Unlocking our understanding of intermittent rivers and ephemeral streams with genomic tools

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Intermittent rivers and ephemeral streams (IRES) – waterways in which flow ceases periodically or that dry completely – are found worldwide, and their frequency and extent are expected to increase in the future in response to global climate change and growing anthropogenic demand for fresh water. Repeated wet–dry cycles generate highly dynamic settings within river networks composed of aquatic and terrestrial habitats, which act as evolutionary triggers for aquatic and terrestrial biota. Drying also alters functions and processes within river networks, with consequences for ecosystem services. Despite the emergence of promising conceptual and methodological developments, our understanding of the occurrence and diversity of organisms in these ecosystems is limited primarily due to their coupled aquatic–terrestrial characteristics. Novel genomic tools based on high-throughput sequencing have the potential to tackle unanswered questions of pivotal importance to predict future change in IRES. Here, we outline why genomic tools are needed to assess these dynamic ecosystems from the population to the metacommunity scale, and their potential role in bridging ecological–evolutionary dynamics.

Front Ecol Environ 2021; doi: 10.1002/fee.2404

Despite increased international efforts and policy agreements, global biodiversity continues to decline as climate change and human disturbance intensifies (Mace *et al*. 2018; Brondizio *et al*. 2019). In this context, understanding how biological communities are organized in the landscape and their underlying assembly mechanisms, as well as inferring the inherent capacity of communities to adapt to changing ecosystems, have become critical (Tonkin *et al*. 2019). Despite several promising conceptual and methodological developments, knowledge remains limited, particularly for ecosystems exhibiting high spatiotemporal variability (Altermatt 2013; Jabot *et al*. 2020). Increased frequency and magnitude of extreme events due to climate change (eg fires, floods, droughts) is projected to have direct and predictable effects on communities (eg changes in richness and biomass; Jacquet *et al*. 2020). These measures are key aspects for determining ecosystem stability; consequently, a better understanding of how biotic communities and associated ecological functions are organized in space and time in dynamic ecosystems is needed (Altermatt *et al*. 2020). Understanding river ecosystems may be particularly relevant because of the growing numbers of species at risk, along with the essential ecosystem services they provide (Tonkin *et al*. 2019).

In the Anthropocene, greater numbers of springs and watercourses worldwide are drying as climate changes and as groundwater abstraction increases (Datry *et al*. 2018a). Naturally prevalent in most biomes and across all continents, intermittent rivers and ephemeral streams (IRES), which periodically dry and/or cease to flow, are becoming increasingly common. IRES range from small channels that flow for several days after heavy rain to large rivers that occasionally recede to little more than isolated pools or dry completely (Figure 1). However, existing science applicable to streams and rivers was largely developed from and for systems with continuous (if not consistent) flow. In contrast, IRES require insight from lotic,

In a nutshell:

- Intermittent rivers and ephemeral streams (IRES) are becoming more common globally due to climate change and anthropogenic pressures
- Current IRES monitoring relies on the flowing phase, which ignores the key dynamics of these systems, including shifts between flowing, non-flowing, and dry phases
- Rapid development of genomic tools offers an unprecedented opportunity to gain insight into the biodiversity of these highly dynamic ecosystems, including their species, gene pools, functions, and adaptations
- We discuss potential breakthroughs and make recommendations to guide the actions of water resource managers

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lentic, and terrestrial sciences, and consequently a transdisciplinary approach to research (Datry *et al.* 2014). While these key features are gradually being incorporated, new approaches are needed to address fundamental questions that advance insight into how biodiversity and ecological functions are organized in IRES. Answering such questions will in turn enhance management of these ecosystems as they adapt to global change and increased pressures during the Anthropocene.

