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Review

Biomonitoring of intermittent rivers and ephemeral streams in Europe: Current practice and priorities to enhance ecological status assessments

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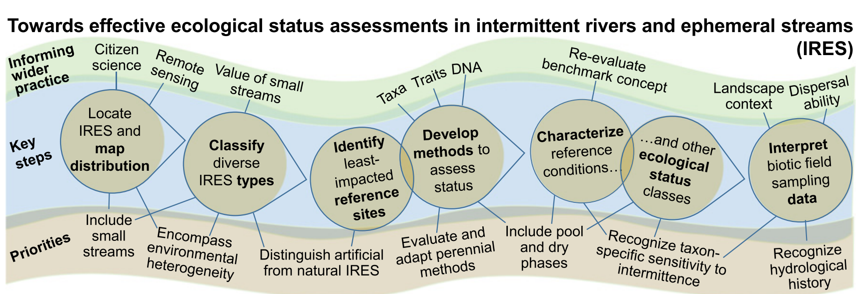
HIGHLIGHTS

- Flow intermittence creates challenges for ecological status assessments.
- Classifying ecologically relevant typologies will underpin future biomonitoring.
- Methods used in perennial rivers need evaluation before use in temporary rivers.
- Metrics may need adaptation due to taxon-specific sensitivity to intermittence.
- Novel biomonitoring tools (e.g. DNA, terrestrial biota) also require development.

GRAPHICAL ABSTRACT



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ABSTRACT

Intermittent rivers and ephemeral streams (IRES) are common across Europe and dominate some Mediterranean river networks. In all climate zones, IRES support high biodiversity and provide ecosystem services. As dynamic ecosystems that transition between flowing, pool, and dry states, IRES are typically poorly represented in biomonitoring programmes implemented to characterize EU Water Framework Directive ecological status. We report the results of a survey completed by representatives from 20 European countries to identify current challenges to IRES status assessment, examples of best practice, and priorities for future research. We identify five major barriers to effective ecological status classification in IRES: 1. the exclusion of IRES from Water

Abbreviations: BQE, biological quality element; COST, European Cooperation in Science and Technology; EU, European Union; GES, good ecological status; GIG, Geographical Intercalibration Group; IRES, intermittent rivers and ephemeral streams; RBD, River Basin District; SMIRES, Science and Management of Intermittent Rivers and Ephemeral Streams; WFD, Water Framework Directive.

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Framework Directive biomonitoring based on their small catchment size; 2. the lack of river typologies that distinguish between contrasting IRES; 3. difficulties in defining the 'reference conditions' that represent unimpacted dynamic ecosystems; 4. classification of IRES ecological status based on lotic communities sampled using methods developed for perennial rivers; and 5. a reliance on taxonomic characterization of local communities. Despite these challenges, we recognize examples of innovative practice that can inform modification of current biomonitoring activity to promote effective IRES status classification. Priorities for future research include reconceptualization of the reference condition approach to accommodate spatiotemporal fluctuations in community composition, and modification of indices of ecosystem health to recognize both taxon-specific sensitivities to intermittence and dispersal abilities, within a landscape context.

Contents

1. Introduction

Temporary rivers and streams, which are defined by periodic flow cessation and often experience loss of some or all surface water (Datry et al., 2014a; Leigh et al., 2015), are common in oceanic and continental temperate regions (Snelder et al., 2013; Stubbington et al., 2017), and can dominate mediterranean-climate and semi-arid river networks (Skoulikidis et al., 2011, 2017; Tockner et al., 2009). These ecosystems are often classified as intermittent rivers or ephemeral streams (IRES), with intermittent rivers typically conceptualized as systems with relatively long, seasonal flowing phases, compared to precipitation-driven hydrological unpredictability in ephemeral streams. However, the intermittent-ephemeral boundary is indistinct and encompasses only two of many possible intermittence regimes, from near-perennial to episodic flow (Cid et al., 2017; Datry et al., 2017a; Uys and O'Keeffe, 1997). Despite this variability, for simplicity, we use *IRES*, *temporary*, and *intermittence* as terms encompassing *all* lotic ecosystems that experience flow cessation and/or drying, and for clarity, we provide descriptive detail in each instance where a particular type of intermittence is considered.

IRES flowing-phase communities can be diverse in multiple aquatic groups including diatoms (Tornés and Ruhí, 2013), macrophytes (Westwood et al., 2006), invertebrates (Bonada et al., 2007; Datry, 2012), and fish (Pires et al., 1999). Although local-scale taxonomic diversity typically declines with increasing intermittence (Davey and Kelly, 2007; Datry et al., 2014b; Tornés and Ruhí, 2013), diversity among sites (i.e. spatial β-diversity) can be higher in IRES compared to perennial systems due to habitat heterogeneity (Schriever and Lytle, 2016; Tornés and Ruhí, 2013; Westwood et al., 2006; but see Datry et al., 2016a) and dispersal limitation, in particular in isolated headwaters (Brown and Swan, 2010; Sarremejane et al., 2017). Equally, taxonomic diversity among times (i.e. temporal β-diversity) can be enhanced in temporary compared with perennial systems due to fluctuations in community composition between lotic, lentic, and terrestrial phases (Bogan and Lytle, 2007; Corti and Datry, 2015; Ruhí et al., 2017). These diversity contributions can result in higher regional-scale biodiversity in networks that include temporary reaches (Katz et al., 2012; Larned et al., 2010; Stubbington et al., 2017).

Recognition of IRES biodiversity and ecosystem service provision across all hydrological phases (Datry et al., 2017b) coincides with increasing anthropogenic alteration of intermittence regimes, with considerable water resource pressures in regions dominated by urban and agricultural land uses (Acuña et al., 2017; Kummu et al., 2016). Increases in the spatiotemporal extent of intermittence reflect over-abstraction (Boix et al., 2010; Jaeger et al., 2014; Mainstone et al., 1999), whereas effluent discharge, water diversions, and releases from impoundments can cause artificial perennialization (Hendriks et al., 2014; Luthy et al., 2015; Morais et al., 2004). These pressures interact within a changing climate that features more extreme events, including hydrological droughts (i.e. surface water deficits; Forzieri et al., 2014; Ledger and Milner, 2015). Anthropogenic activities typically reduce biodiversity by eliminating sensitive taxa, particularly where intermittence increases (Benejam et al., 2010; Datry et al., 2014b; Garcia et al., 2016).

The European Union (EU) Water Framework Directive 2000/60/EC (WFD; EC, 2000) requires EU Member States and other participating countries to achieve at least 'good' ecological status (GES) or 'good ecological potential' in all surface waters (EC, 2003a). Ecological status (hereafter, *status*) is assessed through comparison of 'biological quality elements' (BQEs, for example benthic invertebrate assemblages) with 'reference conditions', i.e. the communities indicative of undisturbed or minimally disturbed sites. Progress towards GES has been limited (EC, 2012; Voulvoulis et al., 2017), and one significant challenge is to incorporate IRES into biomonitoring programmes (Reyjol et al., 2014; Skoulikidis et al., 2017). Other legislative drivers for biomonitoring include the EU Habitats Directive 92/43/EEC (EC, 1992), under which IRES may fall within Special Areas of Conservation monitored to assess the conservation status of habitats and/or species (see Section 4.1). The EU Biodiversity Strategy also provides impetus for the monitoring and protection of ecosystems supporting high biodiversity and robust ecosystem service provision (EC, 2011). In addition, national status assessments have identified rare IRES inhabitants including specialist species (Macadam, 2016), providing impetus for population monitoring and legislative protection (e.g. Službeni list RCG br. 76/06, 2006 in Montenegro).

The EU COST Action CA15113 (*Science and Management of Intermittent Rivers and Ephemeral Streams*; SMIRES; COST, 2015) is seeking to translate increasing understanding of IRES into tangible tools for ecosystem management. The SMIRES Working Group 4: *Community Ecology and Biomonitoring in IRES* (WG4; SMIRES, 2016) is tasked with adapting current biomonitoring methods and developing novel tools to promote effective IRES status assessments. In this paper, we use information gathered from WG4 members to review current practice in IRES biomonitoring. We identify challenges to effective status assessment and, as a result, we establish the need to develop new, specifically designed tools to enable status characterization in IRES across and beyond Europe. To this end, we highlight best practice in IRES status characterization, identify opportunities for its wider adoption, and suggest priorities for future research.

2. Ecological status assessments in European rivers: collation of information

SMIRES WG4 comprises >90 members based in 25 'COST Member Countries' (COST, 2016), including academic researchers, and stakeholders from national 'competent authorities' with responsibility for WFD compliance. WG4 members were asked to collaborate to provide written accounts of national biomonitoring activity. Specifically, information was requested about the biomonitoring conducted to assess status in both IRES and perennial rivers, including the biotic groups used as bioindicators (Appendix A). In addition, qualitative descriptions of perceived issues surrounding, and limitations of, current IRES biomonitoring were sought. This initial survey was supplemented by a second survey focussing on national implementation of WFD stages including "water body" designation; classification of river typologies; reference site identification; characterization of reference conditions; use of perennial status assessment methods; and development of alternative methods for IRES (Appendix B). Information provided in written responses to the initial and second surveys was supplemented by discussion with respondents, both at WG4 meetings and via email.

Representatives from 20 countries contributed biomonitoring information in the initial survey (Fig. 1); in addition, Romania provided limited comments. Of the 20 represented countries, 15 are EU Member States, 13 of which had adopted all of their second WFD 'River Basin Management Plans' by early 2017; the two exceptions are Greece and the archipelago-specific 'River Basin District' (RBD) of the Canary Islands, Spain (EC, 2016). Of the non-Member State respondents, Iceland is also committed to WFD implementation as part of the European Economic Area; Switzerland has set targets comparable to those in the WFD as part of trade agreements; and Macedonia, Montenegro, and Serbia are being supported in implementing the WFD as candidate countries seeking accession to the EU. Mediterranean and other temperate-climate regions were well-represented in both Western and Central Europe, with Finland and Iceland representing northern latitudes. Germany, Norway, and Sweden were the most significant omissions in terms of land area, and regionally, Eastern Europe was poorly represented. All river 'Geographical Intercalibration Groups' (GIGs; formed to promote status benchmarking across countries implementing the WFD; Van de Bund, 2009) were represented, by 3 of 5 *Alpine*; 8 of 18 *Central/Baltic*; 5 of 9 *Eastern Continental*; 8 of 9 *Mediterranean*; and 2 of 5 *Northern* countries, respectively. Representatives from 13 countries (Croatia, Cyprus, Czech Republic, Greece, Hungary, Macedonia, Montenegro, Netherlands, Serbia, Slovakia, Spain, Switzerland, UK) spanning 4 GIGs (Alpine, Central/Baltic, Eastern Continental, Mediterranean) also responded to the second survey.

