A biological tool to assess flow connectivity in reference temporary streams from the Mediterranean Basin

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HIGHLIGHTS

- The effect of flow connectivity on macroinvertebrate communities was tested on temporary rivers.
- Using recursive partitioning, biological metrics were selected to classify flow connectivity.
- Biological trait-based metrics represented antecedent flow conditions of a stream reach.
- A Bio-AS Tool (based on both taxonomy and traits) is proposed.

GRAPHICAL ABSTRACT
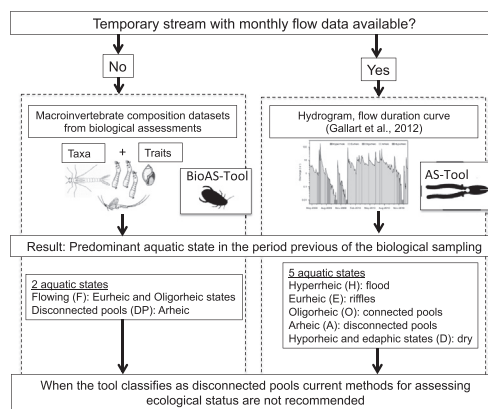
ABSTRACT

Many streams in the Mediterranean Basin have temporary flow regimes. While timing for seasonal drought is predictable, they undergo strong inter-annual variability in flow intensity. This high hydrological variability and associated ecological responses challenge the ecological status assessment of temporary streams, particularly when setting reference conditions. This study examined the effects of flow connectivity in aquatic macroinvertebrates from seven reference temporary streams across the Mediterranean Basin where hydrological variability and flow conditions are well studied. We tested for the effect of flow cessation on two streamflow indices and on community composition, and, by performing random forest and classification tree analyses we identified important biological predictors for classifying the aquatic state either as flowing or disconnected pools. Flow cessation was critical for one of the streamflow indices studied and for community composition. Macroinvertebrate families found to be important for classifying the aquatic state were Hydrophilidae, Simuliidae, Hydropsychidae, Planorbiidae, Heptageniidae and Gerridae. For biological traits, trait categories associated to feeding habits, food, locomotion and substrate relation were the most important and provided more accurate predictions compared to

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taxonomy. A combination of selected metrics and associated thresholds based on the most important biological predictors (i.e. Bio-AS Tool) were proposed in order to assess the aquatic state in reference temporary streams, especially in the absence of hydrological data. Although further development is needed, the tool can be of particular interest for monitoring, restoration, and conservation purposes, representing an important step towards an adequate management of temporary rivers not only in the Mediterranean Basin but also in other regions vulnerable to the effects of climate change.

1. Introduction

Temporary streams are those watercourses that undergo a recurrent cessation of flow or the complete drying of their channel (Williams, 2006), including non-perennial, seasonal, intermittent, episodic or ephemeral flow regimes (Datry et al., 2014a; Datry et al., 2014b). Although temporary streams are very common in many regions of the world, historically they have been neglected by ecologists (Larned et al., 2010) and there remains a general lack of understanding, protecting, and managing this type of freshwater ecosystem (Acuña et al., 2014). In Europe, one of the challenges of the Water Framework Directive (WFD) is the ecological status assessment of temporary streams (Nikolaidis et al., 2013).

The hydrological variability of temporary streams and associated ecological responses has hampered the assessment of their ecological status because tools and metrics have been primarily developed for perennial streams (Sheldon, 2005; Fritz et al., 2008; Munné and Prat, 2011). The cessation of flow represents a critical stage for the river ecosystem where abrupt changes in habitat availability and quality occur, together with associated changes in aquatic communities (Boulton, 2003). Once connectivity is lost and pools become isolated, random and less predictive changes of the environmental conditions may occur, altering biological communities. For this reason, biological quality assessments using available standardized methods should be performed when stream mesohabitats (e.g. riffles, pools) are still connected (Bonada et al., 2006; Buffagni et al., 2009; Buffagni et al., 2010; Prat et al., 2014). In this context, several studies have been devoted to characterizing not only a given hydrological state but also all the different stages that a watercourse may undergo (Boulton, 2003; Williams, 2006). Recently, Gallart et al. (2012) developed a method based on rainfall-runoff models to describe six ecologically relevant aquatic states (AS) representing the different hydrological conditions of a temporary stream reach, ranging from a flood state (i.e. hyperrheic) to the complete absence of surface and subsurface water (i.e. edaphic). This approach resulted in a successful precondition classification tool, named AS-Tool, for the establishment of ecological status, and became a key element of the recently developed integrated assessment protocol for temporary streams in the Mediterranean Basin (i.e. MIRAGE-Toolbox, see Prat et al., 2014). This tool provides information on the frequency of occurrence of aquatic states before the sampling and therefore allows to decide whether biological quality may be measured by the same methods as in permanent streams or not. However, the application of the AS-Tool can be hampered by the absence of gauging stations, the lack of long-term environmental data, or by the influence of local factors such as streambed composition (e.g. bedrock or sand and gravel substrate) not captured by the models. For those cases where the AS-Tool cannot be applied, the MIRAGE-Toolbox aimed at providing an alternative method, named BioAS-Tool, based on the biological assessment of aquatic states (Fig. 1).

On the one hand, macroinvertebrates are widely used as indicators of stream biological quality, including the assessment of flow conditions (Extence et al., 1999; Gore et al., 2001; Suren and Jowett, 2006; Mérigoux et al., 2009; Dunbar et al., 2010). On the other hand, many biomonitoring programmes conducted in streams from the Mediterranean Basin have produced a large number of macroinvertebrate datasets, whereas hydrological data of the stream in question may be absent. Thus, the response of macroinvertebrate communities to

changes in river flow may offer a surrogate method for assessing the aquatic state of temporary streams on the basis that macroinvertebrate communities continuously integrate local flow conditions. For example, the ratio EPT/OCH (i.e. the ratio between Ephemeroptera, Plecoptera and Trichoptera taxa, and Odonata, Coleoptera and Hemiptera taxa) has been used to describe stream flow connectivity in regions with Mediterranean climate (Bonada et al., 2006). Additionally, from a community trait perspective, temporary streams are characterized by macroinvertebrate communities with strategies to cope with extreme hydrological conditions of flooding and drying, as different authors have shown (Williams, 1996; Bonada et al., 2007a; Arscott et al., 2010a; Robson et al., 2011; García-Roger et al., 2013; Vidal-Abarca et al., 2013; Chessman, 2015). These traits explain how organisms respond to environmental constrains and thereby facilitate formulating a priori predictions (Statzner and Bêche, 2010). Thus, biological traits might be useful as indicators of flow connectivity, especially when considering large spatial scales (Statzner et al., 2001; Bonada et al., 2007b).

Climate change predictions in Europe indicate that in particular the Mediterranean region will face severe stream flow deficits (Schneider et al., 2013; Forzieri et al., 2014), increasing the vulnerability of temporary rivers (Acuña et al., 2014; Datry et al., 2014b) and causing permanent ones to become temporary (Datry, 2012; Schneider et al., 2013). Such predicted changes have important implications for the ways in which we currently assess ecological status due to potential shifts in river typology, community composition and, consequently, reference condition baselines (Logez and Pont, 2012; Pace et al., 2013). Thus, there is an urgent need to assess and monitor hydrological and ecological conditions in reference sites in the face of climate change (EC, 2009; Wilby et al., 2010), including reference temporary streams.

The aim of this study was to develop a biological tool (i.e. BioAS-Tool) to classify the aquatic state of temporary streams either as flowing (F) or disconnected pools (DP) using the taxonomic and biological trait composition of the macroinvertebrate community as predictors. Macroinvertebrates from seven reference streams across the Mediterranean Basin were sampled during three years in different seasons, incorporating biogeographic, seasonal, and inter-annual differences in community composition. The rigour of the development of the BioAS-Tool is based on using macroinvertebrate samples from reference streams where hydrological variability and aquatic state classification are well studied (Gallart et al., 2012). The tool aims to enable users to predict the aquatic state that a stream reach has been subjected to before the sample was taken, and thereby to adequately assess the ecological status. Firstly, we assessed the effect of flow connectivity in traditional stream flow indeces. Secondly, we examined shifts in community composition to select those biological predictors that best classified flow connectivity. Finally, we proposed a set of potential novel metrics that constitute the BioAS-Tool.

