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Wetland Ecogenomics – The Next Generation of Wetland Biodiversity and Functional Assessment

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Wetlands are among the most productive and important ecosystems on Earth, yet they have been subject to repeated and dramatic historical losses, and continue to be at risk of degradation and destruction (Millennium Ecosystems Assessment 2005; Cuiabá Declaration 2008). In coastal areas worldwide, it is estimated that 50% of salt marshes and 35% of mangroves have either been lost or degraded, with the proportion exceeding 90% in some areas, such as the West Coast of the United States (Barbier et al. 2011). Arguably, inland areas have seen more rapid and extensive attrition with an areal loss of 64-71% since 1900 (Davidson 2014). Dahl (1990) estimated that over 100 million acres (40 million ha) of wetlands were lost in the coterminous United States between the time of European settlement and 1980, mostly due to ditching, draining and conversion for agricultural purposes. In China, it is estimated that 57% of coastal wetlands and 73% of mangrove forests have been lost since the 1950s, primarily due to land reclamation and other anthropogenic impacts (Qiu 2011). Although loss rates have slowed in some countries (e.g., United States), global wetland loss continues to occur, primarily in less-developed countries (Davidson 2014). For example, 55% of newly urbanized areas in Chile between 1975 and 2000 occurred through wetland conversion (Pauchard et al. 2006). In addition to outright loss, wetland degradation continues as a function of urban and agricultural encroachment, resource extraction, excessive use, pollution, hydrologic alteration, and invasion by aggressive non-native species (Millennium Ecosystem Assessment 2005; Silliman et al. 2009). Looking to the future, climate change effects, particularly sea level rise will pose increasingly significant risks to coastal wetlands. Blankespoor et al. (2014) estimate that a 1m sea-level rise would affect 68% of coastal wetlands in 86 developing countries and territories, with extensive loss occurring in Europe and Central Asia, East Asia, and the Pacific, as well as in the Middle East and North Africa.

International multi-lateral efforts aimed at protection and restoration of wetlands and the promotion of stewardship and conservation include: a) the Ramsar Convention on Wetlands, which provides a framework for national action and international cooperation for the conservation and wise use of wetlands and their resources (McInnes 2014; Box 1) and b) the European Union's Water Framework Directive, which includes a general objective to restore functioning and biodiversity of aquatic ecosystems, with a goal of achieving 'Good Ecological Status' for rivers, lakes, and estuarine and coastal waters. In addition to multi-lateral efforts, many countries have bilateral arrangements that also seek to support the conservation of wetlands. Many individual nations have enacted programs and policies aimed at protecting and managing wetlands in a more sustainable manner. In Australia (Environment Australia 1997), wetlands policy promotes conservation, restoration, and sustainable use. Within North America, individual states, provinces, and territories have legislated to restrict wetlands loss and promote remedial and restoration actions. In China, the development of National Wetland Parks at the county level have been promoted and relevant provisions on wetland park management have been enacted.

Arguably, the hallmark of national wetland regulatory protection is the United States' Federal Clean Water Act, which aims to protect and restore the chemical, physical, and biological integrity of wetlands. Implementing regulations for the Clean Water Act stipulate a process of avoiding and/or minimizing wetland impacts to the maximum extent practicable, and compensating for all unavoidable losses. The Clean Water Act, along with other federal and state regulations, promotes dual goals of short-term no net loss of wetlands and long-term gains in wetlands.

Each of the national and international wetland polices includes language relating to the protection and restoration of not only wetland area, but also wetland functions, values, and services. Despite this universal tenet, no program prescribes *specific* methods for assessing such functions and values, leading to a plethora of options for conducting such assessments.

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Assessing Wetland Ecosystem Functions and Services

Literally hundreds of functional assessment methods have been used in wetlands over the past 30 years (e.g., Bartoldus 1999, Carletti et al. 2004). Functions generally describe the fundamental ecological processes that occur in wetlands (Novitsky et al. 1996; Smith et al. 1995), whereas services specifically refer to ecosystem attributes and processes that support the well-being of human populations (Costanza 2000, MEA 2005). Often, the distinction between functions and services is blurred and, as a result, assessments almost invariably include elements of both function and service evaluation (Table 1). Since functions and services generally involve processes occurring over time, their evaluation requires repeated measurements to quantify process rates. Despite this, most wetland assessment methods measure a combination of cultural, physical, and biological attributes at a single moment in time, providing a snapshot of the status of a wetland that is used to *infer* the degree, or capacity, to which certain functions or services are being performed. To date, an efficient and robust approach to measuring actual functions or services remains one of the most desired, yet elusive goals in wetland management.