3. Ecological status assessments in European rivers: results

Considering all lotic ecosystems, 18 of 20 surveyed countries have established status assessment protocols for perennial rivers, all of which are based on biota characterized at the community level using a taxonomic approach; methods remain in development in Malta and Montenegro (Table 1). Common bioindicator groups comprise the BQEs *benthic invertebrates* (18 countries), *phytobenthos* (17), *macrophytes* (16), *fish* (15), and *phytoplankton* (6); as such, benthic invertebrates are the only BQE used across all surveyed countries with established protocols. This information agrees with and can be supplemented by the comprehensive review of Birk et al. (2012), who reported freshwater monitoring methods used in 28 European countries: those surveyed here, with five exceptions (Iceland, Macedonia, Montenegro, Serbia, Switzerland) and 13 additions (Austria, Belgium, Denmark, Estonia, Germany, Ireland, Latvia, Lithuania, Luxembourg, Norway, Romania, Slovenia, Sweden). We direct readers to Birk et al. (2012) for further information on methods used in perennial freshwater ecosystems, and to Dallas (2013) for information specific to mediterranean-climate rivers; here, we focus on IRES biomonitoring.

Of the 18 surveyed countries with established biomonitoring programmes, 16 (and Romania) include IRES (typically larger systems with predictable intermittence) within sampling networks, whereas perennial rivers are prioritized in Finland and Macedonia (Table 1). In many countries, including Croatia, Czech Republic, Iceland, Poland, Slovakia, Switzerland, and the UK, respondents noted that a 'small' proportion of biomonitoring sites were temporary; poor understanding of the extent of IRES within river networks typically prevented quantification this proportion in relation to IRES occurrence. However, in the Netherlands, the proportion has been quantified as 266 of 6460 sites (i.e. 4%) in total and in the upper reaches, where an estimated two-thirds of the network is temporary, these sites poorly represent IRES compared to 1665 perennial sites. In contrast, in Hungary, an estimated 35% of the WFD-monitored river length is temporary, which effectively represents intermittence at 291 of 923 (i.e. 31%) of monitored water bodies (but see Section 4.1).

Temporary reaches account for a much higher proportion of the total length of river networks in Mediterranean-Basin RBDs, but IRES sometimes remain underrepresented by water body-based biomonitoring networks. For example, >80% of the network length is temporary in the *Algarve*, *Guadiana*, and *Sado and Mira* RBDs of southern Portugal, but only 40% of biomonitoring sites are within IRES. In contrast, in the 16,438 km^2 *Catalan* RBD in north-east Spain, 64% of 248 water bodies (and therefore biomonitoring sites) are temporary, comprising 51% larger rivers with seasonal intermittence and 13% smaller streams with ephemeral flow; this proportion of water bodies exceeds the

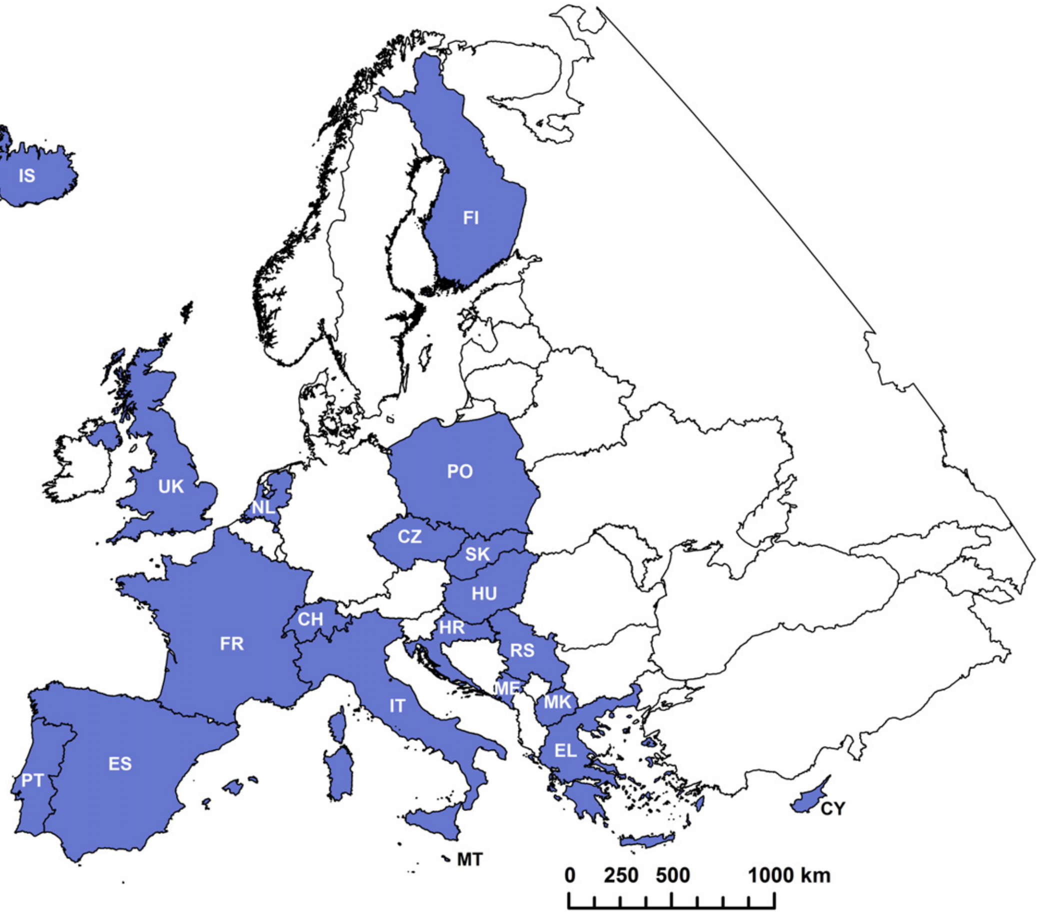

Fig. 1. European countries providing information about national biomonitoring activity in river ecosystems. Abbreviations: CH, Switzerland; CY, Cyprus; CZ, Czech Republic; EL, Greece; ES, Spain; FI, Finland; FR, France; HR, Croatia; HU, Hungary; IS, Iceland; IT, Italy; ME, Montenegro; MK, Republic of Macedonia; MT, Malta; NL, Netherlands; PO, Poland; PT, Portugal; RS, Serbia; SK, Slovakia; UK, United Kingdom.

estimated 58% temporary river length, comprising 50% seasonal, intermittent rivers and 8% ephemeral streams. Similarly, in Cyprus, 87% of water bodies classified for WFD monitoring are IRES, exceeding the 85% estimated temporary river length.

Survey respondents' descriptions of issues surrounding (and limitations of) current IRES biomonitoring, and their responses to the second survey of WFD-focussed questions, are explored in Section 4.

4. Challenges to effective IRES ecological status assessment

Currently, status assessments are routinely conducted for IRES in the Mediterranean regions in which they dominate; elsewhere in Europe, poor recognition of their extent has left IRES excluded from or poorly represented in biomonitoring programmes, which prevents identification of degraded ecosystems that require restoration or management actions. Even where biomonitoring is conducted, poor understanding of IRES may limit its effectiveness. The ongoing challenges of status assessment in IRES have recently been examined by Dallas (2013), Reyjol et al. (2014), Cid et al. (2017), and Skoulikidis et al. (2017); these Mediterranean-focussed reviews raise issues of wider interest, complementing our broader consideration of IRES across European climate zones.

4.1. Legislative definitions may not recognize IRES

The WFD aims to protect "all bodies of surface water", but alongside perennial streams, many IRES (and in particular ephemeral streams) are within catchments that fall below the lowest WFD size typology (10–100 km^2), leaving them without legal recognition as "water bodies". These small systems are typically excluded from biomonitoring networks, or their status is classified based on contiguous perennial reaches. For example, in Hungary, only 1031 of 9800 nationally registered watercourses have catchments >10 km^2, leaving 89.5% of watercourses (equating to 74% of the network length) beyond the remit of WFD-related monitoring. Given the contribution that IRES headwaters make to biodiversity, their inhabitation by rare and endemic IRES specialists (e.g. Macadam, 2016; Matono et al., 2012), and their provision of wider ecosystem services (Datry et al., 2017b), this exclusion is at odds with national and EU-wide commitments to ecosystem protection (EC, 2011), especially in regions where small IRES are numerous (Lazaridou et al., 2016). Skoulikidis et al. (2017) and Stubbington et al. (2017) explore this issue in relation to Mediterranean-region and oceanic-climate IRES, respectively.

Small IRES may fulfil other nationally determined criteria for designation as a WFD water body. For example, some IRES in Cyprus are designated because they support native fish populations of conservation interest. In addition, two IRES types are Habitats Directive Annex I habitats: "water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation", which include the 'winterbourne' headwaters of UK chalk IRES; and "intermittently flowing Mediterranean rivers of the *Paspalo-Agrostidion*", which occur across six Mediterranean countries. Certain UK chalk streams are designated as water bodies regardless of size, because they fulfil a national criterion requiring maintenance and improvement of Special Areas of Conservation (UKTAG, 2003). In contrast, in Spain, many intermittently flowing rivers of the *Paspalo-Agrostidion* remain excluded from WFD consideration because of their small catchment size. For example, in the Catalan RBD, 143 of 248 designated water bodies (equating to

Table 1
Summary of survey responses received from 20 European countries reporting national biomonitoring activity in rivers including intermittent rivers and ephemeral streams (IRES).

Description of national biomonitoring activity	*n* (%) agreeing	Additional information on exceptions
Protocols are established for ecological status assessment in perennial rivers	18 of 20 (90)	No established protocols in: • Malta • Montenegro
Biota are characterized at the community level using a taxonomic approach	18 of 18 (100)	No exceptions; no routine biomonitoring: • is at non-community levels • uses functional approaches • uses metabarcoding of DNA
IRES are included within biomonitoring networks	16 of 18 (89)	IRES not included in networks in: • Finland • Macedonia
Intermittence is recognized in WFD river typologies	9 of 18 (50)	Recognized in: • All 8 Mediterranean GIG countries • Mediterranean regions of Croatia
Status assessment is only done to characterize flowing phases	16 of 16 (100)	No exceptions; no routine biomonitoring: • to characterize pool-phase status • to characterize dry-phase status
IRES status is assessed using protocols developed for perennial rivers	12 of 16 (81)	IRES-specific protocols used in: • Cyprus • Portugal • Spain IRES-evaluated method used in Greece

GIG, Geographical Intercalibration Group; RBD, River Basin District; WFD, Water Framework Directive.