2. Methods

2.1. Study sites

This study was conducted in seven streams throughout the European Mediterranean Basin (Fig. 2). The streams belong to five different circum-Mediterranean eco-regions according to the classification by Illies (1978): Ibero-Macaronesian region (Enxöe and Taibilla), Pyrenees (Vallcebre), Western plains (La Vêne), Italy, Corsica and Malta
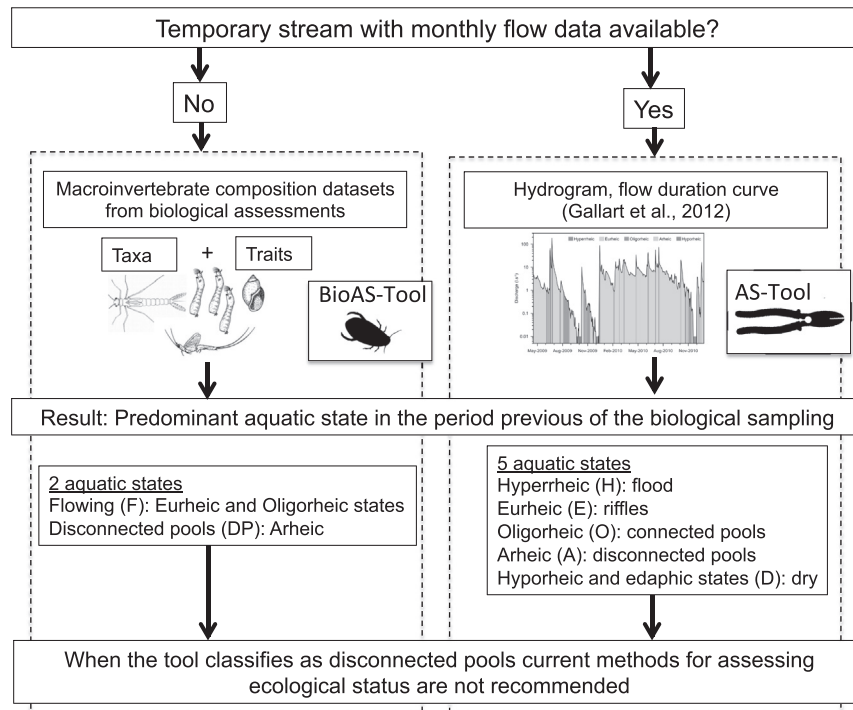
Fig. 1. Flowchart showing the steps to determine the aquatic states of the streams using the AS-Tool and the BioAS-Tool from the MIRAGE toolbox. Adapted from Prat et al., 2014.

region (Biferno and Candelaro), and Hellenic Western Balkan region (Evrotas), thus providing sufficient variation of the aquatic macroinvertebrate communities at regional scale. All streams were subject of study under the EU-funded research project MIRAGE (Mediterranean Intermittent River ManAGEment) and classified as reference sites, supported by Sánchez-Montoya et al. (2012) and Prat et al. (2014). Frequency graphs of the aquatic states for some of streams studied here are described in Gallart et al. (2012) and (De Girolamo et al., 2014). Further details are shown in García-Roger et al. (2013).

2.2. Sample collection and processing

In each stream, we surveyed 1–3 reaches measuring 25–30 m in spring and summer 2009 and 2010, autumn 2010, and winter 2011

Fig. 2. Map of Europe showing the studied streams in the Mediterranean Basin.

Table 1
Summary of location, climate, flow connectivity and invertebrate sampling dates of the temporary streams used in this study (n = 35). F, flowing; DP, disconnected pools.

Country	State/region	Stream	Reach	Year	Season	Flow connectivity	Discharge (l·s^{-1})
France	Languedoc-Roussillon	Vêne	Issanka	2010	Spring	F	495
				2010	Summer	F	83
			Montbazin K	2010	Spring	F	187
			Montbazin R	2010	Summer	DP	0.00
Greece	Laconia	Evrotas	Kolliniatiko	2010	Spring	F	239
				2010	Summer	F	22
				2010	Autumn	F	46
			Rasina	2010	Spring	F	2873
			Sentenikos	2010	Spring	F	1035
				2010	Summer	DP	0.00
				2010	Autumn	DP	0.00
				2011	Winter	F	214
Italy	Molise	Biferno	Riomaio	2010	Spring	F	55
				2010	Summer	DP	0.35
	Puglia	Candelaro	Celone	2010	Spring	F	132
				2010	Summer	F	26
			FM	2010	Spring	F	17
Portugal	Alentejo	Enxöe	Vale do Bargo E	2010	Spring	F	49
				2010	Summer	DP	0.00
Spain	Catalonia	Vallcebre	Cal Rodó	2009	Spring	F	118
				2009	Summer	F	12
				2010	Spring	F	57
				2010	Summer	F	33
			Can Vila	2009	Spring	F	29
				2009	Summer	DP	0.0
				2010	Spring	F	16
				2010	Summer	F	4
	Murcia	Taibilla	Blanco	2009	Spring	F	10
				2009	Summer	F	2
				2010	Spring	F	25
				2010	Autumn	F	13
			Rogativa	2009	Spring	F	2
				2009	Summer	DP	0.08
				2010	Spring	F	11
				2010	Autumn	DP	0.07

(Table 1). In each reach, the sampling integrated all mesohabitats (i.e., riffles and pools) and microhabitats (i.e., different mineral and organic-based substrata) (see García-Roger et al., 2013). We sampled aquatic macroinvertebrate communities using a Surber (dimensions adapted to stream size: ranging from 0.0025 to 0.09 m^2; and 250 μm mesh), and preserved them in 4% formaldehyde to be identified in the laboratory. The identification of macroinvertebrates was done at family level, except for Copepoda, Hydracarina, Ostracoda and Oligochaeta, according to Tachet et al. (2010).
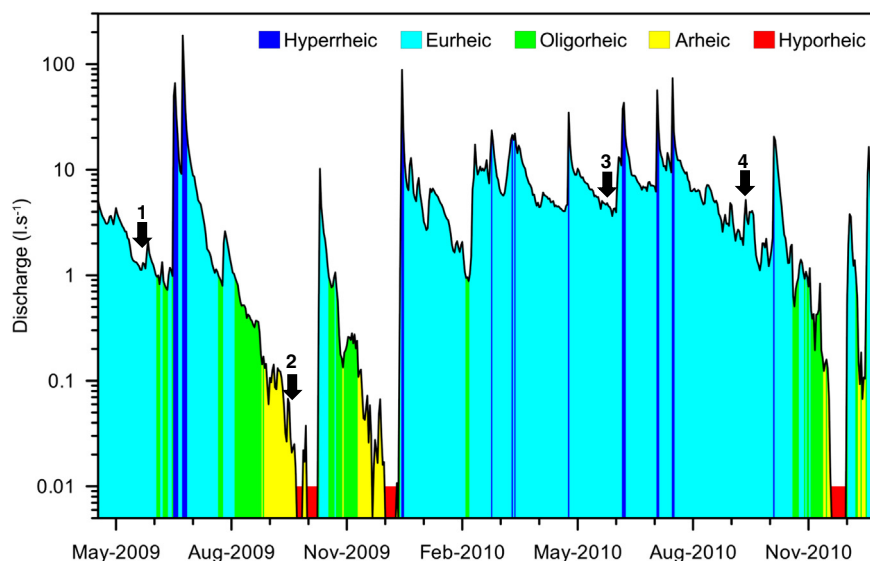

Fig. 3. Hydrograph and aquatic states (F includes the Eurheic and Oligorheic states, while DP corresponds to Arheic) during the biological sampling campaigns (arrows) at Vallcebre, Can Vila.

2.3. Flow connectivity

Following the concept of critical thresholds in flow conditions (Boulton, 2003), the degree of flow connectivity was classified into flowing (F) or disconnected pools (DP) based on the aquatic states obtained from hydrograms in the same streams by Gallart et al. (2012) that were used to develop the AS-Tool (Fig. 3). In the study of Gallart et al. (2012), six ecologically relevant aquatic states were defined to represent the different hydrological conditions of a temporary stream reach (i.e. Hyperrheic, flood state; Eurheic, abundant riffles; Oligorheic, connected pools; Arheic; disconnected pools; Hyporheic, no surface water but wet alluvium; and edaphic, dry alluvium). However, we used a binary aquatic state classification because F and DP encompass the broader aquatic states and indicate the loss of longitudinal flow connectivity. In this sense, F includes the Eurheic and Oligorheic states, while DP corresponds to Arheic. Moreover, well-established aquatic macroinvertebrate communities have been described in F and DP, compared to the heavily disturbed hyperreic state or dry state (Datry, 2012).

To confirm the hydrological classification obtained from hydrograms we used direct field observations on the aquatic states synchronous with water discharge measurements. Discharge (D) was obtained from automatic gauging stations or, alternatively, by measuring channel width and taking water velocity at a minimum of five equal subsections of the reach selected. In total, 27 samples corresponded to F and 8 to DP and at least one stream per country presented one reach in DP conditions (Table 1). Examples of the aquatic state classification and threshold flow values that separate their occurrence at different stream reaches of the present study are shown in Table 2. As a general rule for all the streams not present in Table 2, when $D > 0.5 \ \mathrm{l \cdot s^{-1}}$ the reach was classified into F while if $D \leq 0.5 \ \mathrm{l \cdot s^{-1}}$ into DP.

2.4. Biological traits

Biological trait data were originally obtained from Tachet et al. (2010). We used 61 categories of 11 biological traits, which describe macroinvertebrate morphology (maximum potential size), life-cycle features (duration, number of cycles per year, aquatic stages), reproduction, resilience or resistance abilities (dispersal, resistant forms, locomotion), physiology (respiration) and feeding behaviours (food and feeding habits).

Based on the database at genera level with their affinity to each trait category using a fuzzy coding approach (Chevenet et al., 1994; Bonada et al., 2007a; Mellado-Díaz et al., 2008), we computed and re-scaled (to a 1–5 scale) the average affinity to a family level. Although the common level of taxonomical resolution at which studies on traits in aquatic macroinvertebrates are performed is the genera level, it has been demonstrated that an accurate description of trait abundance and diversity may be achieved by identification at higher taxonomical levels (i.e. family), no matter the spatial scale (Dolédec et al., 2000; Gayraud et al., 2003). Furthermore, the effect of the level of taxonomic resolution (family cf. genus) on the structure of biological trait data corresponding to a subset of the streams used in the present study was previously tested by García-Roger et al. (2013) and was small enough to be neglected.

Considering the purpose of the present study and given that most biomonitoring metrics use family-level identifications, we used family level to compute all community metrics. This coarse approximation will also allow application of the tool developed here to other family-level datasets from biomonitoring programmes in the Mediterranean Basin or other regions (Munné and Prat, 2009, 2011).