The distinct flowing, non-flowing, and dry phases of IRES (Figure 2) challenge traditional approaches to assessing populations and communities. Typically, applied research focuses on flowing phases, and populations and communities are studied morpho-taxonomically to produce taxa lists that enable inference of ecological or evolutionary processes. However, this approach often (1) overlooks taxa, notably lentic and terrestrial species; (2) is not directly applicable to all biotic groups using a unified set of techniques to all biotic groups; (3) fails to characterize underlying genetic variation and responses that may be essential to enable adaptation to environmental conditions; and (4) does not adequately describe the dynamic changes and characteristic state shifts of IRES ecosystems. The rapid development of genomic tools and indicators (eg Pauls *et al.* 2014; Pawlowski *et al.* 2018) now enables ecologists and evolutionary biologists to move beyond taxa lists. Genomic tools improve upon taxonomic information both in terms of higher resolution (Beermann *et al.* 2018; Bush *et al.* 2020) and detection of cryptic, rare, or new non-native or invasive species (Mächler *et al.* 2014), and also deepen insights into eco-evolutionary dynamics at population and community levels (Becks *et al.* 2012). Applying genomics to research on population structure and dynamics will facilitate more accurate characterization of distinctive IRES features, namely that: (1) as dynamic networks of heterogeneous habitats that extend across landscapes, IRES support both aquatic and terrestrial biodiversity; (2) IRES exist in all biogeographical and climatic contexts; (3) IRES experience wide gradients of drying severity; and (4) IRES occur both naturally and as a result of human pressures, allowing exploration of contrasting situations that promote eco-evolutionary processes.

This review highlights key processes that require further understanding of IRES and details how the application of innovative genetic methods could substantially increase our understanding at three levels: populations and communities at

Figure 1. Examples of intermittent rivers and ephemeral streams (IRES) across different continents and climates. (a) Rio Chaki, Bolivia; (b) unnamed stream, New Zealand; (c) La Clauge, France; (d) Sevilleta National Wildlife Refuge, New Mexico.

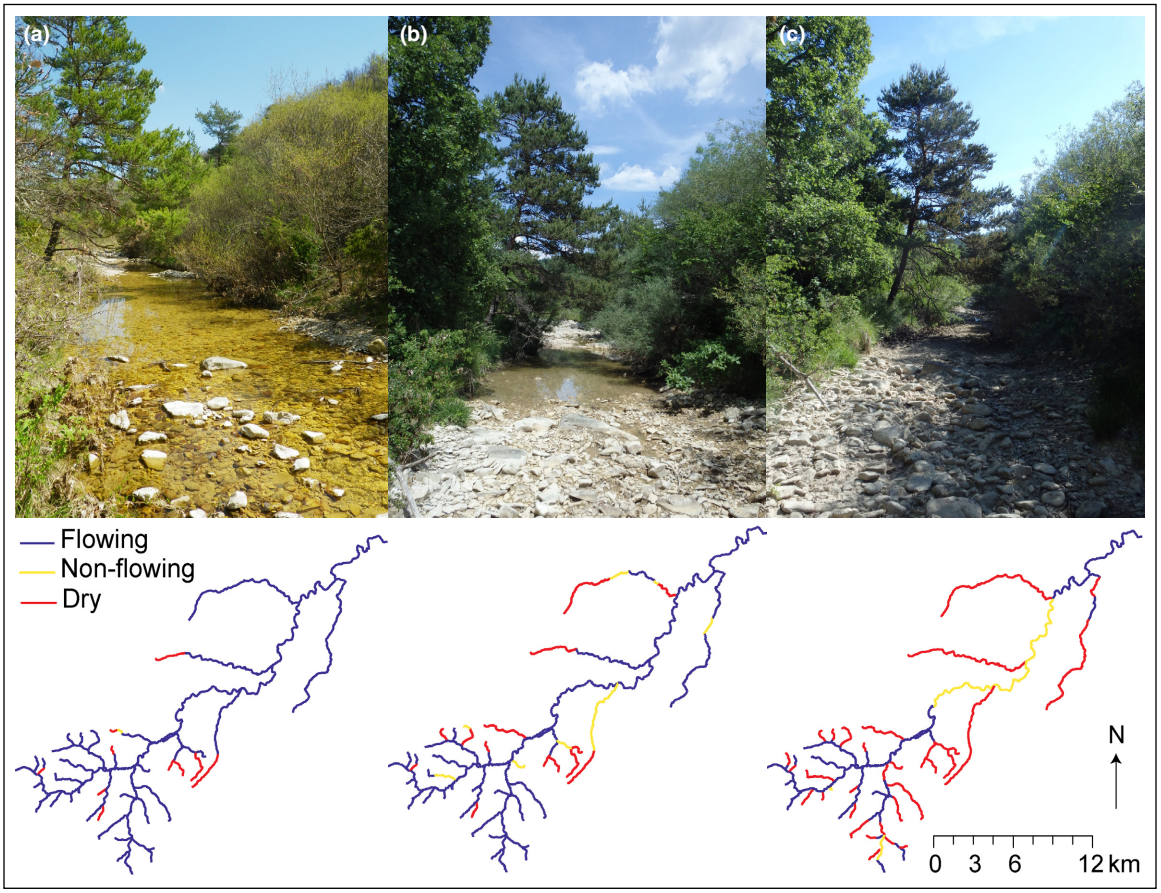

Figure 2. Hydrological phases in a typical IRES, as shown here for the Thouaret catchment, France, in which spatiotemporal dynamics can be very high at the network scale. (a) Flowing phase (7 Jan 2012); (b) non-flowing phase (15 Sep 2012); (c) dry phase (15 Oct 2012).