1059 km of the 3808 km river length, and encompassing all flow permanence regimes) are also 'Natura 2000' protected areas and are monitored, but an additional 7176 km of small systems, including 1750 km located within the Natura 2000 network, are excluded based on their catchment size.

The Habitats Directive provides alternative impetus to assess the conservation status of IRES in designated sites (Fritz et al., 2017). However, different authorities may be responsible for WFD and Habitats Directive implementation, and collaboration is needed to ensure that IRES are not excluded from monitoring programmes. For example, the Environment Agency of England may conduct WFD status assessments in downstream perennial reaches of chalk streams in which the winterbourne headwaters are also protected under the Habitats Directive. A second public body, Natural England, has Habitats Directive responsibility and conducts complementary monitoring that encompasses winterbourne reaches; their assessment of conservation status is also informed by Environment Agency data. Habitat protection may also be afforded by other international- to local-scale site-specific designations, but such legal protection leaves most IRES excluded from monitoring activity.

4.2. Typologies that distinguish between contrasting IRES require development

WFD status assessments are based on robust surface water typologies, and incorporation of IRES into biomonitoring programmes therefore requires classification of IRES types. IRES are recognized by the WFD, which classifies a "temporary" river type that occurs in five Mediterranean countries (Van de Bund, 2009). This classification of one temporary category overlooks profound variability among IRES (Belmar et al., 2011; Cid et al., 2017), variability which is reflected by IRES communities, most strikingly between the fundamental 'intermittent' and 'ephemeral' IRES types (e.g. Argyroudi et al., 2009; Bonada et al., 2007; Stubbington et al., 2009; but see Delgado et al., 2012). This variability (and associated differences in boundaries between status classes) has necessitated flexibility, rather than consistency, in the biomonitoring methods and indices developed for and used within Mediterranean "temporary" rivers (Fritz et al., 2017).

Accordingly, national river classifications in few Mediterranean countries adequately represent IRES. For example, Spain has 37 classified river typologies based on natural variables including climate, geology, geomorphology, and altitude as well as river discharge, with several typologies including but not distinguishing between IRES and perennial systems (BOE, 2015). Similarly, in Portugal, eight of 15 national river classifications encompass both perennial and temporary water bodies. Exceptions include Cyprus, Greece, and Italy, where contrasting intermittence regimes are distinguished (using variable terminology) by the duration and predictability of flowing phases (Lazaridou et al., 2016; Skoulikidis et al., 2017); in Cyprus, this hydrological classification uses the 'temporary stream regime' tool developed by Gallart et al. (2012). In addition, five intermittent river types have been defined in the Mediterranean-climate Dinaric ecoregion of Croatia, spanning small and medium catchment sizes; upland and lowland altitudinal classes; and three sub-ecoregions with climates characterized by year-round precipitation (NN, 2013). These exceptions highlight EU-wide inconsistency in IRES subtype recognition, and a common failure to make even fundamental distinctions, notably between IRES that seasonally recede to chains of pools and those that experience unpredictable periods of complete drying (Argyroudi et al., 2009); these issues are explored by Skoulikidis et al. (2017) and have been addressed by the MIRAGE project (CORDIS, 2015; Nikolaidis et al., 2013; see Section 5.1).

Of the 13 non-Mediterranean surveyed countries in which WFD status assessment methods are established, none recognize flow permanence in river typologies. For example, the UK defined 18 river typologies using WFD "System A", i.e. using prescribed altitude, catchment area, and geology categories; no hydrological parameters inform this classification system (UKTAG, 2003). Discharge categories do, however, inform UK model predictions of community composition across, for example, 43 benthic invertebrate assemblage end groups, but the lowest discharge category is <0.31 m^3 s^{-1} i.e. intermittence is not recognized (UKTAG, 2008). Other countries have used WFD "System B" to define typologies, which allows classification to be informed by additional, optional factors including a "river discharge [flow] category". Despite this, System B classification categories may be reminiscent of those in System A, for example Hungary used slope, catchment area, and geochemistry (comparable to altitude, catchment area, and geology) along with sediment size. In Switzerland, a non-WFD classification system is used, but despite the inclusion of one hydrological category (average annual discharge) along with biogeographical region, altitude, average slope, and geology, none of 54 river typologies are IRES (OFEV, 2015). However, national examples of good practice exist. In particular, the temporary reaches of slow-flowing, sand-bed streams which lose surface water for <10 weeks annually are classified as a type monitored in the Netherlands, but this is in addition to WFD-related activity, because these streams are too small to be deemed "water bodies". Romanian regulatory agencies also distinguish between three intermittence classes based on drying frequency. Similarly, to inform future improvements in regulatory biomonitoring, the Czech BIODROUGHT project (see Section 5.3) distinguishes IRES with annual drying of >1 km for >7 days from near-perennial and perennial systems.

Classification of sufficient IRES typologies to characterize their heterogeneity is needed at the national scales at which regulators operate, with international collaboration desirable from the outset to share best practice and harmonize national typologies. Ideally, quantitative hydrological metrics should be developed to inform classification of ecologically relevant IRES types, with zero-flow periods recognized as primary sources of variation in community composition (Leigh and Datry, 2017; Oueslati et al., 2015). Metrics should incorporate the temporal characteristics of intermittence: the frequency, magnitude, duration, rate of change, timing, and predictability of lotic, lentic, and terrestrial phases (Costigan et al., 2017; Leigh and Datry, 2017; Poff et al., 1997).

Development of quantitative hydrological metrics is hampered by poor IRES representation in gauging station networks, with few continuous hydrological monitoring points located in temporary reaches (Costigan et al., 2017; Leigh and Datry, 2017; Snelder et al., 2013), for example, <5% of the network in Portugal; <1% in Spain and Switzerland; and none in countries including Greece, Hungary, Macedonia, Montenegro, the Netherlands, Poland, and Slovakia. Fewer still coincide with biomonitoring sites, for example, of >5000 gauging stations in France, 123 are in IRES, and at two of these, biotic and hydrological data can be linked – despite intermittence characterizing an estimated 39% of the river network (Snelder et al., 2013). Exceptions to this poor representation include Cyprus, where hydrological monitoring encompasses perennial to episodic systems, with 80 of 104 gauging stations on rivers with intermittent or ephemeral flow, 30 of which correspond with biomonitoring sites; Croatia, where ≥60 of 440 gauging stations are on IRES and hydrological data are collected at nine of 41 IRES biomonitoring points; and Serbia, where 218 flow gauging sites include 54 on IRES, of which 19 are also biomonitored. Even when hydrological data are available, the characteristics of an intermittence regime can be unclear due to difficulties in distinguishing between lentic and dry zero-flow states. To address this limitation of gauging station data, Cypriot authorities also make qualitative observations of aquatic states, following Gallart et al. (2012).

The scarcity of hydrological data necessitates use of other intermittence indicators. Aquatic communities including macroinvertebrates can indicate intermittence, but to date, have only proved able to differentiate between broad, antecedent states i.e. flowing or disconnected pool states (Cid et al., 2016; Řezníčková et al., 2013). Other informative qualitative methods encompass remote sensing using aerial or satellite photography or deployed sensors (González-Ferreras and Barquín, 2017; Spence and Mengistu, 2016) and citizen science initiatives (Datry et al., 2016b; Gallart et al., 2016; Turner and Richter, 2011), and perennial-dominated gauging-station data may allow modelling of IRES distribution (Snelder et al., 2013) and characterization of spatial patterns of intermittence (Larned et al., 2011). Qualitative description of intermittence may enable ecologically relevant classification, with Gallart et al. (2012) defining Mediterranean 'intermittent pool', 'intermittent-dry', and 'episodic or ephemeral' types (Nikolaidis et al., 2013). Equally, Delgado et al. (2012) assigned 60 Mediterranean island 'temporary streams' with overlapping intermittence regimes using non-hydrological criteria, namely altitude, slope, and other geomorphological and topographic discriminators. Beyond the Mediterranean region, broad IRES types such as UK *winterbourne chalk streams* (Westwood et al., 2006) and Dutch *slow-flowing, sand-bed upper reaches* (Van der Molen et al., 2013) have well-characterized environmental characteristics that encompass geomorphological variability, representing a starting point for classification.

4.3. Determination of reference conditions is challenging in dynamic IRES

Only once IRES typologies are classified can undisturbed or minimally disturbed typology-specific reference sites be identified, and their communities then characterized. Accordingly, few surveyed countries have identified IRES-specific reference sites, with most exceptions coming from IRES-rich Mediterranean regions. Relevant research initiatives include the GUADALMED (Sánchez-Montoya et al., 2009; see Dallas, 2013) and MIRAGE projects (Prat et al., 2014; see Section 5.1), which evaluated reference site selection criteria, informed by WFD guidance to use information about anthropogenic pressures to screen potential sites (EC, 2003b). For example, Prat et al. (2014) used threshold values for 37 attributes (relating to catchment land uses, morphological alterations, invasive species, and flow regime modification) to identify pressures and therefore potential reference sites. Similarly, Delgado et al. (2012) used land cover percentages and the absence of other anthropogenic pressures (point sources of pollution, hydromorphological alteration, and significant flow regulation) to select potential IRES reference sites in Spain's Mediterranean Balearic Islands. In addition, predictive models built using perennial reference conditions (e.g. the UK RIVPACS/RICT approach; UKTAG, 2008) warrant exploration to evaluate their applicability to IRES. These broad screening procedures require validation by expert judgement of habitat quality, as well as supplementation by quantitative analysis of chemical variables and biotic assemblages (Chaves et al., 2006; Lunde et al., 2013).

Distinguishing natural from artificial IRES during the site selection process is crucial to inform ecological target-setting based on the natural flow regime (Reyjol et al., 2014) or an alternate desired state (Acreman et al., 2014; Dallas, 2013), but scarcity of hydrological data hampers such differentiation. In response, and building on Richter et al.'s (1996) perennial-focussed 'indicators of hydrologic alteration', the 'MIRAGE toolbox' (Prat et al., 2014) and 'Temporary Rivers Ecological and Hydrological Status' (TREHS) software (Gallart et al., 2017) outline a means of distinguishing natural from artificial IRES (see Section 5.1). Guidance developed for perennial systems (EC, 2003b) may also inform identification of reference sites and subsequent definition of reference conditions in and beyond Mediterranean IRES.