2.5. Community metrics

Two stream flow indices for rivers commonly used in bioassessment in the studied area were calculated: the ratio EPT/(EPT + OCH) (where EPT stands for sum of number of Ephemeroptera, Plecoptera and Trichoptera and OCH the sum of number of Odonata, Coleoptera and Hemiptera, all at family level) and the LIFE-F index (Lotic-invertebrate Index for Flow Evaluation; Extence et al., 1999). The ratio (EPT/(EPT + OCH)) has been used to assess seasonal changes due to flow variation (Bonada et al., 2006). The LIFE-F index is based on British macroinvertebrate preferences to flow velocity and has been used to assess macroinvertebrate community response to flow variability (Extence et al., 1999). These indices were tested for differences between F and DP for each community metric using nonparametric Kruskal–Wallis tests.
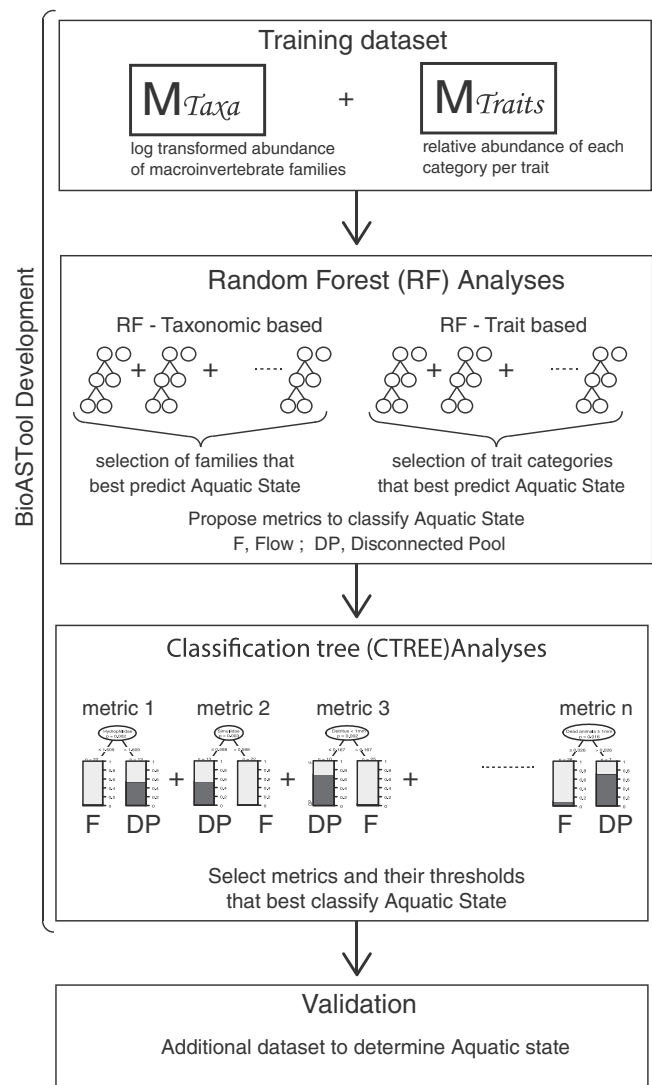

Fig. 4. Diagram showing the different processes for the development of the BioAS-Tool in temporary streams from the Mediterranean Basin.

Table 2
Flow thresholds between aquatic states and F and DP in $\mathrm{l \cdot s^{-1}}$ defined in different stream reaches included in the present study (adapted from Gallart et al., 2012). F, flowing; DP, disconnected pools.

Flow connectivity	Aquatic state	Stream reach			
		Vêne, Montbazin K	Candelaro, Celone	Vallcebre, Can Vila	Taibilla, Rogativa
F	Hyperheic	>800	>1000	>20	>1000
	Eurheic	100–800	30–1000	1–20	10–1000
	Oligorheic	50–100	50–30	0.35–1	3–10
DP	Arheic	10–50	1–10	0.05–0.35	0.05–3
	Hyporheic	<10	<1	<0.05	<0.05

2.6. BioAS-Tool development

The development of the BioAS-Tool consisted of a two-step approach that involved the performance of two different classification methods using macroinvertebrate data as predictors (Fig. 4). Firstly, we used the Random Forest (RF) algorithm (Breiman, 2001) to select the subset of biological predictors that best classified flow connectivity and propose them as potential new metrics. Secondly, we used classification tree (Ctree) analyses (Hothorn et al., 2014) to obtain thresholds for the new metrics and the existing metrics known to respond to flow conditions according to the literature (i.e. LIFE-F index and EPT/(EPT + OCH)).

2.6.1. Random forest analyses

RF is a non-parametric classifier method that uses bootstrap samples of the data with replacement to grow a set of decision trees, allowing selection of the most popular assigned class, and thus producing a significant increase in classification accuracy (Liaw and Wiener, 2002). Compared to other traditional statistical analyses, RF cannot be overfit and are especially useful when there are few sample sites and many possible predictors (Breiman, 2001; Cutler et al., 2007), as is the case in this study. Furthermore, RF provides measures of the importance of predictor variables, which can be used to understand the contribution of specific variables in a classification. Predictors can be considered informative if their variable importance value is above the absolute value of the lowest scoring variable and non-informative if their importance value varies randomly around zero (Strobl et al., 2009). We built two RF models based on taxonomy and traits using the log-transformed macroinvertebrate family abundances and their relative abundance of each category per trait as predictors, respectively, obtaining the predictor importance. The subset of most important macroinvertebrate families and trait categories were therefore proposed as potential novel metrics to classify flow connectivity, as well as a multimetric index based on the sum of the proportion of each selected trait category. Furthermore, for the case of the trait-based RF model, we checked if the subset of trait categories selected as the most

important predictors were within those that we a priori expected to predict flow connectivity (see Table 3).

2.6.2. Classification tree analyses and selection of metrics

Ctree is a non-parametric method of recursive binary partitioning that creates splits in those input variables presenting (individually) the highest P relative to the response variable (Hothorn et al., 2014). Unlike RF, Ctrees provide threshold values for each significant variable, thus facilitating ecological interpretation. We built one Ctree for each metric proposed and also for the existing metrics, and we tested for their performance in classifying flow connectivity. In order to assess each Ctree's performance we calculated: (1) total percentage of classes correctly classified, (2) percentage of F correctly classified (3) percentage of DP correctly classified, and (4) Cohen's kappa. Cohen's kappa (κ) is a measure of the agreement between predictions and actual values, corrected for agreement due to chance alone. For κ, values of 0.0–0.4 are considered to indicate slight to fair model performance, values of 0.4–0.6 moderate, 0.6–0.8 substantial and 0.8–1.0 almost perfect (Manel et al., 2001). We kept only those metrics with at least moderate performance ($\kappa > 0.4$) and use them for validation with the additional dataset (see next section).

2.7. BioAS-Tool validation

The applicability of the BioAS-Tool was tested using external data from other reference temporary streams located in the Mediterranean Basin not initially included in the model building. Data were obtained from different biomonitoring programmes carried out in three 3 regions. The large part of the dataset came from the FEM (Freshwater Ecology and Management) research group in the Department of Ecology at the University of Barcelona (Catalonia) and included records from 189 sites collected between 1998 and 2010 (http://ecobill.diba.cat). Another dataset was obtained from the Istituto di Ricerca Sulle Acque (CNR-IRSA, Italy) and included records from 8 sites sampled in 2004 in Sardinia. Finally, a last dataset was provided by the Department of Ecology and Hydrology at the University of Murcia and included records

Table 3
Biological trait categories expected to predict flow connectivity and to be predominant in flow (F) and disconnected pools (DP).
Rationale formulated after Wallace & Webster (1996), Bonada et al. (2007) and Statzner and Bêche (2010).

Trait	Category	Predicted connectivity	Rationale
Maximal size	>0.5–1 cm	F	The presence of flow requires less resilience capacity and permits larger sizes, but also constrains very large sizes, therefore favouring intermediate sizes in flow conditions.
	>1–2 cm	F	(As above)
	>2–4 cm	DP	Very large sizes not adapted to high hydraulic stress are less constrained in disconnected pools.
	>4–8 cm	DP	(As above)
	>8 cm	DP	(As above)
Aquatic stages	Adult (imago)	DP	Disconnected pools may act as refuge for many aquatic adults that cannot escape by flying (e.g. Mollusca, Crustacea) thus their relative abundance could be increased.
Dispersal	Aquatic passive	F	Flow allows downstream drift.
	Aerial active	DP	Flow cessation favours flying.
Resistance forms	Diapause or dormancy	DP	When flow is disconnected increased resistance against droughts may be favoured.
Respiration	Gills	F	Increased oxygen deficit in disconnected pools may limit aquatic respiration.
	Spiracle (aerial)	DP	Increased oxygen deficit in disconnected pools may favour air breathers.
Locomotion and substrate relation	Flier	DP	When flow is disconnected, strategies to escape may be favoured.
	Surface swimmer	DP	Release from action of flow in disconnected pools favours swimming.
	Full water swimmer	DP	(As above)
	Temporarily attached	F	The action of flow favours organisms that have adaptations for attachment to the substrate.
Food	Detritus <1 mm	F	Higher availability of suspended organic matter (i.e. FPOM) transported by flow
	Dead animal >1 mm	DP	Increased number of dead organisms can be accumulated due to stress as pools decline in water quality
Feeding habits	Filter feeder	F	Higher availability of suspended organic matter under flow conditions

from 21 sites sampled in 2003. In total, the validation dataset consisted of 218 stream reaches with their corresponding aquatic state (F or DP) obtained from direct field observations. The methodology used in 3 regions are similar and the samples were obtained using standardized monitoring protocols (see Sánchez-Montoya et al., 2009), and contained semi-quantitative macroinvertebrate information at the family level.

All statistical analyses and graphs were carried out using R open-source software (R Core Team, 2013) using ade4 (Dray and Dufour, 2007), vegan (Oksanen et al., 2014), party (Hothorn et al., 2014), and vcd (Meyer et al., 2013) packages.

