, the red alga *Bangia atropurpurea* and some cyanobacteria such as the genera *Oscillatoria* and *Lyngbya* are important species of this group. In Group d, streams have small basins (69.0 ± 121.4 km^2), at medium-high altitudes (512.6–258.3 m), with high conductivity (477.8 ± 32.6 μS cm^{-1}), rich in silt, and located in Cyprus and France. They were dominated by helophytes characteristic of nutrient-rich wet meadows and river banks such as the *Equisetum palustre*, *Carex* sp., *Juncus* sp., *Nasturtium officinale*, *Mentha aquatica* and *Mentha longifolia*. Some ephemeral algae such as the genera *Spirogyra* and *Characeae* are also frequent in this group. Finally, Group e included the southeastern Mediterranean (Cyprus and Greece) with small to medium-sized catchments (255.6 ± 98.2 km^2), highly-seasonal hydrological regimes and medium conductivity (389.2 ± 111.3 μS cm^{-1}), leading to the dominance of communities that are highly tolerant to submersion such as the mosses *Cinclidotus* sp. and the emergent species *M. aquatica*, *M. longifolia*, *Rorippa sylvestris*, *Phragmites australis* and *Veronica anagallis-aquatica*. In running waters, the hydrophytes *Ranunculus* sp., *Potamogeton* sp. and *Callitriche* sp. became more abundant; and in pools during summer, the duckweed *Lemna* sp. is frequent along with some macroalgae of the genus *Cladophora*.

4. Discussion

4.1. Least-disturbed conditions in the Mediterranean basin

The high demands of the human population for drinking water, irrigation, fishing or leisure activities in the Mediterranean basin have obvious consequences in the loss of naturalness of streams and rivers (e.g., Hooke, 2006; Prat and Rieradevall, 2006). Our approach revealed the existence of common patterns and problems in Mediterranean least-disturbed streams. We found that hydromorphological changes of human origin were almost always present, as only 30–44% of the national reference samples could be selected in the initial group. Channelization, bank alteration and changes in riparian vegetation affect the majority of small streams. These streams are often close to small residential areas or isolated farms, which commonly use them as water sources for irrigation, tend to reinforce their banks and channels to prevent floods, and constrain the riparian vegetation to enlarge the cultivated area. These problems are therefore interconnected, as loss of riparian vegetation leads to increased bank erosion, which can then be responsible for supplying more than 50% of the sediment in streams, depending on the adjacent land use (Zaimes et al., 2004). On the other hand, streams in medium-sized catchments are more affected by damming to retain water for power production, drinking, fishing and leisure areas, such as fluvial beaches and small boat basins. These alterations have expectable consequences for the aquatic communities. Channelization leads to loss of habitat for feeding, reproduction or protection for aquatic animals, and loss of retentive capacity for allochthonous inputs (Petersen and Petersen, 1991; Allan and Flecker, 1993; Muotka et al., 2002). The changes in natural flow regimes may not be compatible with life cycles of the biota (e.g., Gasith and Resh, 1999; Elosegi et al., 2010). The loss of autochthonous riparian vegetation may reduce litter quality and alter decomposition rates, and increased insolation may result in higher primary production (Gasith and Resh, 1999; Graça, 2001; Ashton et al., 2005). Finally, the introduction of alien invasive species may alter native communities through competition for space and resources (e.g., Moyle and Light, 1996).

4.2. Thresholds

The sites with the best hydromorphological conditions were usually those that also had the best water chemical conditions, which is not surprising, as among other reasons, the natural connectivity leads to better water renewal and aeration, and the riparian corridors provide a natural buffer and filtering capacity. Additionally, it is difficult to find any relatively close source of impact, such as a factory, treatment plant, sewage discharge or agriculture runoff that does not in the same way affect the river channel morphology, bank morphology, flow, connectivity or riparian vegetation (which are controlled in Step I), i.e., without any construction, pipes, crossing structures, or other change being made in the river. But it is still possible that a more-distant source of impact will have some effect on the water quality, and thus potential differences in national standards would lead to the definition of more relaxed thresholds in Step II. Nevertheless we also believe that the use of the 90th percentile, rather than the maximum value observed at the sites selected in Step I, allows for these differences to some degree, and that is was the best practical approach to find common limits without following those used in any of the countries participating in this exercise, which would strongly bias the results.