Identifying undisturbed reference sites may be unfeasible across much of Europe, due to widespread, long-term human influences encompassing land use change, river regulation, and physical habitat modification (EC, 2003b), and in IRES, the problem of reference site identification may be compounded by difficulties in characterizing the natural intermittence regime. Across river ecosystems, 'best available' sites (i.e. those representing the least disturbed conditions; Feio et al., 2014), which may be located across international borders (Nijboer et al., 2004), may provide alternatives. For example, data from pristine Polish sites were used to characterize reference conditions for Dutch communities, due to the common occurrence of most indicator taxa (Nijboer et al., 2006). However, the notion that *any* site remains 'pristine' is questionable, especially due to climate change: the truly pristine benchmark against which to compare sampled sites is shifting (Hawkins et al., 2010), with climate-driven increases in drying exacerbating deviations in IRES (Datry et al., 2014a), especially in Mediterranean regions (Schneider et al., 2013).

Characterization of reference conditions at validated sites is complicated by IRES spatial heterogeneity, with Munné and Prat (2009, p. 659) describing benthic invertebrate assemblages from Catalan IRES reference sites as lacking "a unique typological aggregation". The composition of unimpacted communities partly reflects the spatial arrangement of temporary and perennial reaches (Datry et al., 2016c), and where intermittence varies longitudinally, corresponding variability in community composition may complicate selection of representative sites (Westwood et al., 2006). Community composition also varies between systems within a typology due to environmental heterogeneity among sites with equivalent spatial positions (Schriever and Lytle, 2016) and comparable instream habitats, reflecting the longitudinal, lateral and vertical dimensions of the landscape context in which individual sites are situated (Ward et al., 2002; see Section 4.5.1). Data from multiple sites may therefore require integration to adequately characterize assemblages (Jyrkänkallio-Mikkola et al., 2016; Passy and Blanchet, 2007).

As well as these practical challenges, reference conditions present conceptual difficulties in IRES: they are viewed as a single benchmark against which other states are compared (Hawkins et al., 2010; Stoddard et al., 2006), whereas IRES are ecosystems characterized by spatiotemporal variability (Bonada et al., 2007; Cid et al., 2017; Datry et al., 2016c). Increasing the spatial resolution of sampling networks and/or the temporal resolution of sample collection may therefore be necessary to characterize variability within each classified river type (Dallas, 2013). However, even if such increased biomonitoring activity can be resourced and seasonal variation accounted for (Munné and Prat, 2011), description of one assemblage that characterizes unimpacted conditions may still be compromised by unpredictable variation in assemblage composition (Bunn and Davies, 2000; Datry et al., 2016c). Such variability as well as low taxa richness compared to perennial systems may mean that the aquatic groups defined as WFD BQEs (Van de Bund, 2009) are inappropriate, in particular to represent systems with ephemeral or episodic flow (e.g. Cazemier et al., 2011). Debate is therefore needed to either: (a) select a single state in which status can be adequately represented using an established bioindicator (e.g. the benthic invertebrate BQE sampled late in a flowing phase in a predictable, near-perennial river; Sánchez-Montoya et al., 2012); (b) select a single state in which status may be effectively characterized by a novel bioindicator (e.g. terrestrial biota in an IRES with long, uninterrupted dry phases; see Section 4.4.2); or (c) integrate aquatic and terrestrial assemblage data collected across flowing, pool, and dry phases to provide a holistic picture of ecosystem health.

4.4. Ecological status classification is based on perennial reaches, flowing phases, and perennial indices

4.4.1. Status classification is based on perennial reaches

Small catchment size may result in IRES status classification being based on perennial sites within a contiguous WFD "water body", a practice which assumes that sites with contrasting flow permanence have comparable ecological responses to equivalent anthropogenic pressures. However, contrasting environmental conditions and biotas may render this assumption invalid, and in all situations where one status class does not represent contiguous perennial and temporary reaches, designation as one water body may contravene WFD guidance (EC, 2003c) and monitoring networks may require expansion. For example, IRES may be less impacted by non-native invasive species than perennial rivers, if invaders cannot tolerate flow cessation and/or drying, or more impacted, if invaders are highly adaptable (Larson et al., 2009; Stoffels et al., 2017; Stromberg et al., 2007). Equally, abstraction at a given rate may have minimal ecological impacts in perennial rivers that decrease in depth; moderate impacts in IRES that experience longer dry phases; and severe impacts when perennial flow shifts to a temporary regime that includes dry phases (Skoulikidis et al., 2011).

Polluting effluents can have greater impacts when discharged into IRES. Firstly, an effluent which artificially sustains perennial flow in a natural IRES inherently causes the hydrological regime to fall below GES (Fig. 2). In such situations, water quality is also unlikely to meet WFD physico-chemical status targets, in particular as natural contributions to streamflow decline during dry seasons, reducing effluent dilution (Fig. 2; David et al., 2013; Morais et al., 2004). Biotic communities will respond to fluctuations in physico-chemical water quality and hydrological regime (Fig. 2), but attempting to characterize status using aquatic BQEs is inappropriate during artificial flowing phases. Instead, novel dry-phase biomonitors require development (see Section 4.4.2).

4.4.2. Status classification is based on flowing phases

Where status assessments are conducted in IRES (i.e. in 16 surveyed countries), all restrict biomonitoring activity to wet phases. Sampling is timed to maximize the likelihood of capturing a predictable and stable lotic community that has had sufficient time to establish since the last flow resumption. Such scheduling is primarily feasible in IRES with long, predictable flowing phases, whereas systems with short, unpredictable flowing phases pose considerable challenges, because characterization of an unknown stage in community succession prevents accurate status assessment. Across IRES, if lentic conditions are encountered, samples are typically collected in suboptimal conditions, which may lead to inaccurate status classification (Argyroudi et al., 2009; Buffagni et al., 2009); if a channel is dry, samples are not collected.

Reliance on flowing-phase sampling views ecological quality as temporally stable, but fluctuations between lotic, lentic, and terrestrial habitats are accompanied by changes in biotic communities and so potentially in responses to anthropogenic pressures. A singular focus on lotic assemblages may therefore provide an incomplete characterization of ecosystem health, in particular as lentic and dry phase durations increase. Although acceptable in a WFD-driven context, policy drivers including the EU Biodiversity Strategy (EC, 2011) provide impetus for comprehensive health assessments that encompass lentic and dry phases, to ensure that IRES habitats favour persistence of all life stages of associated rare species, and to verify robust ecosystem service provision. For example, specialist IRES insects may be present as juveniles during flowing phases and as dormant eggs in dry channels, with their persistence depending on environmental conditions during both wet and dry phases (Armitage and Bass, 2013); use of riparian zones by adults also necessitates appropriate habitat availability beyond an IRES channel.

Novel bioindicators may be required to supplement flowing-phase classification if comprehensive assessments encompassing temporal variability in status across lotic, lentic, and dry phases are pursued

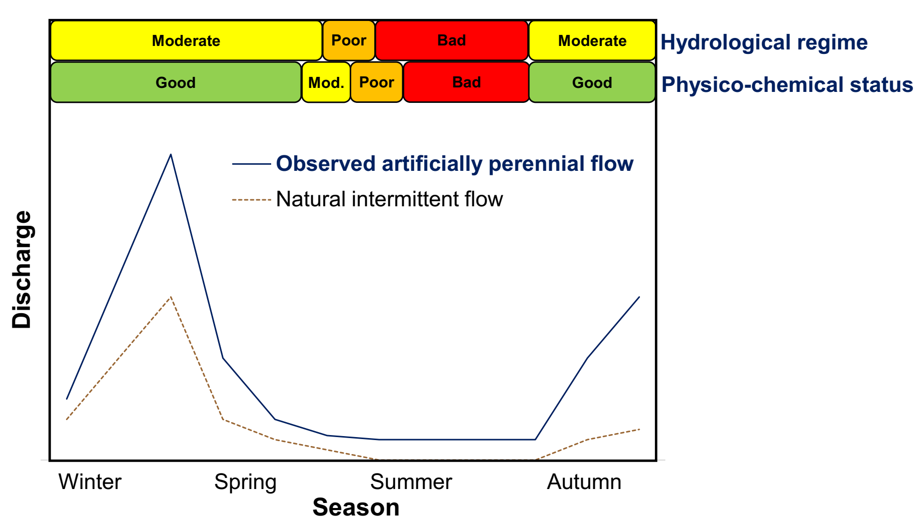

Fig. 2. Hypothesized annual changes in non-biological aspects of ecological status (i.e. physico-chemical quality and hydrological regime) in relation to seasonal changes in discharge in an artificially perennial reach downstream of a sewage treatment plant. The natural, seasonally intermittent flow regime is also indicated. Temporal variation in discharge is informed in part by Morais et al. (2004) and is likely to vary among years and among rivers. Terminology follows Water Framework Directive Section 1.2.1 (EC, 2000). Mod., moderate.

(Skoulikidis et al., 2017). Identification of effective dry-phase biomonitors is a priority, and biotic groups including terrestrial invertebrate communities (Gerlach et al., 2013; Hodkinson and Jackson, 2005), terrestrial carabid beetles (Rainio and Niemelä, 2003), aquatic invertebrate 'seedbanks' (Stubbington and Datry, 2013), dried biofilms including diatoms (Barthès et al., 2015) and/or bacteria (Romaní et al., 2013), and instream vegetation (Westwood et al., 2006) may have the potential to distinguish between sites of contrasting status; different biotic groups will be needed to characterize responses to various hydromorphological and physico-chemical pressures.

4.4.3. Status classification is based on perennial indices

In total, 13 of 16 surveyed countries that characterize IRES flowing-phase status do so using indices developed exclusively for perennial systems (Table 1; Table 2 footnote, plus France and Greece), which assumes the suitability of these approaches for IRES. Although sometimes evaluated and found to be appropriate (e.g. Prat et al., 2014; Table 2; see Section 5.1), the accuracy with which perennial indices characterize IRES flowing-phase status varies (Argyroudi et al., 2009; Morais et al., 2004; Munné and Prat, 2011). For example, Munné and Prat (2011) noted interannual variability in perennial index values calculated to characterize benthic invertebrate communities at temporary reference sites in north-east Spain, reflecting taxonomic differences between high- and low-discharge years. Equally, a perennial macrophyte index (evaluated as appropriate in other Mediterranean countries) is considered unsuitable in IRES in Cyprus due to taxonomic differences in community composition (Papastergiadou and Manolaki, 2012); in contrast, standard benthic invertebrate and phytobenthos indices are effective in Cypriot rivers with seasonal intermittent flow, but not in unpredictable ephemeral streams (Buffagni et al., 2012; Montesantou et al., 2008). In response to evaluation of perennial metrics as inappropriate for IRES, three Mediterranean survey respondent countries (Cyprus, Portugal, and Spain) have developed indices for status classification specifically for IRES, as explored in Section 5. In addition, Greece has developed 'HESY-2', an index evaluated as suitable for the assessment of benthic invertebrate assemblages in both temporary and perennial national river types (Lazaridou et al., 2016).