3. Results

3.1. Effect of flow connectivity on streamflow biological indices

The ratio EPT/(EPT + OCH) presented lower values in DP compared to F, but only the LIFE-F index showed the significant differences between F and DP ($P < 0.01$) (Fig. 5).

3.2. Biological predictors of flow connectivity

In order of importance, the abundance of Simuliidae, Hydrophilidae, Hydropsychidae, Heptageniidae, Planorbidae, Caenidae and Gerridae were the subset of macroinvertebrate families that best predicted flow connectivity in the taxonomy-based RF model (Fig. 6). In the case of biological traits, the proportion of filter feeders, organisms feeding on detritus <1 mm, temporarily attached to the substrate, fliers, with a mean body size between 1 and 2 cm, feeding on dead animals ≥1 mm, with diapause and adult (imago) aquatic stages were prioritized as primary predictors in the trait-based RF model (Fig. 7). Other trait categories a priori expected to be potential predictors of flow connectivity were not selected by the model.

Ctree results for each of the biological predictors selected by RF showed that not all the macroinvertebrate families and not all biological trait categories selected by RF models created splits to classify flow connectivity. Within this group were the abundance of Caenidae family and the trait categories corresponding to organisms with a mean body size between 1 and 2 cm, with diapause and adult (imago) aquatic stages, which were excluded for the subsequent analyses. Nevertheless, most of the taxonomical and biological predictors selected by RF classified flow connectivity into F or DP. For example, higher abundances of Hydrophilidae and Planorbiidae were more likely to be found in DP, with

thresholds of 3.305 and 1.92 log+1 (ind m^{-2}), respectively, together with the presence of Gerridae. In contrast, higher abundances of Simuliidae, with a threshold of 0.588 log+1 (ind m^{-2}), and the presence of Hydropsychidae and Heptageniidae were more representative of F (Fig. 8). As expected from our a priori predictions (Table 3), higher proportions of macroinvertebrates feeding on detritus <1 mm, filter feeders, temporarily attached to the substrate were more likely to be present in F, with thresholds of 16.7%, 7.2%, and 6.6%, respectively (Fig. 9). Also in agreement with our a priori predictions, higher proportions of macroinvertebrates feeding on dead animals ≥1 mm and fliers occurred in DP, with thresholds of 2.6% and 3.5%, respectively (Fig. 9). Consequently, five biological predictors formed the potential metrics to classify flow connectivity. Additionally, based on the performance and sum of the most important trait categories which are Detritus >1 mm, Filter feeder and temporarily attached, a new multimetric index is presented and named hereafter as Metatrait.

In general, the assessments of flow connectivity were more accurate in the trait-based Ctree models compared to the taxonomy-based ones. Three of the 6 biological trait-based Ctree models presented a substantial performance ($\kappa = 0.64$–0.70), whereas the others were moderate ($\kappa = 0.58$–0.59). Within these, the most accurate Ctree model corresponded to detritus >1 mm ($\kappa = 0.7$) and the Metatrait multimetric index ($\kappa = 0.67$), both with 88.6% overall accuracy (Table 4). Within the taxonomy-based metrics, only the Ctree based on the LIFE-F index presented a substantial performance ($\kappa = 0.61$), whereas others performed moderately ($\kappa = 0.40$–0.59) or badly ($\kappa = 0$). Metrics with $\kappa = 0$ (i.e. Heptageniidae abundances) were not considered for validation (see methods). The Ctree performed on the EPT/(EPT + OCH) ratio did not create any split to classify flow connectivity and, therefore, was not included in Table 4.

3.3. Validation

Only the metrics and thresholds that effectively classified into F or DP were calculated for the external data from other reference streams. When validating these metrics, the highest classification success of flow connectivity was obtained for the abundance of Hydrophilidae and the Metatrait multimetric index, respectively with 83.5% and 82.1% of correct classifications (Table 4). However, the classification success predicted with the abundance of Hydrophilidae was much higher for F (91.7%) than for DP (20%). In contrast, within the taxonomy metrics, Simuliidae abundance and the LIFE-F index, performed similarly when classifying DP and F, with correct classifications for both groups around 80%. Within the trait-based metrics, metrics such as organisms that feed on detritus >1 mm and filter feeders followed the same pattern when classifying DP and F. The lowest classification success was obtained for organisms feeding on dead animals of >1 mm and Gerridae abundance, classifying 40.8% and 47.2% of stream reaches correctly, respectively.

4. Discussion

4.1. Macroinvertebrate families and traits as predictors of flow connectivity

The macroinvertebrate families that best predicted flow connectivity in this study agreed with their classification into rheophilic or limnephilic organisms (see Tachet et al., 2010; Mérigoux and Dolédec, 2004; Cid, 2010) and demonstrated a distinct shift of dominant taxa from F (i.e. Hydropsychidae, Simuliidae, and Heptageniidae) to DP (i.e. Hydrophilidae, Planorbidae, Gerridae). Hydrophilids are typically from lentic habitats and were the most important macroinvertebrate family in predicting flow connectivity. These beetles are well adapted to temporary water bodies and have wide distributional ranges (Ribera, 2000). They are also amongst the best indicators of pool habitats in temporary streams not only from the Mediterranean Basin (García-Roger et al., 2011) but also in temporary montane desert streams from Arizona
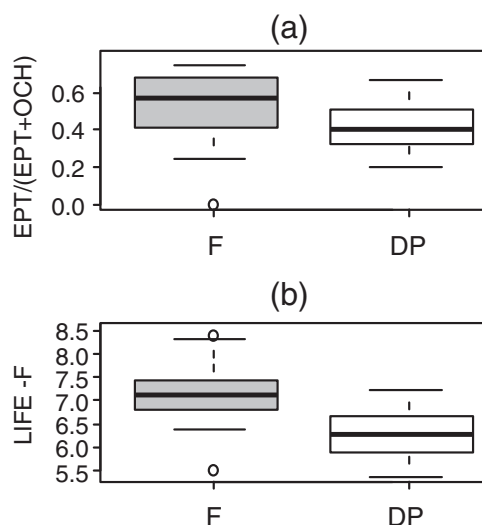
Fig. 5. Box-plots indicating streamflow indices in flowing (F) and disconnected pools (DP) conditions in temporary streams from the Mediterranean Basin.
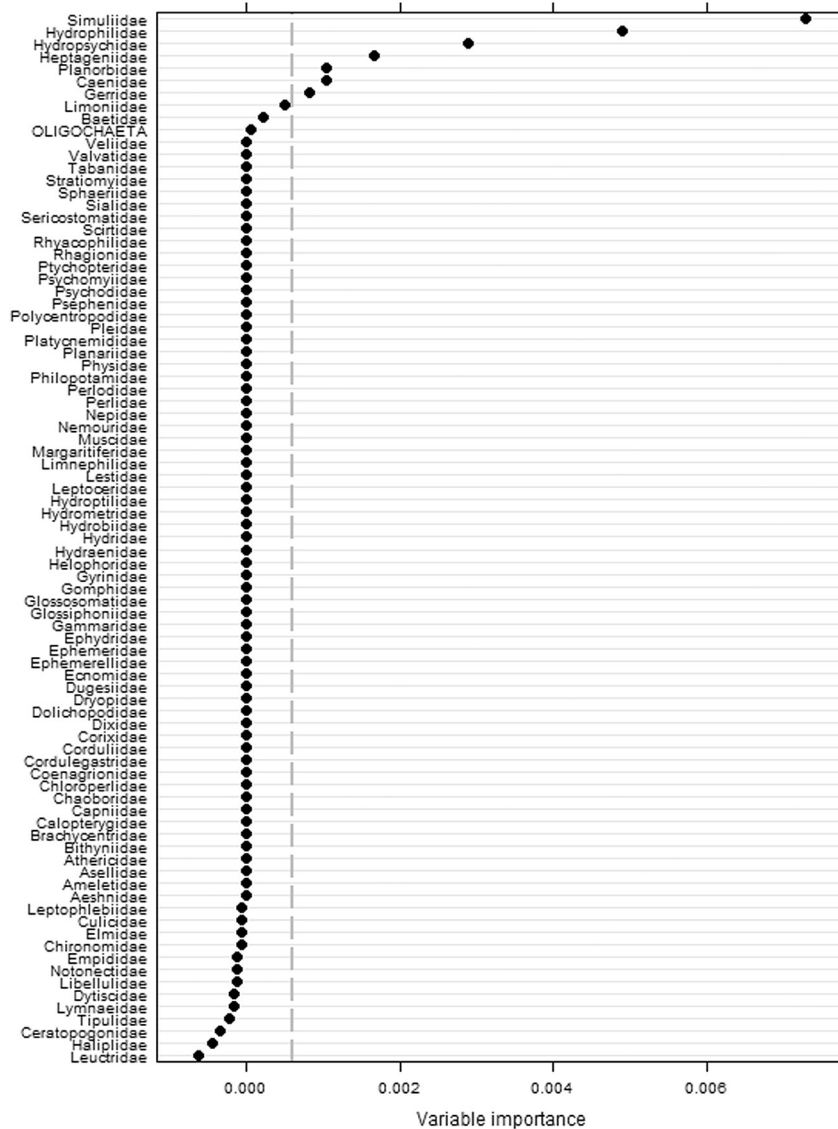
Fig. 6. Importance plot of macroinvertebrate families in predicting flow connectivity obtained from random forest analysis. Higher values indicate higher importance to the random forest classification. The dashed vertical grey line on the plot represents the absolute value of the lowest ranking predictor. Macroinvertebrate families to right of vertical line were significant and were selected as predictors to build the to build the BioAS-Tool (see Methods section).