local scales and their adaptations to the environment, spatial linkage of these populations and communities in the context of metapopulation and metacommunity ecology, and functional linkages across complex aquatic–terrestrial meta-ecosystems. The added value of using genetic tools to infer ecological and evolutionary processes in these unique and extreme ecosystems is also presented through specific examples.

Population structure and dynamics

The population scale is the most relevant level of organization for understanding community change in space and time because multiple populations interact to determine a community-level response. Change in population size is an essential parameter for characterizing resistance (ie persistence in situ as desiccation-resistant forms) and resilience (ie rapid recolonization from refuges when suitable conditions return) to environmental disturbance (Pennekamp *et al.* 2018). Moreover, variation in genotypic traits common to all populations provides the raw material of phenotypic variation for selection to act on; thus, non-random reproduction and survival in a population is a key driver of adaptation to extreme local habitats (Savolainen *et al.* 2013). However, in IRES, systematic evolutionary processes are counterbalanced by stochastic processes (genetic drift), which depend on population size. Genetic drift might be greatly enhanced in IRES given the temporal fluctuations in population abundance and degree of spatial isolation (Bonada *et al.* 2017). For example, wet–dry cycles in IRES may increase the amplitude of population dynamics, or the contraction and expansion of the wet phase may increase the areal extent inhabited by a population. In addition, global climate change is increasing the frequency of flow intermittence and many aquatic populations may be experiencing ongoing population declines. At the same time, the contrary may also be the case, and genetic drift may be low due to temporal changes in habitat. Genetic tools are therefore crucial for studying population dynamics in IRES.

Although conceptual frameworks describing population dynamics of non-model species are well developed, empirical data are difficult to obtain. For the greatest confidence, and to infer a reduction in the effective population size, the number of genetic markers studied is important (Waples *et al.* 2016). Initially, research assessing genetic diversity and turnover due to bottlenecks relied on microsatellites; for example, Shama *et al.* (2011) used microsatellite markers to quantify the impacts of stream drying on populations of an alpine caddisfly. However, such markers can identify only relatively large

changes in population structure. To the best of our knowledge, the population size and dynamics of IRES species have yet to be assessed in detail with genomic markers, despite their ecological relevance and the frequent use of such analyses in perennial flowing and non-flowing ecosystems (eg Roesti *et al.* 2015).

The above approaches used individually sorted and analyzed specimens, typically to examine changes in heterozygosity and allele diversity. More cost-efficient approaches now exist in which either pooled specimen samples per population (eg Pool-seq; Schlötterer *et al.* 2014) or DNA shed by organisms into their environment (ie environmental DNA [eDNA]; Deiner *et al.* 2017) are sequenced. Pool-seq cannot be used to distinguish individual genotypes; that is, no direct information about heterozygosity is obtained, and the method requires a reference genome to first be sequenced and assembled. The analyses can, however, provide insight into allele shifts over time.

Techniques that rely on eDNA, in which DNA is extracted from a sediment and/or water sample (Deiner *et al.* 2017), are particularly useful for species that are difficult to isolate from a habitat (eg due to low abundance, small size, and/or fragility). One method of analyzing eDNA samples is to use a species-specific approach, such as quantitative polymerase chain reaction (qPCR), which can reveal the abundance of the marker gene for a target species (Hernandez *et al.* 2020). This information can reliably infer presence and, with some restrictions, provide information on population size. Studies have linked the DNA copy number (derived from qPCR) or read number (derived from metabarcoding) to biomass of target fish populations (eg brook trout [*Salvelinus fontinalis*; Baldigo *et al.* 2017]; freshwater fish [Di Muri *et al.* 2020]). However, studies of invertebrate population size remain scarce (Blackman *et al.* 2020) and validating single-species assays requires extensive investment (Thalinger *et al.* 2020).