Spatial and temporal characteristics of IRES flow regimes interact to influence the suitability of perennial indices for flowing-phase status assessments (Fig. 3). Spatially, as distance from a temporary reach to upstream and/or downstream perennial recolonist sources increases, differences between temporary and perennial lotic communities become more pronounced (Datry, 2012; Pavićević and Pešić, 2012) and perennial index suitability may decline (Fig. 3, b–d). The spatial arrangement of temporary reaches in relation to perennial reaches also influences IRES community composition; for example, upstream perennial reaches promote recolonization by drifting aquatic taxa (Fritz and Dodds, 2004; Fig. 3, a,f). Time is a crucial modifier of the resultant compositional differences between perennial and temporary communities: as flowing phase duration increases, differences decline then disappear, as taxa with varying dispersal abilities form a recovering assemblage originating from catchment-wide recolonist sources (Datry et al., 2014b; Leigh et al., 2016). Therefore, as spatial isolation increases, the period in which perennial metrics are suitable for IRES status assessments may decrease (Fig. 3, a–d). Sampled assemblages should therefore be explored in a landscape context and informed by taxon-specific dispersal abilities (see Section 4.5.1).

Broad-scale ecohydrological analyses of macroinvertebrate biomonitoring data have identified drying events as a primary determinant of community composition in IRES, with taxon-specific responses to intermittence reflecting trait variation (Leigh and Datry, 2017). Biotic sensitivity to anthropogenic stressors and to flow intermittence often covary (Hughes et al., 2009), meaning that a taxon's absence from IRES may reflect either deviation from GES or an antecedent flow-cessation or drying event. For example, many mayfly and stonefly juveniles are sensitive to both environmental degradation (Paisley et al., 2014) and intermittence (Boulton and Lake, 2008; Chadd et al., 2017; see Section 5.3). As a result, indices developed to assess the status of perennial rivers may perform poorly in IRES, and require adaptation to recognize the influence of taxon-specific sensitivities to flow cessation and drying on the occurrence and abundance of individual taxa. With short-term deviation from GES permissible under the WFD if deterioration reflects natural events such as hydrological drought, such adapted indices are needed to prevent inaccurate claims that the absence of intermittence-sensitive taxa indicate a legislative breach.

4.5. All biomonitoring is restricted to community-level, taxonomic characterization

4.5.1. Metacommunity dynamics require recognition

Biomonitoring reported by all 20 survey respondents uses a taxonomic approach to characterize biota at a local community level in *all* water bodies, including both temporary and perennial systems. This indicates EU-wide collection, analysis, and interpretation of biomonitoring data according to the 'species sorting' perspective, which assumes that taxa differ in their responses to environmental variation, and that

Table 2
Evaluation of biotic indices developed for perennial rivers to determine their suitability for intermittent rivers characterized by long, seasonal flowing phases[a]. Only 'replacements' that are being tested by or are in use by WFD competent authorities are listed. Indices have not been evaluated in other surveyed countries in which IRES biomonitoring is conducted[b].

WFD GIG	Country	Index	Biotic group	Suitable?	Replacement	Source
Eastern Continental	Croatia	IBMWP, SI_{HR}	MIV	Yes		Mihaljević et al. (2011)
		IPS	Diatoms	No	TDI	Mihaljević et al. (2011)
Mediterranean[c]	Cyprus	STAR_ICMi	MIV	Yes		Buffagni et al. (2012)
		IPS	Diatoms	Yes		Montesantou et al. (2008)
		IBMR	Macrophytes	No	MMI	Papastergiadou and Manolaki (2012)
	Greece	HESY-2	MIV	Yes		Lazaridou et al. (2016)
	Portugal	IPtIS, IPtIN	MIV	Yes		INAG (2009)
		IPS	Diatoms	Yes		EC (2012), in Skoulikidis et al. (2017)
	Spain – Catalan RBD	IBMWP	MIV	No	IMMi-T; IMMi-L	Munné and Prat (2011)
		IPS	Diatoms	Yes		Burfeid et al. (2017)
Northern; Central/Baltic	France	I_2M_2	MIV	No	Required	Pelte et al. (2012, 2014)

[a] No indices have been evaluated as suitable for IRES with short, unpredictable (i.e. ephemeral or episodic) flowing phases; caveats to use in IRES with seasonal intermittence may apply.
[b] I.e. Croatia, Czech Republic, Hungary, Iceland, Italy, the Netherlands (using an adapted species list), Poland, Serbia, Slovakia, Switzerland, and the UK.
[c] Spain is also in the Central/Baltic GIG but examples are relevant only to the Mediterranean GIG.
Abbreviations: HESY-2, Hellenic Evaluation System-2; I_2M_2, French macroinvertebrate multimetric index (Mondy et al., 2012); IBMR, L'Indice Biologique Macrophytique en Rivière; IBMWP, Iberian Biological Monitoring Working Party; IMMi-L, Iberian Mediterranean Multimetric Index (IMMi) – qualitative; IMMi-T, IMMi – quantitative; IPS, Specific Polluosensitivity Index; IPtIN, Invertebrate Index for northern Portugal; IPtIS, Invertebrate Index for southern Portugal; MIV, macroinvertebrates; MMI, Multimetric Macrophyte Index; RBD, River Basin District; SI_{HR}, Croatian index; STAR_ICMi, Standardization of River Classifications Intercalibration Common Metric index; TDI, Trophic Diatom Index.

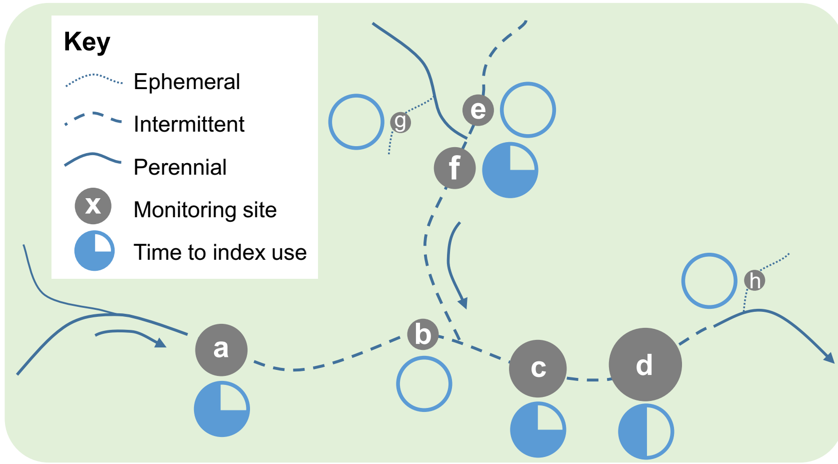

Fig. 3. Plan view of a hypothetical river network, indicating spatial and temporal influences on the suitability of a perennial biotic index for use at temporary sites. Size of symbols *a* to *h* is proportional to index 'suitability', where suitability is based on similarity in community composition between perennial and temporary sites during periods of peak diversity. Fill of partial circles indicates the period of continuous flow needed before the index becomes suitable: in *intermittent* reaches with seasonal, predictable flow cessation and drying, site *d* and sites *a*, *c* and *f* require 6 and 9 months of continuous flow, respectively, before index use is valid; and at sites *b* and *e*, differences in community composition persist throughout an annual cycle, meaning that the perennial index is unsuitable and requires modification to recognize taxon-specific intermittence sensitivities. Perennial indices are unlikely to be suitable at sites *g* and *h* within an annual cycle, since *ephemeral* reaches experience flowing phases that are unpredictable and often short.

local abiotic conditions are the primary determinants of community composition (Leibold et al., 2004). However, local communities connected by dispersal form metacommunities structured by the wider processes that influence dispersal (Heino, 2013; Sarremejane et al., 2017). Ignoring metacommunity dynamics may impede accurate status classification; for example, due to dispersal limitation, sites isolated by distance or by natural landscape features may support fewer taxa (a, Fig. 4) than sites of equivalent status that are longitudinally, laterally, and vertically connected to many recolonist sources (b, Fig. 4). A metacommunity perspective is particularly important in IRES, where transitions between flowing, pool, and dry phases necessitate repeated recolonization from instream, riparian, and more distant refuges (Cañedo-Argüelles et al., 2015; Datry et al., 2016c).

Taxon-specific dispersal abilities require consideration within a landscape framework that recognizes the spatial arrangement of temporary reaches (Fig. 4): (1) longitudinally, in particular the occurrence of and distance to connected perennial upstream and downstream refuges (Larned et al., 2011); (2) laterally, including the availability of terrestrial habitat to support aerial adult insects (Stubbington et al., 2016) and the distance to other recolonist sources (White et al., 2017); and (3) vertically, including the quality of subsurface sediments as a refuge for recolonists, potentially including saturated hyporheic sediments (Stubbington, 2012; Vander Vorste et al., 2016a) and/or drying sediments that support an invertebrate 'seedbank' (Stubbington and Datry, 2013). This landscape influence requires interpretation of sampled assemblages in a context of temporal variability: local communities will achieve peak diversity more quickly after a hydrological transition (i.e. pool formation, drying, or flow resumption) where distance to recolonist sources is lower.