(Bogan and Lytle, 2007). The amphibious character of Hydrophilidae has been widely described, giving them the capacity to escape from pools when they completely dry out (Larned et al., 2010). While adults are herbivorous, the larvae are carnivorous and can also be cannibalistic, feeding almost on anything available as prey (Richmond, 1920). Other studies have shown that they can avoid oviposition when predatory fish are present in pools (Resetarits, 2001). Recently, Picazo et al. (2012) found that Hydrophilidae were the most diverse Coleoptera in terms of biological traits in Mediterranean freshwater ecosystems, confirming the high adaptation capacity of this family. Other lentic macroinvertebrates, such as Planorbidae (Gastropoda), were more abundant in DP, in agreement with the results in Bonada et al. (2006). Gastropoda were also more abundant in rewetted sediments from temporary streams compared to flowing streams in a study performed by Storey and Quinn (2013). Reophilic taxa, such as Simuliidae, Hydropsychidae and Heptageniidae, were more abundant in F, as shown by other studies (Bonada et al., 2006; Bogan and Lytle, 2007). However, within these reophilic families, Simuliidae was the best reophilic predictor of flow connectivity. Overall, our results complement the classical view that EPT and OCH taxa are the best indicators of flow connectivity (Bonada et al., 2006; Bonada et al., 2007a).

Of the five biological trait categories that best predicted flow connectivity, only two (i.e. organisms feeding on dead animals ≥1 mm and fliers) were associated to DP, while others were associated to F (i.e. organisms feeding on detritus <1 mm, filter feeders, and temporarily attached). As expected, macroinvertebrates temporarily attached to the substrate were very abundant in F as a result of morphological adaptation to flow (Statzner and Bêche, 2010). The same was observed for the proportion of filter feeders (Lamouroux et al., 2004; Mérigoux and Dolédec, 2004), which could be related with the increased abundances of reophilic families, such as Hydropsychidae and Simuliidae. In general, biological traits provide a mechanistic response to direct stressors related with water discharge (e.g. near bottom flow), whereas indirect flow effects such as temporality may produce more diverse and equivocal responses (Statzner and Bêche, 2010). As expected, those biological trait categories from our a priori hypotheses that are strictly linked to the action of flow were the best predictors of flow connectivity. This suggests that the observed shifts in the macroinvertebrate community are due to the selective loss of taxa susceptible to drying rather than selection for desiccation-resistant specialists, in agreement with other studies (Arscott et al., 2010a; Datry, 2012; Vidal-Abarca et al., 2013). However, other trait categories may become more important as a result of
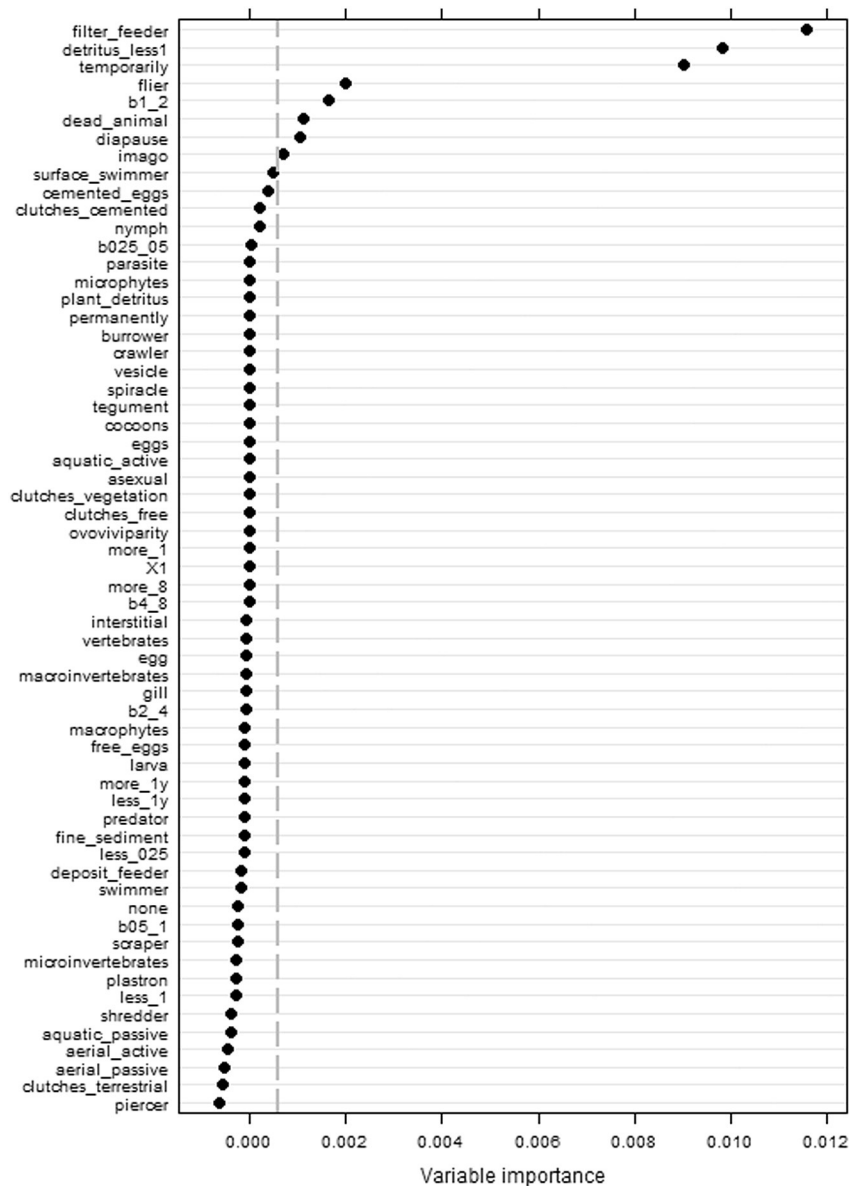
Fig. 7. Importance plot of macroinvertebrate biological trait categories in predicting flow connectivity obtained from random forest analysis. Higher values indicate higher importance to the random forest classification. The dashed vertical grey line on the plot represents the absolute value of the lowest ranking predictor. Biological traits to right of vertical line were significant and were selected as predictors to build the BioAS-Tool (see Methods section).

opportunism. For instance, the higher proportion of macroinvertebrates feeding on dead animals found in DP could be related with an increased mortality during drought (Ledger et al., 2011). On the other hand, the higher proportion of fliers in DP suggests that aquatic invertebrates inhabiting temporary streams also have avoidance strategies to escape immediately to neighbouring water bodies (Bonada and Resh, 2013). The fact that other a priori predicted traits (e.g. life history traits) were not linked to flow connectivity could be explained by the Mediterranean behaviour of all these streams as a result of filters acting at larger spatial and temporal scales (Bonada et al., 2007a, 2007b).