In spite of potential problems of our method and although the different systems and approaches to select reference sites are not fully comparable, we reached criteria and values that are close to those proposed by other authors within and outside Europe (e.g., Camargo et al., 2005; Smith et al., 2007; Pardo et al., 2012; Smith and Tran, 2012; Sanchéz-Montoya et al., 2012). Camargo et al. (2005) proposed a maximum value of 2 mg NO_3-N/l to protect sensitive freshwater animals from nitrate pollution. The threshold that we found here is even lower than this value (1.15 mg/l) and stricter than the mean values indicated by Pardo et al. (2012) for reference sites of Central-Baltic rivers, for both diatoms and invertebrates (between 2 and 6 mg/l). Lower values were proposed by Smith et al. (2007; 0.98 mg/l) and Smith and Tran (2012; 0.3 mg/l) for wadeable rivers, based on the responses of invertebrates of large rivers and on diatoms and invertebrates, respectively. Skoulikidis et al. (2006; 0.22 mg/l) found an even lower value for invertebrates of high-quality streams in Greece. An excess of nitrate can lead to waters clogged with fast-growing algae and macrophytes (Hilton et al., 2006), while for animals the main toxic action of nitrate is the conversion of oxygen-carrying pigments, such as hemoglobin and hemocyanin, to forms incapable of carrying oxygen (Camargo et al., 2005). Long-term exposure to a concentration of 10 mg/l N-NO_3 can adversely affect freshwater invertebrates, fishes and amphibians (Camargo et al., 2005). Our value is therefore reasonable, but could ideally be lower to protect the aquatic communities.

Phosphorous is naturally low in streams, and its concentrations depend on the local soil and bedrock; however, it can be increased anthropogenically as a result of wastewater discharge, runoff from fertilized land or forest fires, among others. As phosphorus is often a limiting factor in freshwaters (Reddy et al., 1999), increased concentrations stimulate the growth of phytoplankton and aquatic plants (Bornette and Puijalon, 2011) and eventually lead to a decrease in the dissolved-oxygen content. Here we proposed the value of 0.07 mg/l as a maximum threshold for total phosphorus. This value is identical to the values proposed by Smith et al. (2007) for wadeable rivers based on the responses of invertebrate communities to nutrients, but higher than the 0.03 mg/l proposed by Smith and Tran (2012). For orthophosphates our threshold (0.06) is slightly higher than the value proposed by Pardo et al. (2012) for the Central-Baltic European streams (0.04 mg/l) but close to the one proposed for Spanish Mediterranean streams (0.052) by Sanchéz-Montoya et al. (2012) and lower than the 0.1 mg/l proposed by Birk et al. (2012) for large rivers in good condition. Conversely, Skoulikidis et al. (2006) found a much higher threshold value of 0.125 mg/l.

Low oxygen concentrations (<35%) induce sublethal effects on macroinvertebrates, such as suppressed drift, but usually not mortality, which only occurs at very low saturation levels (<10%) for most insects and <20% for mayflies (Connoly et al., 2004). We determined a threshold interval of 76–128% oxygenation for Mediterranean streams. This interval is wider than but not far from the proposed intervals of between 85–115% and 90–110% proposed by Pardo et al. (2012) for Central-Baltic European streams (10–90th percentile). Our values are also wider than those of Sanchéz-Montoya et al. (2012; 91–115%), which is probably due to the use of the 5th percentile in our case, vs the 25th percentile in that referred study. The major difference lies in the values proposed for temporary streams, where the 10th percentile was lower (60%). Low flow during summer periods may lead to extremely low oxygen concentrations (ca. 22%) and therefore we recommend that summer months not be used for routine monitoring, as the communities will be affected by these anomalous values. The temporary streams studied here were among the least affected by organic and chemical pollution, even in summer, and therefore the lower oxygen concentrations can be attributed to natural conditions in spite of the higher percentage of intensive agriculture found for these sites. In fact, intensive agriculture apparently did not affect water quality, probably because in most cases this intensive agriculture consists of subsistence agriculture with very little impact on the ecosystems.