4.5.2. Functional approaches may complement taxonomic community characterization

Taxon-specific responses to environmental changes make taxonomy-based indices of environmental degradation spatially and temporally

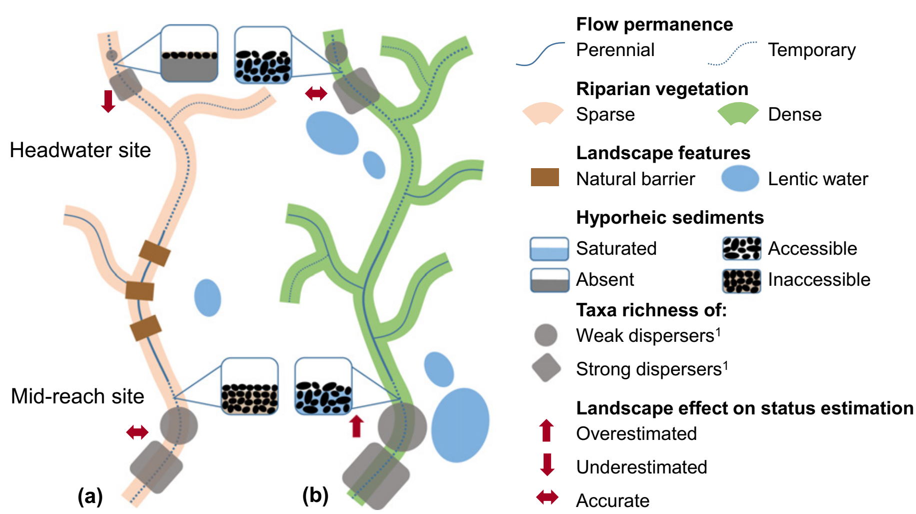

Fig. 4. Longitudinal, lateral, and vertical dimensions of the landscape influence lotic community taxa richness, which can result in overestimation or underestimation of ecological status at temporary river sites of equivalent status. Two possible scenarios are shown: plan view of (a) a catchment with sparse riparian vegetation, natural barriers (e.g. waterfalls), few lentic surface waters, a low drainage density, and fine-sediment-clogged (light brown) or absent (dark grey) hyporheic sediments; here, status may be underestimated in the headwaters; (b) a catchment with dense riparian vegetation, no natural barriers, abundant lentic surface waters, a high drainage density, and saturated, accessible hyporheic sediments; here, status may be overestimated in the mid-reaches. [1]Symbol size is proportional to richness; differences between weak and strong dispersers will decrease as flowing phase duration increases (temporal changes not shown).

variable, which can impede accurate status assessment (Bady et al., 2005). Functional approaches can complement taxonomic community characterization by exploring the biological and ecological traits possessed by a sampled assemblage. Despite limitations of biological trait databases (Wilkes et al., 2017), measures of functional diversity can be reliable indicators of human impacts in perennial rivers (Charvet et al., 2000; Dolédec et al., 1999), and Bruno et al. (2016) found functional redundancy (i.e. the number of taxa performing a comparable ecosystem function) of woody riparian vegetation lining seasonal Mediterranean intermittent rivers to be a sensitive indicator of anthropogenic alteration that remained stable among sites of equivalent status. In Hungarian rivers, metric screening during development of new macroinvertebrate-based multimetric indices identified a combination of ecological traits and taxonomic descriptors as the most effective discriminator among status classes (Várbíró et al., 2011, 2015).

Traits of aquatic biota can also complement taxonomic descriptors to indicate intermittence regimes, distinguishing among perennial, intermittent, and ephemeral systems (Bonada et al., 2007; Giam et al., 2017), between flowing and pool conditions (Cid et al., 2016), and identifying antecedent dry phases (Pařil et al., 2015). By acting as a proxy for hydrological data in IRES, functional characterization of aquatic communities can inform description of IRES reference conditions (Cid et al., 2016). However, differences among functional assemblages from sites with contrasting flow regimes can be obscured by high variability within a regime (Leigh et al., 2016) and by high functional redundancy (Vander Vorste et al., 2016b), and as for taxonomic assemblages, compositional differences among temporary and perennial sites decline then disappear as flowing phase duration increases (see Section 4.4.3; Vander Vorste et al., 2016b).

Where development of new IRES-specific status assessment methods is required, functional approaches warrant consideration alongside taxonomic approaches, their potential to distinguish between sites exposed to different natural and anthropogenic stressors having been demonstrated in IRES for aquatic biota including macroinvertebrates (Bonada et al., 2007; Cid et al., 2016; Mondy et al., 2016; Suárez et al., 2017) and biofilms (including bacteria, algae, and diatoms; Acuña et al., 2015; Amalfitano et al., 2008; Romaní et al., 2013). Functional aspects of terrestrial biota remain poorly characterized in dry channels (but see Corti and Datry, 2014; McCluney and Sabo, 2012) but evidence from other habitats indicates their potential, with the functional traits of riparian arthropod assemblages demonstrated as sensitive to habitat conditions including flooding regime (Lambeets et al., 2009).

4.5.3. Molecular approaches may provide an integrated picture of IRES ecological health

Molecular characterization of sampled assemblages may also have advantages over morphology-based taxonomic approaches, with genetic tools receiving increasing attention by academic researchers (Leese et al., 2016) and regulatory agencies (Vasselon et al., 2017). DNA sequences act as species-level 'barcodes' (Hebert et al., 2003), and metabarcoding allows automated identification of the species in bulk samples that comprise assemblages of whole organisms, or from degraded environmental DNA in water or sediment samples (Baird and Hajibabaei, 2012; Elbrecht et al., 2017; Taberlet et al., 2012). Metabarcoding and other molecular approaches overcome the taxonomic limitations of standard biomonitoring in which reliance on morphology can achieve too coarse an identification level to characterize responses to environmental drivers (Cardoso et al., 2011; Macher et al., 2016; Pešić et al., 2017). For example, identification of Chironomidae (Diptera) to family and *Deleatidium* (Ephemeroptera) to genus prevents quantification of their biodiversity contributions and obscures species-level responses to environmental variability (Cañedo-Argüelles et al., 2016; Macher et al., 2016; Stubbington et al., 2016). Species-level identification makes DNA-based tools particularly promising for application to such taxonomically demanding IRES biota, especially in regulatory biomonitoring programmes where financial constraints promote uptake of cost-effective solutions (Elbrecht et al., 2017). Through its integration of freshwater and terrestrial data from multiple sites, metabarcoding may prove particularly appropriate for catchment-scale characterization of IRES biodiversity (Deiner et al., 2016).

Although metabarcoding is time-effective in its concurrent identification of most common taxa, it may fail to identify rare species (i.e. those occurring at low abundance, which may also be of conservation interest), even in bulk samples (Hajibabaei et al., 2011). Molecular approaches may therefore complement but should not replace traditional biomonitoring activity (Hajibabaei et al., 2016). Other molecular tools with potential to characterize biotic responses to status variability include transcriptomics, proteomics, and metabolomics (Colin et al., 2016). In particular, transcriptomics (i.e. characterization of gene expression using RNA transcripts) may complement taxonomic biomonitoring by determining the metabolic activity (and therefore, potentially, the physiological health) of IRES communities – as well as presenting novel opportunities to explore ecological quality using the expression of genes responsible for important ecosystem processes (Poretsky et al., 2005; von Schiller et al., 2017).

5. Best practice in IRES ecological status assessment

Despite the described challenges of IRES status characterization, innovative examples of recent work to improve practice exist, notably in Mediterranean countries where IRES dominate lotic networks and are consequently central to routine biomonitoring. The wider applicability of this best practice requires exploration across regions with different climates and therefore, potentially, contrasting IRES typologies.

5.1. The MIRAGE and LIFE+ TRivers projects: defining a sampling time window

Within the Mediterranean Basin, advances have been made to improve biomonitoring of intermittent rivers with long seasonal flowing phases, through adaptation of methods developed for perennial rivers. The *Mediterranean Intermittent River ManAGEment* project (MIRAGE; CORDIS, 2015) developed an integrated 'toolbox' to improve IRES biomonitoring, with classification of the hydrological regime (including identification of artificial IRES) emphasized as a pre-requisite for effective status assessment (Gallart et al., 2016; Prat et al., 2014). The MIRAGE project tested macroinvertebrate-based methods developed for perennial rivers in IRES, demonstrating their suitability for some IRES, if flowing phases are long-lasting and sampling is conducted long enough after flow resumption for aquatic diversity to peak (García-Roger et al., 2011; Prat et al., 2014); Mazor et al. (2014) reached comparable conclusions in Californian mediterranean-climate IRES. Equally, Burfeid et al. (2017) determined that status can be assessed in IRES using indices developed for perennial-river diatom assemblages, but only during flowing phases (Table 2); once flow between connected pools ceases, index performance declines considerably.

To implement MIRAGE recommendations, the *LIFE+ TRivers* project (TRivers, 2014) has developed TREHS software, which uses quantitative gauging station data and qualitative information from interviews, aerial photographs, and site visits to characterize temporary flow regimes, including anthropogenic alteration of natural regimes and differentiation of natural and artificial IRES (Gallart et al., 2017). The resultant flow regime classification can inform selection of the most appropriate time window for collection of lotic biomonitoring samples and also identifies systems in which perennial sampling protocols are inappropriate. TREHS is currently (in 2016–17) being tested by the Catalan (http://aca-web.gencat.cat/aca) and Júcar River Basin (http://www.chj.es) Water Agencies in Spain, and this evaluation will inform improvements in hydrological regime classification, environmental objective setting, and status assessments in Mediterranean IRES and more widely.

5.2. Development of new biotic indices for intermittent Mediterranean rivers

Evaluation of biotic indices designed for perennial rivers as unsuitable for IRES has provided impetus for index adaptation and for the development of new approaches, most commonly for lotic macroinvertebrate communities in seasonally intermittent rivers. For example, the IRES-specific multimetric indices IMMi-T and IMMi-L outperform the standard perennial-river IBMWP metric in their detection of environment impacts in Spanish IRES (Munné and Prat, 2009; Sánchez-Montoya et al., 2010; also see Dallas, 2013), leading to their incorporation into regulatory biomonitoring in the Catalan region (Munné et al., 2016). Similarly, García et al. (2014) developed the multimetric INVMIB index for Balearic Island IRES and demonstrated its ability to distinguish between status classes using macroinvertebrate assemblage data; this index has been adopted by regulators. Finally, Morais et al. (2004) and Pinto et al. (2004) established that, compared to perennial metrics, a specifically designed multimetric macroinvertebrate-community index was more sensitive to organic pollution and more robust to seasonal variability in siliceous catchments of IRES-dominated southern Portugal, contributing to the subsequent development of official indices (Table 2).

In Cyprus, the Multimetric Macrophyte Index (MMI) was developed to assess water quality in intermittent rivers, after Papastergiadou and Manolaki (2012) showed no response of a standard, perennial index to the pressure gradient characterized for the Mediterranean "temporary" river type (Van de Bund, 2009; Table 2); testing is underway to ensure national reliability of this index.