4.2. Predicting flow connectivity using biological metrics: Bio-AS tool

The selected metrics and thresholds proposed in this study were able to successfully classify flow connectivity in most of the reference temporary streams analysed. This provides a potential new method to determine flow connectivity at the reach scale that can be particularly relevant when hydrological data are missing and the AS-Tool developed under the MIRAGE project cannot be applied. Even when gauging stations are present, they may under-represent temporary river segments (Snelder et al., 2013) or provide stream flow statistics that could be a poor surrogate for ecologically significant reach-scale hydraulic metrics (Turner and Stewardson, 2014). Therefore, the combination of metrics proposed by the BioAS-Tool can be an alternative method for predicting hydrological conditions of an temporary stream reach (e.g. AS-Tool, see Prat et al., 2014), thereby becoming another important element of the integrated assessment for temporary streams provided by the MIRAGE Toolbox (Prat et al., 2014). In order to exemplify the application of the BioAS-Tool in ecological status assessments, we used a subset of the validation samples from one temporary reference stream taken in different seasons (spring and summer during 10 years) from which data on IBMWP index (Iberian Biological Monitoring Working Party, Alba-Tercedor et al., 2004) was available. The IBMWP index is commonly used for water quality bioassessment in rivers from the Mediterranean Basin (Munné and Prat, 2009). On the one hand, these samples were classified as F or DP by direct field observations, and on the other hand, the same classification was used using the BioAS-Tool. The highest variability of IBMWP is displayed in the
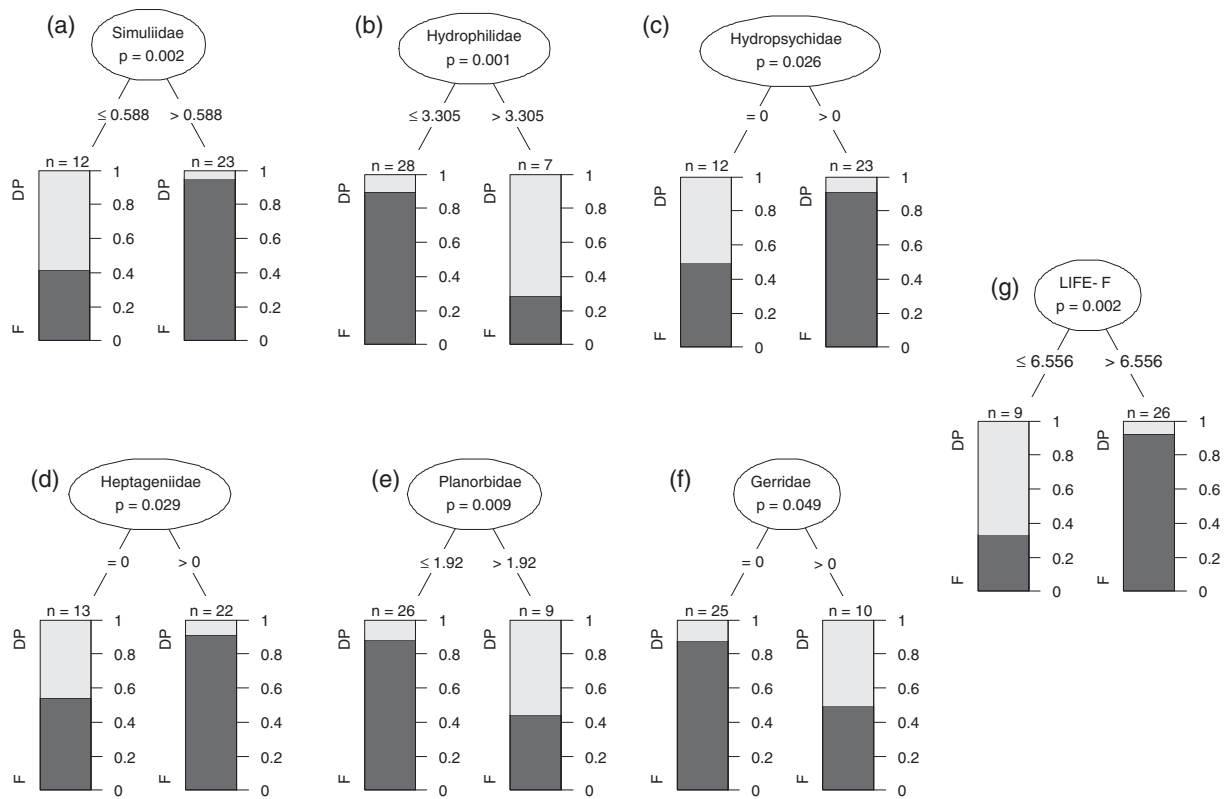
Fig. 8. Conditional inference trees (Ctree) showing macroinvertebrate families that significantly classified stream reaches into flowing (F) and disconnected pools (DP) (a–e). The last Ctree (f) was performed using the LIFE-F index. For each inner node, the Bonferroni-adjusted *P* is given and the fraction of F and DP is displayed for each terminal node.
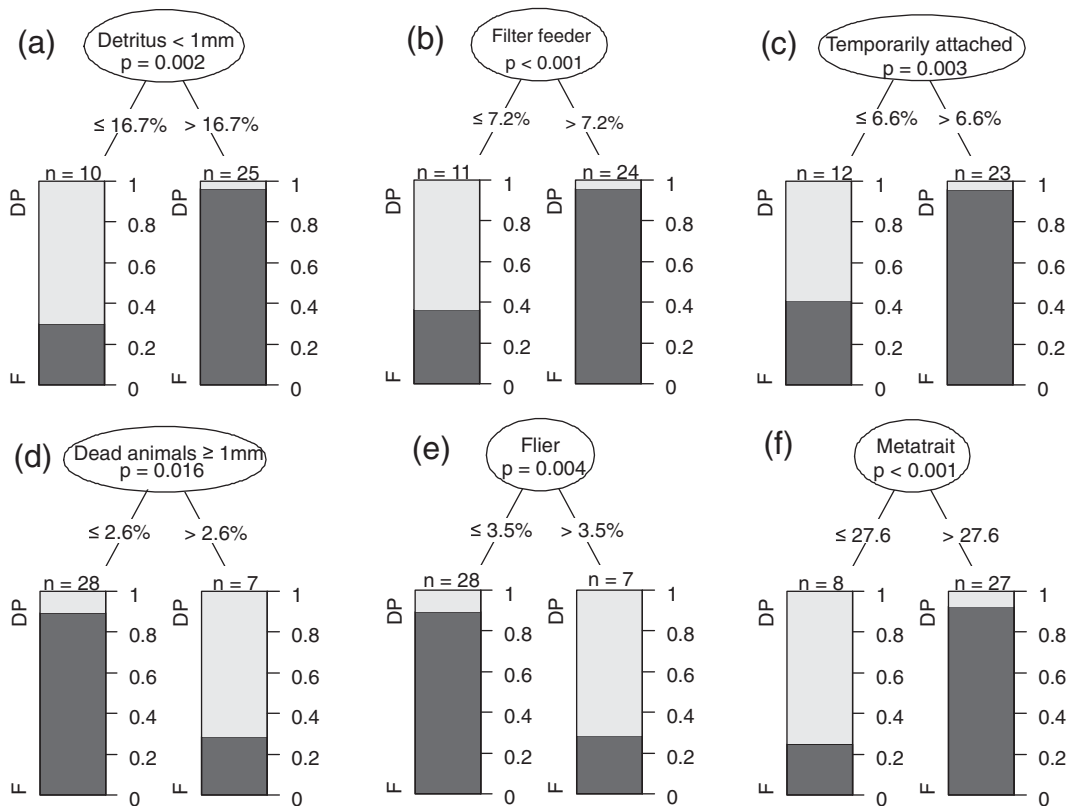

Fig. 9. Conditional inference trees (Ctree) showing biological trait categories and thresholds that significantly classified stream reaches into flowing (F) and disconnected pools (DP) (a–f). The last Ctree (g) was performed on the sum of these significant trait categories. For each inner node, the Bonferroni-adjusted *P* is given and the fraction of F and DP is displayed for each terminal node.

Table 4

Results of the classification trees built using each of the most important macroinvertebrate families and biological trait categories selected from the random forest model models (see Fig. 6 and Fig. 7). Classification trees were also built using the Lotic Invertebrate index for Flow Evaluation (LIFE-F) and novel index based on the sum of the above mentioned trait categories (Metatrait). Threshold values for each macroinvertebrate family refer to the log-transformed family abundances and threshold values for each biological trait category refer to the proportion per trait.

	a. Taxonomy-based metrics							b. Trait-based metrics					
	Hydrophilidae	Simulidae	Hydropsychidae	Planorbidae	Heptageniidae	Gerridae	LIFE-F	Detritus < 1 mm	Filter feeder	Temporarily attached	Dead animals > 1 mm	Flier	Metatrait
Threshold	3.305	0.588	0	1.92	0	0	6.556	16.7	7.2	6.6	2.6	3.5	27.6
Unit	Log+1 (ind/m²)	Log+1 (ind/m²)	Log+1 (ind/m²)	Log+1 (ind/m²)	Log+1 (ind/m²)	Log+1 (ind/m²)	score	%	%	%	%	%	Score
P	0.001	0.002	0.026	0.009	0.029	0.049	0.002	0.002	<0.001	0.003	0.016	0.004	<0.001
Training dataset (n = 35)													
Total correctly classified (%)	80.0	82.9	77.1	80	77.1	77.1	85.7	88.6	85.7	82.8	85.7	85.7	88.6
F correctly classified (%)	77.7	81.5	77.7	85.2	100	81.5	88.8	88.8	85.2	81.5	92.6	92.6	92.6
DP correctly classified	87.5	87.5	75.0	62.5	0	62.5	75.0	87.5	87.5	87.5	62.5	62.5	75.0
Cohen's Kappa	0.58	0.59	0.45	0.46	0	0.4	0.61	0.7	0.64	0.59	0.58	0.58	0.67
Test dataset (n = 218)													
Total correctly classified (%)	83.5	79.8	58.7	62.4	–	47.2	78.9	76.1	72.9	70.6	40.8	71.6	82.1
F correctly classified (%)	91.7	79.8	54.9	68.4	–	45.6	80.3	76.7	73	68.4	37.8	69.9	85.0
DP correctly classified	20	80	88	16	–	60	68	72	72	88	64	84	60

DP classification with direct field observation (Fig. 10a), and corroborates the difficulty of evaluating antecedent flow conditions in DP. In contrast, the variability of the IBMWP index for the same samples was reduced when F or DP was classified using the Metatrait multimetric index (Fig. 10b). Those samples presenting higher IBMWP values would have a macroinvertebrate community typical of F according to the BioAS-Tool, although DP was registered at the moment of sampling. Therefore, the aquatic community may be more representative of antecedent conditions than conditions present at the time the sample was collected. This can occur when sampling takes place just after the fracture into several pools and the probability of finding predictors of F can be still high before the succession to a pool-dominated community takes place. In this sense, the classification of DP or F by direct observation in the field could be biased to any side whereas precision increases using the BioAS-Tool. This can be very relevant when setting reference conditions in temporary rivers. For instance, Sánchez-Montoya et al. (2009) were not able to validate the reference condition criteria using biological communities from temporary streams due to the high variability of reference values of biological indices. When only spring samples were used to establish reference conditions to minimize the effect of summer drought, a high variability was also observed depending on the index used (Sánchez-Montoya et al., 2010; Munné and Prat, 2011). Therefore, rather than seasonality, between-site variability attributed to different frequencies and durations of connections and disconnections is indeed a key aspect to be considered (Larned et al., 2010). In this sense, the BioAS-Tool can contribute to predict hydrologic conditions independently of the season, and, for instance, be applied in those samples taken in the past and used for setting reference conditions.

Although the methods used in this study offer robust and accurate classifications (Cutler et al., 2007), several limitations were identified. Firstly, different percentages of DP correctly classified compared to F were observed for the different metrics, which could be associated to a high variability of ecological responses in each pool. For instance, the presence of riparian forest could buffer temperature increase through its shading effect (Beschta, 1997; Kristensen et al., 2013) and contribute towards maintaining the level of dissolved oxygen in the pool. Similar effects could be observed if the pool is connected to the groundwater. In contrast, if the pool remains exposed to direct solar irradiation the contraction process goes faster (Lake, 2011). Depending on the time elapsed since disconnection took place, different environmental conditions may exert greater or lesser effects on biological communities because of changes in water quality (Lake, 2011), available mesohabitats and microhabitats (García-Roger et al., 2011, 2013), and biological interactions (Acuña et al., 2005). Secondly, the imbalance between the number of stream reaches in DP (n = 8) and F (n = 27) in the training dataset could have caused some level of bias in the classification, underpredicting the minority class (Evans and Cushman, 2009). Still, the classification performance using most of the trait-based metrics was higher compared to the taxonomy-based metrics. Therefore, a taxonomy-based approach could be limiting when large spatial scales are compared due to the influence of historical or phylogenetic factors (Bonada et al., 2008), whereas traits may be less affected (Bonada et al., 2007b).