Species-specific IRES adaptations

Specific adaptations to variation in natural flow regimes have been reported in river organisms, among which adaptations to extreme droughts and floods are the most conspicuous (Lytle and Poff 2004). IRES are extreme ecosystems that impose a strong adaptive pressure on the taxa persisting in a habitat across wet–dry cycles (Bonada *et al.* 2017). This raises an important question: can species adapt to the increasing prevalence and severity of drying in a global change context? Genomic tools offer approaches to mechanistically relate population- or species-specific adaptations to changing environmental conditions (Rudman *et al.* 2018). Most importantly, the toolbox to identify the genomic regions involved in the adaptation to new environmental conditions is available for non-model organisms that typically inhabit IRES (Weigand and Leese 2018). Using the genomic sequence information of closely related species, comparative genomic tools are also available to pinpoint genes involved in adaptation and to assess the degree to which adaptive evolution has shaped a species' trajectory (eg Moutinho *et al.* 2019).

Comparative genomics and transcriptomics can also reveal the molecular mechanisms supporting adaptation to an IRES lifestyle. For example, Gusev *et al.* (2014) demonstrated that in the chironomid species *Polypedilum vanderplanki*, late embryogenesis abundant protein genes, which promote homeostasis in cells under desiccation, are highly upregulated under dry conditions. Further comparative genomic research confirmed that this upregulation is due to the species-specific co-option of heat shock regulatory system DNA motifs in promoter regions of desiccation-induced genes (Mazin *et al.* 2018). Whether or not comparable evolutionary and functional mechanisms enable other IRES taxa to cope with dry conditions, and how quickly this adaptation developed, are key topics for future studies. IRES occur across regions, which could enable identification of general principles underlying adaptation to wet–dry conditions, and might therefore serve as a useful natural laboratory for studying adaptation and molecular convergence of global change. The main factor limiting research on such adaptations is the lack of available genomes for species pairs exclusively occurring in either IRES or perennial streams, but the advent of new high-throughput and long-read technologies should increase the number of genomes available for investigating such changes in the future. Projects should specifically target IRES specialist species to reveal comparative genomics and their relative speed of adaptation to desiccation (Table 1).

Community composition

The communities present at the boundary of aquatic–terrestrial habitats consist of a characteristic set of species due to strong environmental filtering, which represents an intrinsically valuable research area. However, these communities are hard to study in the context of river assessments, and therefore dry-phase IRES communities have been less studied than their aquatic counterparts (Steward *et al.* 2012). Whereas different traditional sampling approaches are needed to assess IRES biodiversity across hydrological phases, eDNA collection and analyses via metabarcoding could be used to integrate information from across wet and dry phases. Extracting DNA from a sediment sample collected during a dry phase and/or a water sample taken during a wet phase (non-flowing or flowing) can effectively encompass the hydrological phases of IRES (Figure 3). Applying a metabarcoding approach to eDNA samples (eg identification at the community level rather than a species-specific approach) can reveal alpha diversity to an unprecedented degree (Blackman *et al.* 2017) and is increasingly being used in a metacommunity context (eg Bush *et al.* 2020).

Perhaps the Achilles heel of any eDNA sample is determining the location of the original source from which the DNA molecule was shed (eg DNA from a living organism currently

Table 1. Proposed model organisms for further study of IRES (species occur in a range of IRES types) with available genome and transcriptome IDs or ongoing genome project

Group	Subgroup/Family	Example taxa	Adaptations	Genome ID	Transcriptome ID
Crustacea	Ostracoda	*Heterocypris incongruens*	Desiccation-resistant eggs	–	TSA:ICLE00000000
		Darwinula stevensoni	Drought resilient	BP:PRJNA515625	–
Diptera	Chironomidae	*Belgica antarctica*	Drought resilient	G:14659	TSA:GAAK01000000
		Cardiocladius sp	Desiccation-resistant eggs	–	TSA:GGBD00000000
		Cricotopus draysoni	Desiccation-resistant eggs	–	TSA:GFNI00000000
		Cricotopus parbicinctus	Desiccation-resistant eggs	–	TSA:GFNF00000000
		Cricotopus albitarsis	Desiccation-resistant eggs	–	TSA:GFNG00000000
		Polypedilum vanderplanki	Anhydrobiosis	BP:PRJDB1558	TSA:GGBC00000000
	Simuliidae	*Simulium* sp	Early colonizer	–	TSA:GGBP00000000
	Ceratopogonidae	*Culicoides sonorensis*	Multiple resistance forms	G:67281	TSA:GAWM00000000
Ephemeroptera	–	*Cloeon dipterum*	Drought resilient	G:88976	BP:PRJEB35103
Mollusca	–	*Radix balthica*	Drought resilient	BP:PRJNA52079	BP:PRJNA79893
Plecoptera	–	*Isoperla grammatica*	Desiccation-resistant eggs	G:45288	–
		Brachyptera risi	Desiccation-resistant eggs	–	TSA:GDBN00000000
		Nemoura cinerea	Desiccation-resistant eggs	–	TSA:GDCQ00000000
Trichoptera	Limnephilidae	*Limnephilus lunatus*	Desiccation-resistant eggs	G:17773	–
		Stenophylax sp	Adult diapause	–	BP:PRJNA380791
		Micropterna lateralis	Adult diapause	–	TSA:GELV01000000