5.3. Recognizing and characterizing responses to intermittence

Beyond the Mediterranean Basin, research initiatives examining variation in IRES biota have focussed on responses to intermittence, rather than to ecological quality, in oceanic-climate temperate regions. For example, the Czech Republic BIODROUGHT project (Polášek, 2013a) used taxonomic and functional analyses to develop a suite of 370 macroinvertebrate indicator taxa (primarily species) that can be used to calculate the probability that a stream (sampled to represent peak lotic diversity) has experienced no drying, a < 7 day drying event, or a longer dry period in the preceding year (Polášek, 2013b; Pařil et al., 2015). Quantifying such initiatives, a system developed in the Netherlands has determined the sensitivity of 2236 invertebrate species to drying (Verberk et al., 2012). 'Affinity scores' from 0 (none) to 10 (very high) are assigned to species according to their association with one perennial class and each of four intermittence classes (that describe IRES with typical annual dry periods of <6 weeks to >5 months); for example, *Gammarus pulex* (Amphipoda) scores 8 in the *perennial* class, 2 in the *<6 weeks intermittence* class, and 0 in other classes. A comparable Dutch system that assigns scores based on species-specific affinities for lotic and lentic habitats (Verberk et al., 2012) can characterize invertebrate community responses to flow cessation.

A complementary index has been developed to describe macroinvertebrate community responses to hydrological drought disturbances in near-perennial UK IRES (Chadd et al., 2017). Building on work in Australia (Boulton and Lake, 2008), 92 families/genera have been assigned to six habitat types based on their occurrence (but not abundance) and scored to reflect their sensitivity to loss of this habitat: 1. flow reduction (scores 10, 9); 2. loss of lateral connectivity (8, 7); 3. flow cessation (6, 5); 4. disconnected pool formation (4, 3); 5. disconnected pool contraction (2, 1); and 6. complete surface drying (0). The resultant Drought Effect of Habitat Loss on Invertebrates (DEHLI) index is calculated as the average score per taxon (Chadd et al., 2017).

National assignment of taxon-specific scores indicating sensitivity to flow cessation and drying has considerable potential to enhance IRES biomonitoring, and development of these national initiatives at the EU level is a priority of SMIRES WG4 (SMIRES, 2016). Firstly, the presence and/or abundance of scored taxa could be integrated within an index indicating a site's natural drying or flow cessation regime to inform classification of river typologies. Scores could then inform expectations of community composition during reference condition characterization and subsequent status assessments, including identification of deviations from GES caused by anthropogenic alteration of natural flow regimes. In addition, interpretation of environmental degradation indices in light of intermittence sensitivity scores may demonstrate that a short-term status deterioration does not constitute a legislative breach, for example in near-perennial rivers during drought disturbances.

Community-level approaches are complemented by recent exploration of specific taxa as potential status indicators, in particular, mites (Acari, which are recognized as perennial-river bioindicators; Goldschmidt, 2016) in Mediterranean regions of the Balkan Peninsula. Firstly, the ratio of Oribatida (Acari) to Ostracoda (Crustacea) can be used to differentiate perennial from temporary streams (Pešić, unpublished). Secondly, the 'PTH_{fam}' index (informed by Plecoptera, Trichoptera, and Hydrachnidia [Acari] families; Miccoli et al., 2013) may be a sensitive status indicator in IRES compared to other taxa combinations (Pozojević and Pešić, unpublished). Research is ongoing to evaluate and test the PTH_{fam} index, prior to potential recommendation for wider use.

Community-level and taxon-specific intermittence sensitivity scoring can be informed by research initiatives compiling IRES biodiversity information. Notably, the Intermittent River Biodiversity Synthesis (IRBAS) project (www.irbas.cesab.org/irbas) has created an international, open-access database of hydrological and biological data, primarily reporting aquatic invertebrate assemblages sampled from IRES during flowing phases (Leigh et al., 2017). The database currently comprises >2500 samples from European and other countries, and is expected to grow considerably due to input from ongoing initiatives including the SMIRES consortium (COST, 2015).

6. Conclusions: priorities to address the challenges to IRES ecological status assessment

A fundamental first step to enable establishment of IRES biomonitoring programmes is to map IRES occurrence across Europe (1, Fig. 5; 1, Table 3; SMIRES, 2016). Next, typologies that represent IRES natural heterogeneity (in particular, their contrasting intermittence regimes) and that recognize anthropogenic hydrologic alterations (in particular, discrimination of natural and artificial IRES) need classification; exclusion of typologies with small catchments is unlikely to be justifiable (2, Fig. 5; 2–3, Table 3). Ideally, classification should be informed by quantitative, long-term hydrological data that encompass natural inter-annual variability (Dallas, 2013), although qualitative, expert description of intermittence regimes may provide an informative preliminary classification (2, Fig. 5; 3, Table 3).

Following typology classification, reference sites require identification and validation during field visits to candidate sites identified by pressure-based screening (3a, Fig. 5; 4, Table 3; EC, 2003b). Characterization of reference site communities for each typology is the next step towards status assessment, although the benchmark definition of the reference condition approach may require adaptation to provide a more flexible view of unimpacted IRES (4, Fig. 5; 6, Table 3) and to encompass lentic and dry as well as flowing phases (5a, Table 3). To characterize reference conditions (and lower status class boundaries) effectively, status assessments methods require development, starting with evaluation of those used in perennial rivers (3b, Fig. 5; 5b, Table 3). Where these methods need adaptation or replacement, the accuracy of modified or novel approaches will be enhanced by recognizing taxon-specific intermittence sensitivities and dispersal abilities (5, Fig. 5; 5c, Table 3). Mediterranean regions have spearheaded adaptation of indices, and new, IRES-specific methods (e.g. García et al., 2014; Munné

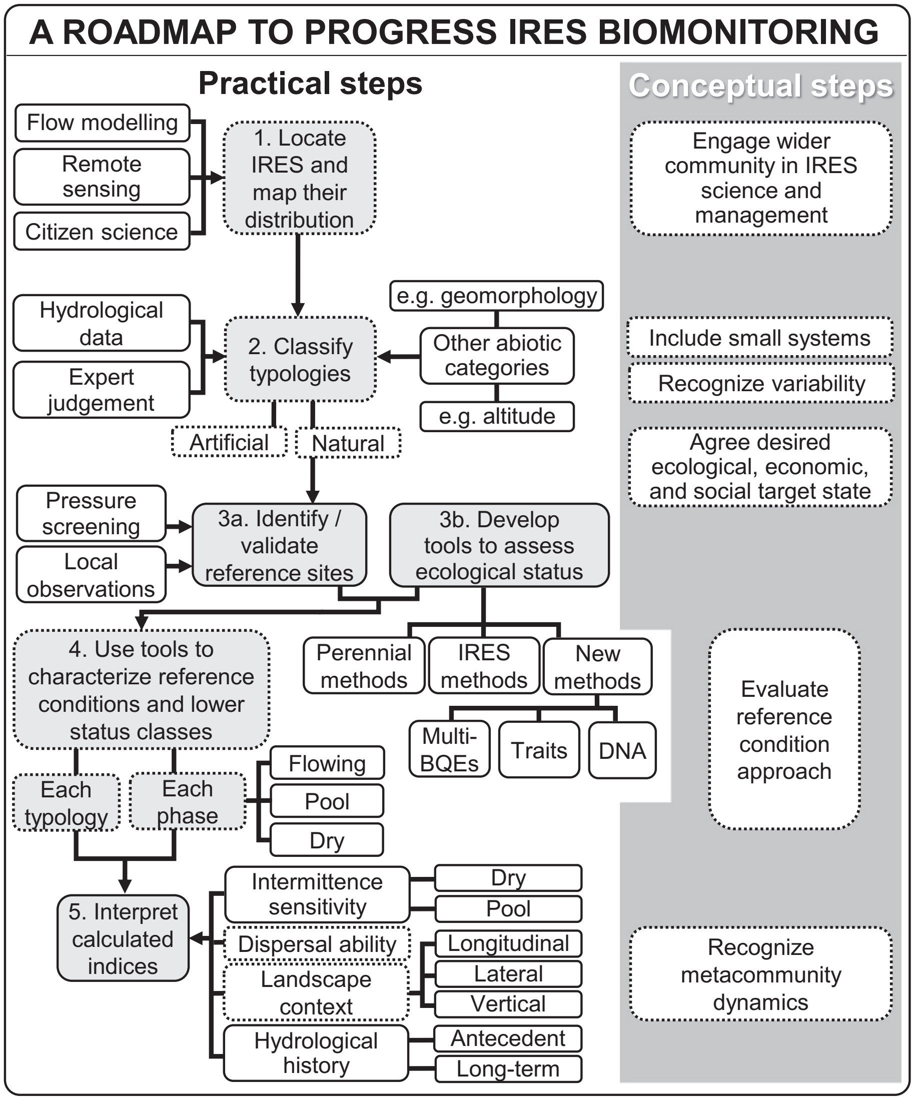

Fig. 5. A roadmap for the development of effective ecological status assessments in intermittent rivers and ephemeral streams (IRES). Filled boxes indicate key stages; vertical arrows navigate between stages; horizontal arrows indicate aspects informing each stage; dotted lines in the *Conceptual steps* box align vertically with related *Practical steps*. BQE, biological quality element (EC, 2000).

and Prat, 2009; 5b, Table 3) warrant evaluation to establish their potential for wider adoption, or to inform flexible development of comparable tools elsewhere.

In interpreting sampled assemblages, regulators should recognize a site's spatiotemporal context: its hydrological history, and the availability of recolonists from the wider metacommunity (Fig. 4; 5, Fig. 5; 5c, Table 3). Beyond the taxonomic classification of site-specific communities, integration of structural and functional aspects of multiple biotic groups could promote sensitive characterization of ecological responses to interacting anthropogenic stressors (Hughes et al., 2009; 5d, Table 3). In addition, molecular approaches including DNA metabarcoding and transcriptomics could transform biomonitoring by integrating taxonomic, functional, and phylogenetic diversity information (Yu et al., 2012) from the lotic, lentic, and terrestrial assemblages (Deiner et al., 2016; 5d, Table 3) that collectively respond to interacting anthropogenic pressures. Newly developed methods – potentially spanning characterization of dry-phase biota and quantification of ecological processes, using traditional and molecular approaches (von Schiller et al., 2017) – will require rigorous testing and validation to inform implementation of standardized protocols by regulatory agencies, and these protocols must be efficient, to facilitate uptake. Where novel biotic groups that fall outside of WFD BQEs prove informative, debate will be needed to justify their use (Jeppesen et al., 2011).