Finally, by the end of Step III, not all national reference sites were accepted as benchmarks according to the MedGIG criteria. This means that some of the national standards could be lower than others, leading to the classification of more sites as "in good condition" than the number thus classified in other countries. On the other hand, we cannot form conclusions about which countries have lower or higher standards, as in fact the pool of reference sites provided by the countries is only a sample of the reference sites that exist and are not necessarily those used to develop the classification systems. The comparison of boundaries predicted by the Intercalibration Exercise constitutes a subsequent step to this work, and aims to compare of classification systems. However, these common benchmark thresholds can be directly compared to those used at the national level, and can be used to improve national standards, aiming toward more stringent definitions of the values.

4.3. Biological communities

Biologically, we found consistent groups for the different biological elements. However, the number of groups varied between the invertebrates, diatoms and macrophytes, which is in agreement with previous studies that found no concordance in classifications of fish, invertebrates and bryophytes (Paavola et al., 2003) or invertebrates and diatoms (Passy et al., 2004). The groups were also not coincident with the IC types for any of the biological elements: for example, temporary streams (type 4) were spread into at least three biological groups for all elements. Differences in classification approaches (abiotic vs biological) were also found by other authors (Hawkins and Vinson, 2000; Sanchéz-Montoya et al., 2007). However, here, the broad scale of the variables that define the IC types and their relatively small number can contribute to explain the differences. River size, geology and hydrological regime (used in IC types) are indeed frequently cited as important for plant and invertebrate communities. However, other variables that also strongly affect the relevant structuring of primary producers and invertebrates and translating more local conditions were not accounted for, such as current velocity, substrate, alkalinity, harness or light availability (e.g., Soininen, 2004; Feio et al., 2007, 2012; Franklin et al., 2008; Bornette and Puijalon, 2011). This means that, as they were defined, the IC types are important for the communities and do not need to be treated differently to compare the boundaries and quality classes, except for temporary streams because of the differences in reference criteria.

Biogeographically, we found some biological differences between countries, especially regarding Cyprus, where the samples appear isolated in a group for both diatoms and invertebrates. This is probably expectable, as the countries represented in this study are distributed along a longitudinal gradient over the Mediterranean basin, reflecting, among other aspects, climatic and orographic differences (Jalut et al., 2009). Additionally, Cyprus is characterized by a very distinct geology, the ophiolites, which are fragments of oceanic crust on land, associated with moderately alkaline surface waters (Neal and Shand, 2002). Even though it is possible, we do not believe that the influence from national sampling protocols was strong, as the methods were compared prior to beginning the analyses and were generally similar (same sampling gear, habitats sampled, substrates) in the different countries.

Finally, we determined the indicator taxa that distinguished each of the natural groups for each element. These lists may constitute a useful basis for future studies in the area of biodiversity and conservation, as they indicate exclusive taxa (or at least taxa that are present in relatively high abundance) in a certain group of streams. Nevertheless, in some cases further work with a larger dataset should be carried out in order to obtain more-restricted groups with a higher indicator value for the most important species.

4.4. Final remarks

This was a very comprehensive study that: 1) reviewed the major sources of impact on Mediterranean streams, 2) contributed to the future selection of benchmarks in this region, and 3) set biological and abiotic targets for the improvement of these streams. This work could be improved through the expansion of the database, especially for temporary rivers, and the inclusion of more detail, especially on hydromorphological variables. Better uniformity in the methods used to measure stressors within Europe, as well as in the biological assessment methods, also proved to be important, as more-uniform methods would improve the comparability of abiotic evaluations and consequent joint efforts toward the recovery of streams and rivers, especially in the catchments that extend over national borders.