Notes: IDs are prefixed with a code indicating the corresponding NCBI database: G = genome (www.ncbi.nlm.nih.gov/genome); BP = bioproject (www.ncbi.nlm.nih.gov/bioproject); TSA = nucleotide transcriptome shotgun assembly database (www.ncbi.nlm.nih.gov/nuccore).

inhabiting – or no longer present in – the sampled habitat, or DNA transported from another habitat; Deiner *et al.* 2017). It is even more critical in IRES when determining the fate of aquatic organisms persisting in situ during dry phases (eg does the DNA signal in a sediment sample reflect current occupancy?). When coupled with environmental RNA (eRNA) approaches, eDNA metabarcoding can distinguish contemporary from older signals, but the use of eRNA to detect contemporary species signals is rare. Fundamental questions relating to factors that influence eRNA persistence and the ease with which it can be used in the field are current research priorities (Cristescu 2019). IRES represent ideal systems for developing eRNA methods as a necessary step toward the separation of dead cells left by organisms from those still living as desiccation-resistant life stages.

DNA metabarcoding of bulk samples (tissue and biofilm) has also revealed previously undetected species diversity (eg by identifying cryptic or overlooked species; Blackman *et al.* 2017). This is especially relevant for IRES systems with characteristic but understudied organisms. Insights from studies exploring dominant IRES taxa such as the Chironomidae family (eg Datry *et al.* 2014) suggest that although the vast diversity of ecologically important taxa is poorly known, they can be described using the operational taxonomic unit (OTU) concept, and that in some cases OTUs can be used as a proxy for biological species. The power of this approach has recently been demonstrated by Beermann *et al.* (2018), who studied chironomid responses to multiple stressors in a stream; while the authors could not morphologically identify taxa below the family level, DNA metabarcoding at 3% and 5% OTU clustering thresholds revealed 183 and 142 distinct OTUs, respectively. Although many taxa could not be molecularly assigned to species due to missing taxonomic information in DNA barcode reference databases, the study revealed distinct ecological profiles linked to multiple stressors. Similar patterns have been documented for other taxa abundant in IRES such as oligochaetes (Vivien *et al.* 2020). Microbial biofilms are also powerful indicators of aquatic ecosystem health that could easily be investigated in both wet and dry phases using metabarcoding (Kermarrec *et al.* 2013), allowing characterization of both heterotrophic and autotrophic communities. Such studies highlight the potential of DNA metabarcoding to provide a comprehensive census of IRES biodiversity, stressor-specific responses, and turnover across wet–dry cycles.

Metapopulation and metacommunity dynamics

Community ecology theory acknowledges that dispersal – the movement of individuals between local populations and communities within a landscape – is a pivotal regional process determining metapopulation and metacommunity dynamics within meta-ecosystems (Leibold *et al.* 2004). Dispersal can promote or even be essential for the persistence of a species in landscapes composed of heterogeneous