Collaboration is key to transforming IRES status assessment: between academics and river managers, to promote implementation of research recommendations; between countries, to disseminate best practice and enact new practices in a way that balances standardization with flexibility; between aquatic and terrestrial ecologists, to develop suites of bioindicators that represent both wet and dry phases; and between ecologists and geneticists (including DNAqua-Net COST Action members; Leese et al., 2016), to promote incorporation of IRES into emerging metabarcoding approaches from an early stage. The goal of these collaborations is to explicitly recognize the extent and value of IRES; to develop effective status assessment methods for use in biomonitoring schemes that adequately represent IRES and their diverse communities; and ultimately, to promote protection of IRES as they become an increasingly common landscape feature.

Table 3
The current state of biomonitoring in intermittent rivers and ephemeral streams (IRES) across Europe, including challenges limiting progress; highlights to date (i.e. best practice examples); overarching priority actions; and steps towards achieving these actions. Numbered points reflect a logical sequence of priority actions, with scope for concurrent progress to be made towards multiple actions; in particular, characterization of reference conditions and development of status assessment methods are complementary activities. Numbered citations are provided separately for each row.

	Current state	Highlights to date	Priority actions	Steps towards action completion	Sources
1	Mapping of IRES distribution hampered by poor representation in gauging station networks, in particular for small, headwater IRES.	Citizen scientists mapped >4000 km of flow states in France[1]; these data have been explored by the IRBAS project[2], leading to a national citizen science network[3].	Locate and map distribution of European IRES.	Develop a citizen science network to supplement hydrological monitoring data[4] and support Europe-wide mapping.	[1]Datry et al. (2016c); [2]Leigh et al. (2017); [3]ONEMA (2017); [4]SMIRES (2016).
2	IRES with catchments <10 km^2 are too small to be defined as WFD "water bodies" that require status assessment[1]; resources often restrict biomonitoring to "water bodies".	Non-WFD biomonitoring done by national agencies e.g. in slow-flowing sand-bed Dutch IRES[2]. National criteria define some small IRES as WFD water bodies e.g. in the UK[3].	Expand biomonitoring networks to encompass small streams including IRES, motivated by non-WFD drivers e.g.[4].	Identify national priorities to start expansion of biomonitoring networks.	[1]EC (2000); [2]Van der Molen et al. (2013); [3]UKTAG (2003); [4]EC (2011).
3	National IRES typologies are non-existent or fail to reflect IRES dynamism and heterogeneity. Hydrological data to inform development of IRES typologies are scarce.	Five WFD typologies distinguished in Croatia[1]. Official distinction of contrasting IRES in Cyprus, Greece, and Italy[2–3]. Classification of reach-scale Mediterranean IRES flow regimes[4–6].	Classify national typologies, including distinction between IRES with flowing phases that contrast in their predictability and duration.	Build on qualitative IRES descriptions in local use. Evaluate ability of new tools to inform typology classification more widely, including differentiation of natural and artificial IRES[4–7].	[1]NN (2013); [2]Skoulikidis et al. (2017); [3]Lazaridou et al. (2016); [4]Gallart et al. (2012); [5]Prat et al. (2014); [6]Gallart et al. (2017); [7]Polášek (2013a).
4	Reference sites identified in few countries. Absence of unimpacted sites in some countries.	Pressure criteria to screen sites developed for perennial rivers[1] and for Mediterranean IRES[2–4]. MIRAGE toolbox[4] and TREHS software[5] identify natural IRES. Expert judgement used to identify reference sites for 3 Croatian WFD typologies[6].	Screen and validate reference sites for each classified typology (see point 3), using sufficient sites to represent spatial variability[7].	Explore wider use of pressure criteria used to screen Mediterranean IRES and perennial rivers. Validate screened sites using expert judgement of field conditions.	[1]Wallin et al. (2003); [2]Sánchez-Montoya et al. (2009); [3]Delgado et al. (2012); [4]Prat et al. (2014); [5]Gallart et al. (2017); [6]NN (2013); [7]Munné and Prat (2009).
5	If assessed, IRES status is classified:				
	(a) during only wet, typically flowing, phases, whereas dry phases are excluded	Initial exploration of dry-phase communities[1]. Characterization of pool-phase communities[2].	Characterize variability in status among phases. Develop tools to assess status during each phase. Define a time window in which each tool is effective.	Evaluate potential of dry-phase biotas to indicate status. Evaluate ability of DNA-based tools to integrate catchment-wide biotic information from all phases[3].	[1]Corti and Datry (2015); [2]Cid et al. (2016); [3]Deiner et al. (2016).
	(b) using (often unevaluated) perennial methods	Perennial methods validated for assessment of flowing phases in IRES with long, predictable flowing phases[1–2]. New IRES-specific indices developed for flowing phases[3–6] when perennial methods have been deemed unsuitable[7].	Evaluate suitability of perennial methods for flowing phase assessments across Europe. Adapt or replace unsuitable methods, recognizing covarying biotic responses to degradation and intermittence. Develop new tools for IRES with short/unpredictable flowing phases.	Assign taxon-specific intermittence sensitivity scores[8–9] to inform expectations of community composition in IRES with different flow permanence regimes.	[1]Prat et al. (2014); [2]Morais et al. (2004); [3]Munné and Prat (2011); [4]Papastergiadou and Manolaki (2012); [5]Sánchez-Montoya et al. (2010); [6]García et al. (2014); [7]Munné et al. (2016); [8]Verberk et al. (2012); [9]Chadd et al., 2017.
	(c) using only community-level methods	Recognition that biomonitoring across freshwaters should incorporate metacommunity dynamics[1–3].	Interpret communities in a landscape context that recognizes the spatial arrangement of perennial and temporary reaches and wider recolonist sources.	Assign taxon-specific dispersal weightings, to inform expectations of community composition in IRES in different landscape contexts.	[1]Heino (2013); [2]Datry et al. (2016c); [3]Ruhí et al. (2017).
	(d) using only taxonomic methods.	Trait-based communities more stable than taxonomic communities within status classes[1] (but see[2–3]).	Explore functional approaches (trait-based and ecosystem services[4]) to status assessment.	Explore the potential of molecular approaches e.g. DNA metabarcoding and transcriptomics to overcome taxonomic limitations of traditional biomonitoring[5].	[1]Bruno et al. (2016); [2]Leigh et al. (2016); [3]Vander Vorste et al. (2016b); [4]von Schiller et al. (2017); [5]Leese et al. (2016).
6	Reference conditions characterized in few countries. Single benchmark concept	Diatom[4] and invertebrate[5] assemblages characterized in Mediterranean IRES with long, predictable flowing phases.	Develop conceptual alternatives to defining a single benchmark in dynamic ecosystems.	Recognize influence of metacommunity dynamics and landscape context on reference conditions.	[1]Bunn and Davies (2000); [2]Datry et al. (2016c); [3]Ruhí et al. (2017); [4]Delgado et al. (2012); [5]Munné and Prat (2009); [6]NN (2013).

Table 3 (*continued*)

Current state	Highlights to date	Priority actions	Steps towards action completion	Sources
inappropriate for dynamic ecosystems with unpredictable taxonomic communities[1–3].	'Best' and 'worst' values calculated for macrophytes, macroinvertebrates, and phytobenthos in Croatia[6].		Characterize reference conditions and class boundaries for each typology, informed by development of status classification methods.	

WFD, Water Framework Directive (EC, 2000).

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Appendix A. Initial survey used to request information about national biomonitoring activity from Working Group 4 members in the COST Action CA15113 Science and Management of Intermittent Rivers and Ephemeral Streams

SECTION 1 – RESPONDENT DETAILS
Your **name:**
Other Action participants involved in preparing this document**:**
Non-Action participants involved in preparing this document**:**
Country you represent**:**

SECTION 2 – BIOINDICATORS AND BIOMONITORING PROTOCOLS IN YOUR COUNTRY

Numbers reflect the order of tasks listed in CA15113 Memorandum of Understanding.

WG4 task 1*: "*List the different bioindicators and protocols used in the participating countries to assess the ecological status of rivers, including IRES when relevant*"

2.1 State the biotic groups used as bioindicators/biomonitors (including those used in **perennial** systems).

2.2 For each group listed in (2.1), describe the protocols used, *and/or* provide links to online descriptions, *and/or* provide protocols as attachments.

WG4 task 3*: "*identify issues and limitations [of current bioindicators and biomonitoring protocols]*".

2.3 Briefly note any issues and limitations for further discussion at WG meetings (optional section, leave blank if you have no comments at this stage).

SECTION 3 – RESEARCH ACTIVITY* INVOLVING YOUR COUNTRY

Including MSc, PhD, post-doc and other projects or activities, and including collaborations with other countries

3.1 Give details of any research activity relating to WG4 activities currently being conducted.
[Hyperlink to online shared spreadsheet]

3.2 Give details of any planned future research activity relating to WG4 activities.
[Hyperlink to online shared spreadsheet]

Appendix B. Second survey used to request further information about national biomonitoring activity from Working Group 4 members in the COST Action CA15113 Science and Management of Intermittent Rivers and Ephemeral Streams

In your country:

1. Have WFD typologies been developed that distinguish between different types of IRES? If "yes", provide details of classification criteria/each IRES type. If "no", state any descriptions that are in common use (e.g. in the UK, 'winterbourne chalk streams/rivers').

2. Are river typologies (including perennial typologies) classified using WFD System A or B (see WFD Annex II, Sections 1.1 and 1.2.1)? If System B is used, which "optional factors" are used in addition to the obligatory factors?

3. Have reference sites been identified for IRES typologies? If so, provide details of the site identification process (e.g. pressure criteria for site screening, modelling, expert judgement).

4. Have reference conditions (and boundaries between ecological status classes) been described for IRES typologies? If so, give details of classification criteria (e.g. quality elements considered).

5. Are *any* flow gauging stations located on IRES? If so, how many are on IRES and of these, how many match biomonitoring sites? What % of the *gauging station network* is located on IRES - and how does this relate to the % of the *river network* that is intermittent?

6. Are river flows (or pool/dry states) characterized by regulatory agencies using any method other than hydrological monitoring at gauging stations (e.g. citizen science initiatives, models)?

7. If perennial methods are used to assess IRES ecological status, has their suitability been evaluated? If so, state the methods/metrics tested and the results of the evaluation (suitable/not suitable).

   [Responses used to inform Table 1]

8. If specific methods/indices have been developed for IRES (or IRs; e.g. because perennial methods were evaluated and found to be unsuitable), provide details.

[Responses used to inform Table 1]

9. Are any non-taxonomic (e.g. DNA metabarcoding, trait-based) approaches used to assess the ecological status of any rivers (including perennial rivers)?

10. Do any IRES that are too small to be designated as WFD water bodies based on catchment size fulfil *other* criteria for designation as a WFD water body?

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