The Mediterranean Basin is considered a hotspot of biodiversity with high rates of endemism (Myers et al., 2000; Bonada and Resh, 2013) but also with highly threatened biota (Filipe et al., 2013). Studies analysing climate change effects on the distribution of freshwater organisms and biodiversity have shown that southern European regions will be the most affected (Domisch et al., 2013; Markovic et al., 2014). In this context, given that long-term datasets on macroinvertebrates can be obtained from biomonitoring programmes usually identified at the family level, the BioAs-Tool could provide a site-specific history in flow connectivity by using samples collected in the past and contribute towards assessing temporal and spatial climate change effects in reference streams from the Mediterranean Basin.
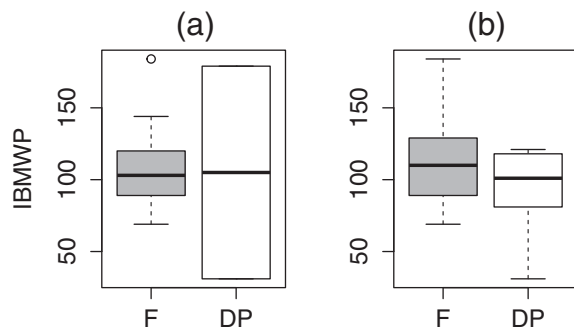
Fig. 10. Box-plots of IBMWP indices of a temporary stream from the Mediterranean Basin in flowing (F) and disconnected pools (DP) calculated in two ways: (a) based on field observation or (b) calculated using the Metatrait multimetric index from the BioAS-Tool.

This study is the first in providing a methodological framework for predicting aquatic states using both taxonomic and biological trait composition of macroinvertebrate communities from temporary streams. Although further development is needed, identifying the relevant metrics responding to flow cessation and their thresholds at the reach scale is of particular interest for monitoring, restoration and conservation purposes, especially knowing that the absence of hydrological data is a common problem in Mediterranean temporary rivers. This represents an important step towards an adequate management of rivers in the Mediterranean Basin as well as towards increasing their recognition in policies related to water and biodiversity.

4.3. Future perspectives

Changes in biotic communities with increasing flow intermittence are generally gradual, with no clearly defined thresholds (Datry et al., 2014a). Due to the management focus of our study, we defined thresholds of flow discharge (F and DP) and obtained the response of particular macroinvertebrate groups and biological traits. However, a detailed analysis of the gradual changes of biotic communities along the different hydrological conditions that the river ecosystem undergoes (from a flood state to complete drying) would be needed. This would allow the development of more precise tools needed to predict past and future hydrological conditions using biological data and advance in the science of temporary rivers.

Acknowledgements

References

Acuña, V., Muñoz, I., Giorgi, A., Omella, M., Sabater, F., Sabater, S., 2005. Drought and postdrought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. J. North Am. Benthol. Soc. 24, 919–933.
Acuña, V., Datry, T., Marshall, J., Barceló, D., Dahm, C.N., Ginebreda, A., et al., 2014. Why should we care about temporary waterways? Science 343, 1080–1081.
Alba-Tercedor, J., Jáimez-Cuéllar, P., Alvarez, M., Avilés, J., Bonada, N., Casas, J., et al., 2004. Caracterización del estado ecológico de los ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP'). Limnetica 21, 175–185.
Arscott, D.B., Larned, S., Scarsbrook, M.R., Lambert, P., 2010. Aquatic invertebrate community structure along an intermittence gradient: Selwyn River, New Zealand. J. North Am. Benthol. Soc. 29, 530–545.
Beschta, R.L., 1997. Riparian shade and stream temperature: an alternative perspective. Rangelands 19, 25–28.
Bogan, M.T., Lytle, D., 2007. Seasonal flow variation allows "time-sharing" by disparate aquatic insect communities in montane desert streams. Freshw. Biol. 52, 290–304.
Bonada, N., Resh, V.H., 2013. Mediterranean-climate streams and rivers: geographically separated but ecologically comparable freshwater systems. Hydrobiologia 719, 1–29.
Bonada, N., Rieradevall, M., Prat, N., Resh, V.H., 2006. Benthic macroinvertebrate assemblages and macrohabitat connectivity in Mediterranean-climate streams of northern California. J. North Am. Benthol. Soc. 25, 32–43.
Bonada, N., Rieradevall, M., Prat, N., 2007a. Macroinvertebrate community structure and biological traits related to flow permanence in a Mediterranean river network. Hydrobiologia 589, 91–106.
Bonada, N., Dolédec, S., Statzner, B., 2007b. Taxonomic and biological trait differences of stream macroinvertebrate communities between Mediterranean and temperate regions: implications for future climatic scenarios. Glob. Chang. Biol. 13, 1658–1671.
Bonada, N., Rieradevall, M., Dallas, H., Davis, J., Day, J., Figueroa, R., et al., 2008. Multi-scale assessment of macroinvertebrate richness and composition in Mediterranean-climate rivers. Freshw. Biol. 53, 772–788.
Boulton, A.J., 2003. Parallels and contrasts in the effects of drought on stream macroinvertebrate assemblages. Freshw. Biol. 48, 1173–1185.
Breiman, L., 2001. Random forests. Mach Learn 45, 5–32.
Buffagni, A., Armanini, D.G., Erba, S., 2009. Does the lentic–lotic character of rivers affect invertebrate metrics used in the assessment of ecological quality? J. Limnol. 68, 92–105.
Buffagni, A., Erba, S., Armanini, D.G., 2010. The lentic–lotic character of Mediterranean rivers and its importance to aquatic invertebrate communities. Aquat. Sci. 72, 45–60.
Chessman, B., 2015. Relationships between lotic macroinvertebrate traits and responses to extreme drought. Freshw. Biol. 60, 50–63.
Chevenet, F., Dolédec, S., Chessel, D., 1994. A fuzzy coding approach for the analysis of long-term ecological data. Freshw. Biol. 31, 295–310.
Cid, N., 2010. Ecology of the Benthic Macroinvertebrates in the lower Ebro River, Community Characterization, Population Dynamics and Bioaccumulation of Pollutants in Response to Environmental Factors. (PhD Thesis). University of Barcelona, Catalonia.
Cutler, D.R., Edwards, T.C., Beard, K.H., Cutler, A., Hess, K.T., Gibson, J., et al., 2007. Random forests for classification in ecology. Ecology 88, 2783–2792.
Datry, T., 2012. Benthic and hyporheic invertebrate assemblages along a flow intermittence gradient: effects of duration of dry events. Freshw. Biol. 57, 563–574.
Datry, T., Larned, S.T., Fritz, K.M., Bogan, M.T., Wood, P.J., Meyer, E.I., et al., 2014a. Broad-scale patterns of invertebrate richness and community composition in temporary rivers: effects of flow intermittence. Ecography 37, 94–104.
Datry, T., Larned, S.T., Tockner, K., 2014b. Intermittent rivers: a challenge for freshwater ecology. Bioscience 64, 229–235.
De Girolamo, A.M., Lo Porto, A., Pappagallo, G., Tzoraki, O., Gallart, F., 2014. The hydrological status concept: application at a temporary river (Candelaro, Italy). River Res. Appl. http://dx.doi.org/10.1002/rra.2786.
Dolédec, S., Olivier, J.M., Statzner, B., 2000. Accurate description of the abundance of taxa and their biological traits in stream invertebrate communities: effects of taxonomic and spatial resolution. Arch. Hydrobiol. 148, 25–43.
Domisch, S., Araújo, M.B., Bonada, N., Pauls, S.U., Jähnig, S.C., Haase, P., 2013. Modelling distribution in European stream macroinvertebrates under future climates. Glob. Chang. Biol. 19, 752–762.
Dray, S., Dufour, A.B., 2007. The ade4 package: implementing the duality diagram for ecologists. J. Stat. Softw. 22, 1–20.
Dunbar, M.J., Pedersen, M.L., Cadman, D., Extence, C., Waddingham, J., Chadd, R., Larsen, S.E., 2010. River discharge and local scale physical habitat influence macroinvertebrate LIFE scores. Freshw. Biol. 55, 226–242.
EC (European Commission), 2009. River basin management in a changing climate. Common Implementation Strategy for the Water Framework Directive Guidance Document Number. 24.
Evans, J.S., Cushman, S.A., 2009. Gradient modelling of conifer species using random forests. Landsc. Ecol. 24, 673–683.
Extence, C., Balbi, D., Chadd, R., 1999. River flow indexing using British benthic macroinvertebrates: a framework for setting hydroecological objectives. Regul. River 15, 545–574.
Filipe, A., Lawrence, J., Bonada, N., 2013. Vulnerability of stream biota to climate change in mediterranean climate regions: a synthesis of ecological responses and conservation challenges. Hydrobiologia 719, 331–351.
Forzieri, G., Feyen, L., Rojas, R., Flörke, M., Wimmer, F., Bianchi, A., 2014. Ensemble projections of future streamflow droughts in Europe. Hydrol. Earth Syst. Sci. 18, 85–108.
Fritz, K.M., Johnson, B.R., Walters, D.M., 2008. Environmental indicators of hydrologic permanence in forested headwater streams. J. North Am. Benthol. Soc. 27, 690–704.
Gallart, F., Prat, N., García-Roger, E.M., Latron, J., Rieradevall, M., Llorens, P., et al., 2012. A novel approach to analysing the regimes of temporary streams in relation to their controls on the composition and structure of aquatic biota. Hydrol. Earth Syst. Sci. 16, 3165–3182.
García-Roger, E.M., Mar Sánchez-Montoya, M., Gómez, R., Suárez, M.L., Vidal-Abarca, M.R., Latron, J., et al., 2011. Do seasonal changes in habitat features influence aquatic macroinvertebrate assemblages in perennial versus temporary Mediterranean streams? Aquat. Sci. 73, 567–579.
García-Roger, E.M., Sánchez-Montoya, M.M., Cid, N., Erba, S., Karaouzas, I., Verkaik, I., et al., 2013. Spatial scale effects on taxonomic and biological trait diversity of aquatic macroinvertebrates in Mediterranean streams. Fundam. Appl. Limnol. 183, 89–105.