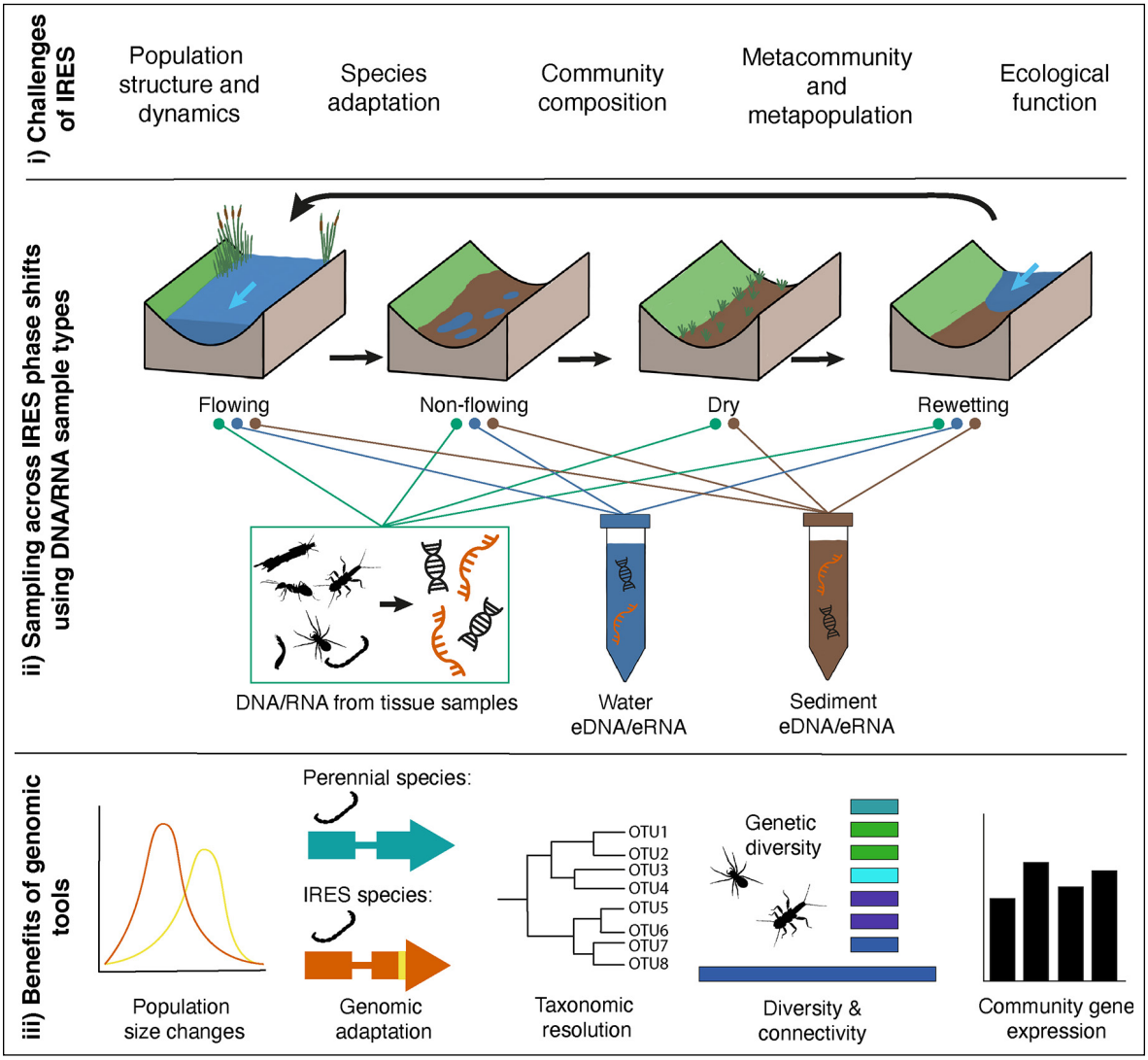

Figure 3. Summary of the (i) key challenges of IRES as discussed in this article, (ii) sampling across IRES phase shifts using DNA/RNA sample types, and (iii) the benefits of using genomic tools.

habitat patches: local deterioration of environmental conditions or loss of patches can be counteracted by dispersal and colonization of new patches. This is especially relevant for IRES, in which habitat patches fluctuate between wet and dry states. While some species persist locally, most occur at a regional scale and track habitats matching their environmental preferences in space (Sarremejane *et al.* 2020). This patchiness and the local deterioration of habitat patches have been well studied in classic metapopulation cases in which hydrological changes are common, such as rock and tide-pool ecosystems (Altermatt and Ebert 2008), and geological outcrops supporting specific grasslands (Thomas and Hanski 2004).