Box 1

The Ramsar Convention on Wetlands

The Ramsar Convention on Wetlands has designated over 1,900 wetlands on its List of Wetlands of International Importance. These wetlands are found in 160 different nations and total over 186 million hectares. As a part of its Strategic Plan for 2009-1015, the Ramsar Convention outlined a number of strategies to achieve its overarching goal of ensuring the Wise Use of wetlands (Ramsar Convention Secretariat 2010). These strategies include “wetlands inventory and assessment”, “global wetland information”, “science-based management of wetlands”, “wetland restoration”, and “control of invasive alien species” in order to maintain the ecological character of all wetlands. Each of the strategies proposed by this international body require large volumes of high-resolution biodiversity data.

In particular, ecogenomics can contribute to the requirements under the Convention to list wetlands of international importance based on the composition and abundance of their biodiversity, as well as tools to assess changes in the ecosystem functions and biogeochemical processes that support the biodiversity and the ecosystem services that they provide. The absence of adequate data on the biodiversity and functions and processes that characterize wetlands is seen as a major limitation on maintaining their ecological character, including measuring the success of restoration efforts.

Table 1: Commonly assessed wetland services (left) and functions (right). Although developed somewhat independently, there is substantial overlap between functions and services.

Wetland Ecosystem Services (MEA 2005, Barbier et al. 2011)	**Wetland Functions (Smith et al. 1995, Novitsky et al. 1996)**
Provisioning Services Food production Fresh water storage and retention Fiber and fuel production Biochemical extraction of medicines and other materials Genetic materials **Regulating Services** Source of and sink for greenhouse gases, carbon sequestration Water regulation (hydrological flows) groundwater recharge/discharge Water purification and waste treatment retention Erosion regulation, retention of soils and sediments Natural hazard regulation flood control, storm protection, coastal erosion Pollination- habitat for pollinators **Cultural Services** Spiritual and inspirational source Recreational and tourism opportunities Aesthetic values Educational opportunities **Supporting Services** Soil formation sediment retention and accumulation of organic matter Nutrient cycling storage, recycling, processing, Fisheries maintenance	**Functions associated with Water Quality Improvement** Removal/transformation of nutrients Removal of metals and toxic organics Removal of sediment **Functions associated with Habitat** Habitat for plant communities Invertebrate species habitat Vertebrate species habitat Maintenance of wildlife diversity and abundance Support primary production and export **Functions associated with Hydrology/Water Quantity** Reduction in peak flows Decrease in downstream erosion, sediment stabilization Maintenance of low flows to streams during dry season Ground water and aquifer recharge

Although simple in concept, functional assessment is a challenging proposition. Existing structural measures remain limited in scope, and do not fully reflect the dynamic processes that occur in wetlands. By nature, wetlands are temporally variable and spatially heterogeneous largely due to variations in biotic communities and hydrologic conditions that can fluctuate over diel, tidal, seasonal, inter-annual, and decadal time scales. Many assessment methods rely on measurement of "characteristic" or "diagnostic" plant or animal communities, such as plants, amphibians, fish, birds, or invertebrates as indicators of condition. Despite their utility and some success at large scales, surveys of multiple taxonomic groups across ecosystems have suggested that no single group can be used effectively to predict variation in the biodiversity of other taxonomic groups, thereby undermining the assumptions of indicator taxa as measures of overall ecosystem condition or function (Heino 2010; Mandelik et al. 2012). Moreover, many groups involved in the performance of key functions (e.g., microbial assemblages) are poorly captured in conventional assessments, as no simple, practical methods exist for their observation and quantification of their functional attributes.

Advances in wetland functional assessment depend on our ability to develop tools that can capture the trophic interactions, food web complexity/diversity, and biogeochemical processes that drive wetland health/condition. These tools should support routine application in a consistent and repeatable manner so that they can be easily incorporated into regulatory and management programs. They must be relatively simple and inexpensive to facilitate their application at scales that capture spatial and temporal patterns in wetland condition. In addition, they should be taxonomically and phylogenetically more 'complete', extending beyond a limited number of indicator taxa.