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Appendix A. Supplementary data

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References

Aguiar FC, Ferreira MT, 2005. Human-disturbed landscapes: effects on composition and integrity of riparian woody vegetation in the Tagus River basin, Portugal. Environ Conserv 2005;32:30–41.
Allan JD, Flecker AS, 1993. Biodiversity conservation in running waters. Bioscience 1993;43:32–43.
Ashton IW, Hyatt LA, Howe KM, Gurevitch J, Lerday MT, 2005. Invasive species accelerate decomposition and litter nitrogen loss in a mixed deciduous forest. Ecol Appl 2005;15:1263–72.
Birk S, Van Kouwen L, Willby N, 2012. Harmonising the bioassessment of large rivers in the absence of near-natural reference conditions — a case study of the Danube River. Freshw Biol 2012;57:1716–32.
Bonada N, Dolédec S, Statzner B, 2007. Taxonomic and biological trait differences of stream macroinvertebrate communities between Mediterranean and temperate regions: implications for future climatic scenarios. Glob Chang Biol 2007;13:1658–71.
Bornette G, Puijalon S, 2011. Response of aquatic plants to abiotic factors: a review. Aquat Sci 2011;73:1–14.
Buffagni A, Erba S, Armanini DG, 2010. The lentic–lotic character of Mediterranean rivers and its importance to aquatic invertebrate communities. Aquat Sci 2010;72:45–60.
Camargo JA, Alonso A, Salamanca A, 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 2005;58:1255–67.
Clarke KR, Warwick RM, 2001. Change in marine communities: an approach to statistical analysis and interpretation. 2nd ed. Plymouth, UK: PRIMER-E Ltd, Plymouth Marine Laboratory; 2001.
Connoly NM, Crossland MR, Pearson RG, 2004. Effect of low dissolved oxygen on survival, emergence, and drift of tropical stream macroinvertebrates. J N Am Benthol Soc 2004;23:251–70.
Dufrene M, Legendre P, 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol Monogr 1997;67:345–66.
Elosegi A, Díez J, Mutz M, 2010. Effects of hydromorphological integrity on biodiversity and functioning of river ecosystems. Hydrobiologia 2010;657:199–215.
EN 13946, 2003. Water quality — guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers. Brussels: CEN; 2003.
EN 14184, 2003. Water quality — guidance for the surveying of aquatic macrophytes in running waters. Brussels: CEN; 2003.
EN 14407, 2004. Water quality — guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters. Brussels: CEN; 2004.
EN 14996, 2006. Water quality — guidance on assuring the quality of biological and ecological assessments in the aquatic environment. Brussels: CEN; 2006.
Environmental Agency, 2003. River habitat survey in Britain and Ireland. Field survey guidance manual: 2003 version. UK: EA, SEPA, Environment and Heritage Service; 2003.

European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. Off J Eur Communities 2000;L327:1–73.

Feio MJ, Almeida SFP, Craveiro SC, Calado AJ, 2007. Diatoms and macroinvertebrates provide consistent and complementary information on environmental quality: a predictive model approach. Fundam Appl Limnol/Arch Hydrobiol 2007;169:247–58.

Feio MJ, Coimbra CN, Graça MAS, 2010. The influence of extreme climatic events and human disturbance on macroinvertebrate community patterns of a Mediterranean stream over 15 y. J N Am Benthol Soc 2010;29:1397–409.

Feio MJ, Aguiar FC, Almeida SFP, Ferreira MT, 2012. AQUAFLORA: a predictive model based on diatoms and macrophytes for streams water quality assessment. Ecol Indic 2012;18:586–98.

Ferreira MT, Albuquerque A, Aguiar FC, Catarino LF, 2002a. Seasonal and yearly variations of macrophytes in a Southern Iberian River. Proc Int Assoc Theor Appl Limnol 2002a;27:3833–7.

Ferreira MT, Albuquerque A, Aguiar FC, Sidorkewicz N, 2002b. Assessing reference sites and ecological quality of river plant assemblages from an Iberian basin using a multivariate approach. Arch Hydrobiol 2002b;155:121–45.

Franklin P, Dunbar M, Whitehead P, 2008. Flow controls on lowland river macrophytes: a review. Sci Total Environ 2008;400:369–78.

Gasith A, Resh VH, 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annu Rev Ecol Syst 1999;30:51–81.