Habitat patchiness is especially pronounced in IRES, both with respect to organisms' occurrence and the underlying habitat dynamics (Datry *et al.* 2014). The dynamics of dispersal in such habitats are theoretically well described (eg Reigada *et al.* 2015), but most empirical studies have considered only a few select species and fail to represent the considerable biodiversity of IRES. To advance knowledge, scientists must monitor species' spatiotemporal dynamics, tracking variation in instream conditions that generate highly dynamic settings for IRES biota (Figure 2). Such dynamics may affect both the occurrence and spatial organization of habitat patches in general, and also the population structures of their aquatic and terrestrial inhabitants (eg due to death from desiccation or inundation, respectively). For perennial rivers, the influence of dispersal on metapopulation diversity and stability is well understood (eg Terui *et al.* 2018), but these studies assume the metapopulation to be of a fixed structure and size, and to be continuous (Altermatt 2013). In contrast, drying is a predominant factor structuring IRES metapopulations (Phillipsen *et al.* 2015) and metacommunities (Crabot *et al.* 2019). Drying therefore alters the size of the network, causes its temporal fragmentation, and influences species' coexistence and stability (Crabot *et al.* 2019).

Genomic tools will be critical for addressing two key issues during future exploration of IRES metapopulations and metacommunities. First, such tools will be essential for describing the physically interlinked, but temporally separated, metacommunities of organisms inhabiting dry channels compared to those present during flowing phases. Fingerprinting the occurrence of organisms based on eDNA from water and sediments will allow reconstruction of metacommunity spatial-temporal dynamics. Second, dispersal greatly influences metacommunity stability, and genomic tools will facilitate (1) detection and quantification of dispersal propagules in both wet and dry states, with many aquatic organisms having desiccation-resistant dispersal stages that persist in the dry phase, whereas the dispersal stages of many terrestrial organisms are passively transported by water; (2) reconstruction of the genetic connectivity of populations; and (3) identification of dispersal barriers and corridors as well as their underlying environmental variables.

A central question in freshwater ecology concerns the realized dispersal of aquatic species in landscapes and riverscapes (Leibold *et al*. 2004). Information about gene flow and community fragmentation can be obtained with the aid of genetic markers like microsatellites and single nucleotide polymorphisms (SNPs) (eg Weiss and Leese 2016). In IRES, an additional key question relates to species-specific capacities for resistance and resilience. Some species-specific adaptations to flow intermittence may impact strongly on a population's genetic structure, adding another level of complexity to two-dimensional stream network patch hypotheses; moreover, strong dispersers can quickly recolonize rewetted habitats, even if they are poorly connected. Examples of this recolonization have been documented in IRES using traditional morphological methods, but these approaches tend to exclude poorly described taxa, such as acarids, chironomids, and oligochaetes (eg Datry *et al*. 2014). For all taxonomic groups, genetic markers could be used to assess the complex interplay between resistance and resilience as opposed to extinction and recolonization. For example, Phillipsen *et al*. (2015) analyzed multiple diploid nuclear microsatellites to study an aquatic species *Abedus herberti* (Hemiptera) in desert streams in Arizona and compared patterns to semi-aquatic species. Assessments of gene flow directionality revealed that *A. herberti* populations were extremely isolated, even at very small scales (several hundreds of meters). Two populations separated by an intermittent stretch in one stream showed strong subdivision and asymmetric gene flow, indicating that even at small, local scales, flow intermittence can inflate the separation of gene pools. At the same locations, genetic signatures of isolation for the stonefly *Mesocapnia arizonensis* were much less pronounced. In contrast to *A. herberti*, *M. arizonensis* is adapted to flow intermittence and survives long dry periods as dormant eggs and juveniles in subsurface sediments (Bogan 2017). Phillipsen *et al*. (2015) found that local-scale habitat connectivity remained high even where intermittent stream stretches were present, whereas regional-scale isolation increased greatly in the absence of perennial or even intermittent stretches.

Ecological function

Understanding the mechanisms through which biodiversity drives ecosystem functioning remains a central challenge in ecology (Loreau *et al*. 2001). Genomic tools might provide fruitful insights into the strength of biotic interactions in IRES communities and the functional traits of ecologically important taxa (Pauls *et al*. 2014). Statistical methods are increasingly applied to infer species interactions from their abundance and co-occurrence patterns, as obtained with DNA metabarcoding approaches (Vacher *et al*. 2016; Derocles *et al*. 2018). The inferred ecological networks offer a means of assessing the contribution of biotic interactions to diversity patterns and community assembly while providing a representation of matter and energy flow from basal resources to higher trophic levels (Ohlmann *et al*. 2018). Although traditional ecological network reconstruction requires morphological identification of taxa and characterization of their interactions (Evans *et al*. 2016), approaches allowing inference without assumptions of the network structure or a priori dependence among taxa are particularly appropriate for IRES, which often have poorly characterized taxa. These approaches might also benefit from the development of taxonomic, trait, and trophic interaction databases that allow restriction of the inferred networks to the most probable interactions (Brose *et al*. 2019; Djurhuus *et al*. 2020).