Gayraud, S., Statzner, B., Bady, P., Haybachp, A., Schöll, F., Usseglio-Polatera, P., et al., 2003. Invertebrate traits for the biomonitoring of large European rivers: an initial assessment of alternative metrics. Freshw. Biol. 48, 2045–2064.

Gore, J.A., Layzer, J.B., Mead, J., 2001. Macroinvertebrate instream flow studies after 20 years: a role in stream management and restoration. Regul. River 17, 527–542.

Hothorn, T., Hornik, K., Strobl, C., Zeileis, A., 2014. A laboratory for recursive part(y)itioning. R Package.

Illies, J., 1978. Limnofauna europaea. G. Fischer Verlag, Amsterdam.

Kristensen, P.B., Kristensen, E.A., Riis, T., Baisner, A.J., Larsen, S.E., Verdonschot, P.F.M., et al., 2013. Riparian forest as a management tool for moderating future thermal conditions of lowland temperate streams. Hydrol. Earth Syst. Sci. 10, 6081–6106.

Lake, P.S., 2011. Drought and Aquatic Ecosystems: Effects and Responses. John Wiley & Sons, Chichester.

Lamouroux, N., Dolédec, S., Gayraud, S., 2004. Biological traits of stream macroinvertebrate communities: effects of microhabitat, reach, and basin filters. J. North Am. Benthol. Soc. 23, 449–466.

Larned, S.T., Datry, T., Arscott, D.B., Tockner, K., 2010. Emerging concepts in temporary-river ecology. Freshw. Biol. 55, 717–738.

Ledger, M.E., Edwards, F.K., Brown, L.E., Milner, A.M., Woodward, G., 2011. Impact of simulated drought on ecosystem biomass production: an experimental test in stream mesocosms. Glob. Chang. Biol. 17, 2288–2297.

Liaw, A., Wiener, M., 2002. Classification and regression by randomForest. R. News 2, 18–22.

Logez, M., Pont, D., 2012. Global warming and potential shift in reference conditions: the case of functional fish-based metrics. Hydrobiologia 704, 417–436.

Manel, S., Williams, H.C., Ormerod, S.J., 2001. Evaluating presence–absence models in ecology: the need to account for prevalence. J. Appl. Ecol. 38, 921–931.

Markovic, D., Carrizo, D., Freyhof, J., Cid, N., Szabolcs, L., Scholz, M., et al., 2014. Europe's freshwater biodiversity under climate change: distribution shifts and conservation needs. Divers. Distrib. 20, 1097–1107.

Mellado-Díaz, A.M., Suárez-Alonso, M.L., Vidal-Abarca, M.R., 2008. Biological traits of stream macroinvertebrates from a semi-arid catchment: patterns along complex environmental gradients. Freshw. Biol. 53, 1–21.

Mérigoux, S., Dolédec, S., 2004. Hydraulic requirements of stream communities: a case study on invertebrates. Freshw. Biol. 49, 600–613.

Mérigoux, S., Lamouroux, N., Olivier, J.M., Dolédec, S., 2009. (2009) Invertebrate hydraulic preferences and predicted impacts of changes in discharge in a large river. Freshw. Biol. 54, 1343–1356.

Meyer, D., Zeileis, A., Hornik, K., 2013. Visualizing categorical data. Package Version 1. 3-1.

Munné, A., Prat, N., 2009. Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: an intercalibration approach with the IBMWP index. Hydrobiologia 628, 203–225.

Munné, A., Prat, N., 2011. Effects of Mediterranean climate annual variability on stream biological quality assessment using macroinvertebrate communities. Ecol. Indic. 11, 651–662.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Nikolaidis, N.P., Demetropoulou, L., Froebrich, J., Jacobs, C., Gallart, F., Prat, N., et al., 2013. Towards sustainable management of Mediterranean river basins: policy recommendations on management aspects of temporary streams. Water Policy 15, 830–849.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, RGe, Simpson, G.L., et al., 2014. Vegan: community ecology package. R Package Version 2.0-9 (http://CRAN.R-project.org/package=vegan).

Pace, G., Bonada, N., Prat, N., 2013. Long-term effects of climatic-hydrological drivers on macroinvertebrate richness and composition in two Mediterranean streams. Freshw. Biol. 58, 1313–1328.

Picazo, F., Millán, A., Dolédec, S., 2012. Are patterns in the taxonomic, biological and ecological traits of water beetles congruent in Mediterranean ecosystems? Freshw. Biol. 57, 2192–2210.

Prat, N., Gallart, F., Von Schiller, D., Polesello, S., García-Roger, E.M., Latron, J., et al., 2014. The Mirage Toolbox: an integrated assessment tool for temporary streams. River Res. Appl. 30, 1318–1334.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria URL http://www.R-project.org/.

Resetarits, W., 2001. Colonization under threat of predation: avoidance of fish by an aquatic beetle, Tropisternus lateralis (Coleoptera: Hydrophilidae). Oecologia 129, 155–160.

Ribera, I., 2000. Biogeography and conservation of Iberian water beetles. Biol. Conserv. 92, 131–150.

Richmond, E.A., 1920. Studies on the biology of the aquatic Hydrophilidae. B. Am. Mus. Nat. Hist. 42, 1–94.

Robson, B.J., Chester, E.T., Austin, C.M., 2011. Why life history information matters: drought refuges and macroinvertebrate persistence in non-perennial streams subject to a drier climate. Mar. Freshw. Res. 62, 801–810.

Sánchez-Montoya, M.M., Vidal-Abarca, M.R., Puntí, T., Poquet, J.M., Prat, N., Rieradevall, M., et al., 2009. Defining criteria to select reference sites in Mediterranean streams. Hydrobiologia 619, 39–54.

Sánchez-Montoya, M.M., Vidal-Abarca, M.R., Suárez, M.L., 2010. Comparing the sensitivity of diverse macroinvertebrate metrics to a multiple stressor gradient in Mediterranean streams and its influence on the assessment of ecological status. Ecol. Indic. 10, 896–904.

Sánchez-Montoya, M.D.M., Arce, M.I., Vidal-Abarca, M.R., Suárez, M.L., Prat, N., Gómez, R., 2012. Establishing physico-chemical reference conditions in Mediterranean streams according to the European Water Framework Directive. Water Res. 46, 2257–2269.

Schneider, C., Laizé, C.L.R., Acreman, M.C., Flörke, M., 2013. How will climate change modify river flow regimes in Europe? Hydrol. Earth Syst. Sci. 17, 325–339.

Sheldon, F., 2005. Incorporating natural variability into the assessment of ecological health in Australian dryland rivers. Hydrobiologia 552, 45–56.

Snelder, T.H., Datry, T., Lamouroux, N., Larned, S.T., Sauquet, E., Pella, H., et al., 2013. Regionalization of patterns of flow intermittence from gauging station records. Hydrol. Earth Syst. Sci. 17, 2685–2699.

Statzner, B., Bêche, L.A., 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshw. Biol. 55, 80–119.

Statzner, B., Bis, B., Dolédec, S., Usseglio-Polatera, P., 2001. Perspectives for biomonitoring at large spatial scales: a unified measure for the functional composition of invertebrate communities in European running waters. Basic Appl. Ecol. 2, 73–85.

Storey, R.G., Quinn, J.M., 2013. Survival of aquatic invertebrates in dry bed sediments of intermittent streams: temperature tolerances and implications for riparian management. Freshw. Sci. 32, 250–266.

Strobl, C., Hothorn, T., Zeileis, A., 2009. Party on! R. J. 1, 14–17.

Suren, A.M., Jowett, I.G., 2006. Effects of floods versus low flows on invertebrates in a New Zealand gravel-bed river. Freshw. Biol. 51, 2207–2227.

Tachet, H., Richoux, P., Bournard, M., Usseglio-Polatera, P., 2010. Invertébrés d'eau douce: systématique, biologie, écologie. CNRS Éditions, Paris.

Turner, M., Stewardson, M., 2014. Hydrologic indicators of hydraulic conditions that drive flow–biota relationships. Hydrol. Sci. J. 59, 659–672.

Vidal-Abarca, M.R., Sánchez-Montoya, M.M., Guerrero, C., Gómez, R., Arce, M.I., García-García, V., et al., 2013. Effects of intermittent stream flow on macroinvertebrate community composition and biological traits in a naturally saline Mediterranean stream. J. Arid Environ. 99, 28–40.

Wilby, R.L., Orr, H., Watts, G., Battarbee, R.W., Berry, P.M., Chadd, R., et al., 2010. Evidence needed to manage freshwater ecosystems in a changing climate: turning adaptation principles into practice. Sci. Total Environ. 408, 4150–4164.

Williams, D.D., 1996. Environmental constraints in temporary fresh waters and their consequences for the insect fauna. J. North Am. Benthol. Soc. 15, 634–650.

Williams, D.D., 2006. The Biology of Temporary Waters. Oxford University Press, New York.