Gonçalves Jr, Graça MAS, Callisto M, 2006. Leaf-litter breakdown in 3 streams in temperate, Mediterranean, and tropical Cerrado climates. J N Am Benthol Soc 2006;25:344–55.

Graça MAS, 2001. The role of invertebrates on leaf litter decomposition in streams — a review. Int Rev Hydrobiol 2001;86:383–93.

Hawkins CP, Vinson MR, 2000. Weak correspondence between landscape classifications and stream invertebrate assemblages: implications for bioassessment. J N Am Benthol Soc 2000;19:501–17.

Hawkins CP, Olson JR, Hill RA, 2010. The reference condition: predicting benchmarks for ecological water-quality assessments. J N Am Benthol Soc 2010;29:312–43.

Hering D, Buffagni A, Moog O, Sandin L, Sommerhaüser M, Stubauer I, et al, 2003. The development of a system to assess the ecological quality of streams based on macroinvertebrates — design of the sampling programme within the AQEM project. Int Rev Hydrobiol 2003;88:345–61.

Hilton J, O'Hare M, Bowes MJ, Jones JI, 2006. How green is my river? A new paradigm of eutrophication in rivers. Sci Total Environ 2006;365:66–83.

Hooke JM, 2006. Human impacts on fluvial systems in the Mediterranean region. Geomorphology 2006;79:311–35.

Jalut G, Dedoubat JJ, Fontugne M, Otto T, 2009. Holocene circum-Mediterranean vegetation changes: climate forcing and human impact. Quat Int 2009;200:4–18.

Kahlert M, Kelly M, Albert R, Almeida SFP, Bešta T, Blanco S, et al, 2012. Identification versus counting protocols as sources of uncertainty in diatom-based ecological status assessments. Hydrobiologia 2012;695:109–24.

Kelly MG, Cazaubon A, Coring E, Dell'Uomo A, Ector L, Goldsmith B, et al, 1998. Recommendations for routine sampling of diatoms for water quality assessment in Europe. J Appl Phycol 1998;10:215–24.

Köppen W, 1923. De Klimate der Erde. Berlin: Bornträger; 1923.

Landres PB, Morgan P, Swanson FJ, 1999. Overview of the use of natural variability concepts in managing ecological systems. Ecol Appl 1999;9:1179–88.

Lytle DA, Poff NL, 2004. Adaptation to natural flow regimes. Trends Ecol Evol 2004;19:94–100.

Moss B, 2008. The Water Framework Directive: total environment or political compromise. Sci Total Environ 2008;400:32–41.

Moyle PB, 1995. Conservation of native freshwater fishes in the Mediterranean-type climate of California, USA: a review. Biol Conserv 1995;72:271–9.

Moyle PB, Light T, 1996. Biological invasions of freshwater empirical rules and assembly theory. Biol Conserv 1996;78:149–61.

Munné A, Prat N, Solà C, Bonada N, Rieradevall M, 2003. A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. Aquat Conserv Mar Freshw Ecosyst 2003;13:147–63.

Muotka T, Paavola R, Haapala A, Novikmec M, Laasonen P, 2002. Long-term recovery of stream habitat structure and benthic invertebrate communities from in-stream restoration. Biol Conserv 2002;105:243–53.

Neal C, Shand P, 2002. Spring and surface water quality of the Cyprus ophiolites. Hydrol Earth Syst Sci 2002;6:797–817.

Nijboer RC, Johnson RK, Verdonschot PFM, Sommerhäuser M, Buffagni A, 2004. Establishing reference conditions for European streams. Hydrobiologia 2004;516:91–105.

Paavola R, Muotka T, Virtanen R, Heino J, Kreivi P, 2003. Are biological classifications of headwater streams concordant across multiple taxonomic groups? Freshw Biol 2003;48:1912–23.

Pardo I, Gómez-Rodríguez C, Wasson J-G, Owen R, van de Bund W, Kelly M, et al, 2012. The European reference condition concept: a scientific and technical approach to identify minimally-impacted river ecosystems. Sci Total Environ 2012;420:33–42.