Metagenome and metatranscriptome sequencing are also promising methods for gaining a more complete picture of the metabolic processes within IRES communities, compared to that provided by traditional targeted laboratory assays that focus on a restricted number of enzymatic reactions (Manoharan *et al*. 2015). Such approaches could determine the relative abundance of multiple functional genes at the community level and could derive proxies of community-weighted mean traits without a priori knowledge of the genes carried by individual taxa (Fierer *et al*. 2014). The identification of traits related to substrate utilization, nutrient acquisition, or stress tolerance (Manoharan *et al*. 2015) could promote the inclusion of IRES communities in a response–trait framework and could provide a more mechanistic understanding of the role of IRES biodiversity in processes including organic matter decomposition and nutrient cycling. Improved understanding of the mechanisms of trait selection through environmental filtering might also help to explain IRES community assembly under aquatic or terrestrial phases. For example, genome analysis showed that members of the Actinobacteria – a bacterial phylum whose species play key roles in soil carbon storage – carry genes promoting resistance to an environmental stressor (Trivedi *et al*. 2013), which could account for their high relative abundance in dry IRES (Gionchetta *et al*. 2019).

Molecular approaches, such as metatranscriptomics (targeting the active fraction of the community with high temporal resolution) or stable isotope probing, might also be useful to enhance understanding of the response of IRES microbial communities during drying or rewetting phases. Rewetting

events can drive large shifts in microbial community composition (Gionchetta *et al*. 2020), also representing a critical moment of biogeochemical cycling in riverine networks (Datry *et al*. 2018b). Identifying organisms that are active when flow resumes could indicate the fraction of a community that (1) has remained active, (2) reactivates from dormant life stages, (3) immigrates from adjacent perennial freshwater habitats, or (4) enters from groundwater aquifers. However, use of such approaches remains expensive and is hampered by several logistical constraints, which currently restrict their application primarily to laboratory settings or a handful of large field studies (eg Carradec *et al*. 2018). Although rates of ecosystem processes are not always reflected by the abundance of the corresponding functional genes or their transcripts (Rocca *et al*. 2015), approaches coupling field measurement of ecosystem process rates and genomic tools might provide valuable insights into the potential mechanisms driving responses to intermittent flow regimes, such as physiological acclimation or changes in community structure (Hall *et al*. 2018).

Conclusions

The global extent of IRES is growing, but our understanding of the total biodiversity (ie aquatic and terrestrial) within these ecosystems and the functional roles of their species remains limited. IRES research requires integration of several conceptual and methodological approaches that have yet to be used to investigate the processes shaping these dynamic systems. However, innovative genetic tools could potentially be employed to characterize these systems across the aquatic–terrestrial continuum. In addition, IRES and their associated species are prime candidates for these exploratory genetic methods. We encourage the next generation of ecologists and evolutionary biologists to embrace genomic tools as a means of advancing our understanding of IRES biodiversity patterns and processes, especially the underlying adaptations that enable species to persist in these highly dynamic ecosystems across wet–dry phases (Figure 3). Exploiting the full potential of these tools will require investment of both time and money by researchers, but the benefits they offer are substantial. This investment will facilitate the establishment of fit-for-purpose IRES monitoring approaches incorporating the development of new sampling methods, and will maximize knowledge of ecological functioning and how these systems are responding to global change.

Acknowledgements

This manuscript represents an international collaboration between SMIRES (Science and Management of Intermittent Rivers and Ephemeral Streams, CA15113, https://www.smires.eu) and DNAqua-Net (Developing new genetic tools for bioassessment of aquatic ecosystems in Europe, CA15219, https://dnaqua.net), funded by the EU COST (European Cooperation in Science and Technology) program. This research was also supported by the H2020 European Research and Innovation action Grant Agreement N°869226 (DRYvER). FA acknowledges funding from the Swiss National Science Foundation Grants 31003A_173074 and PP00P3_179089, and the University of Zurich Research Priority Programme "URPP Global Change and Biodiversity".

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