Passy SI, Bode RW, Carlson DM, Novak MA, 2004. Comparative environmental assessment in the studies of benthic diatom, macroinvertebrate, and fish communities. Int Rev Hydrobiol 2004;89:121–38.

Petersen L-BM, Petersen RC, 1991. Short term retention properties of channelized and natural streams. Verh Int Ver Theor Angew Limnol 1991;24:1756–9.

Prat N, Rieradevall M, 2006. 25-years of biomonitoring in two Mediterranean streams (Llobregat and Besòs basins, NE Spain). Limnetica 2006;25:541–50.

Reddy KR, Kadlec RH, Flaig E, Gale PM, 1999. Phosphorus retention in streams and wetlands: a review. Crit Rev Environ Sci Technol 1999;29(1):83–146.

Resh VE, Jackson JK, Mc Elravy EP, 1990. Disturbance, annual variability, and lotic benthos: examples from a California stream influenced by a Mediterranean climate. Mem Ist Ital Idrobiol 1990;47:309–29.

Reynoldson TB, Norris RH, Resh VH, Day KE, Rosenberg DM, 1997. The reference condition: a comparison of multimetric and multivariate approaches to assess water quality impairment using benthic macroinvertebrates. J N Am Benthol Soc 1997;16:833–52.

Sabater S, Elosegi A, Acuña V, Basaguren A, Muñoz I, Pozo J, 2008. Effect of climate on the trophic structure of temperate forested streams. A comparison of Mediterranean and Atlantic streams. Sci Total Environ 2008;390:475–84.

Sanchéz-Montoya MM, Vidal-Abarca MR, Puntí T, Poquet JM, Prat N, Rieradevall M, et al, 2009. Defining criteria to select reference sites in Mediterranean streams. Hydrobiologia 2009;619:39–54.

Sanchéz-Montoya MM, Punti T, Suárez ML, Vida-Abarca MR, Rieradevall M, Poquet JM, et al, 2007. Concordance between ecotypes and macroinvertebrate assemblages in Mediterranean streams. Freshw Biol 2007;52:2240–55.

Sanchéz-Montoya MM, Arce MI, Vidal-Abarca MR, Suárez ML, Prat N, Gómez R, 2012. Establishing physico-chemical reference conditions in Mediterranean streams according to the European Water Framework Directive. Water Res 2012;46:2257–69.

Santos MJ, 2010. Encroachment of upland Mediterranean plant species in riparian ecosystems of southern Portugal. Biodivers Conserv 2010;19:2667–84.

Skoulikidis NTh, Amaxidis Y, Bertahas I, Laschou S, Gritzalis K, 2006. Analysis of factors driving stream water composition and synthesis of management tools — a case study on small/medium Greek catchments. Sci Total Environ 2006;362:205–41.

Smith AJ, Tran CP, 2012. A weight-of-evidence approach to define nutrient criteria protective of aquatic life in large rivers. J N Am Benthol Soc 2012;29:875–91.

Smith AJ, Bode RW, Kleppel GS, 2007. A nutrient biotic index (NBI) for use with benthic macroinvertebrate communities. Ecol Indic 2007;7:371–86.

Soininen J, 2004. Assessing the current related heterogeneity and diversity patterns of benthic diatom communities in a turbid and a clear water river. Aquat Ecol 2004;38:495–501.

Stoddard JL, Larsen DP, Hawkins CP, Johnson RK, Norris RH, 2006. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecol Appl 2006;16:1267–76.

Stromberg JC, Boudell JA, Hazelton AF, 2008. Differences in seed mass between hydric and xeric plants influence seed bank dynamics in a dryland riparian ecosystem. Funct Ecol 2008;22:205–12.

Zaimes GN, Schultz RC, Isenhart TM, 2004. Stream bank erosion adjacent to riparian forest buffers, row-crop fields, and continuously-grazed pastures along Bear Creek in central Iowa. J Soil Water Conserv 2004;59:19–27.

Zeder MA, 2008. Domestication and early agriculture in the Mediterranean basin: origins, diffusion, and impact. Proc Natl Acad Sci U S A 2008;105:11597–604.