Wetland Ecogenomics

Existing and newly developed molecular tools provide promise for fulfilling the needs for "next generation wetland functional assessment." The use of standardized DNA sequence markers – DNA barcodes – has become a common, standard practice in many areas of biodiversity assessment (Hajibabaei et al. 2007a, 2007b). Customized, public databases of DNA barcodes and other marker gene sequences (e.g., BOLD, GenBank) contain representative DNA barcodes for hundreds of thousands of animal, plant, fungal, and microbial taxa. Comparison of DNA barcodes recovered from unidentified specimens can be used to provide species-level identification for a wide range of organisms. In addition to DNA barcode regions, molecular methods have also been employed to investigate functional gene regions of both prokaryotes and eukaryotes (Pujolar et al. 2012; Mason et al. 2012).

Next-generation sequencing (NGS) has been established as a powerful and practical means for generating millions of DNA sequences across broad phylogenetic groups from bulk environmental samples (Hajibabaei et al. 2011; Shokralla et al. 2012; Gibson et al. 2014; Shokralla et al. 2014). This use of NGS to extract DNA sequence data for biodiversity analysis from mixed environmental samples has been termed metasystematics (Hajibabaei 2012). The types of environmental samples that have been employed in metasystematic research include organisms such as benthic invertebrates (Hajibabaei et al. 2011), terrestrial insects (Gibson et al. 2014), and diatoms (Kermarrec et al. 2014).

Table 2: Potential ecogenomic approaches that could be used to assess various wetland functions

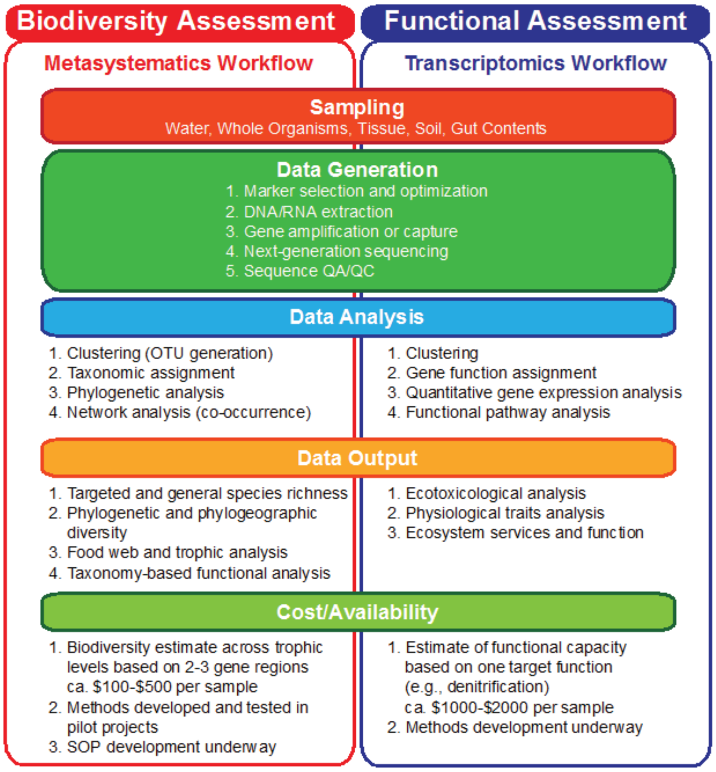
Wetland Functions (Smith et al. 1995, Novitsky et al. 1996)	Potential Wetland Ecogenomics Assessment Approach
Functions associated with Water Quality Improvement	
Removal/transformation of nutrients	Microbial diversity; functional gene and metatranscriptome expression
Removal of metals and toxic organics	Microbial diversity; active microbial community
Removal of sediment	Detection of presence of sediment tolerant or intolerant taxa
Functions associated with Habitat	
Habitat for plant communities	Plant diversity via tissues or propagules
Invertebrate species habitat	Invertebrate diversity
Vertebrate species habitat	Vertebrate diversity via tissue or eDNA
Maintenance of wildlife diversity and abundance	Pan-taxonomic diversity and/or phylogenetic diversity
Support primary production and export	Microbial and plant diversity
Functions associated with Hydrology/Water Quantity	
Reduction in peak flows	Detection of taxa adapted to specific flow or inundation conditions
Decrease in downstream erosion, sediment stabilization	Detection of presence of sediment tolerant or intolerant taxa
Maintenance of low flows to streams during dry season	Detection of taxa adapted to specific flow or inundation conditions
Ground water and aquifer recharge	

Other metasystematic studies have been able to extract DNA from fluids such as ethanol preservative used for sampling benthic invertebrates (Hajibabaei et al. 2012) and filtered water samples (Pilliod et al. 2013; Turner et al. 2014). The use of any of these sources of environmental DNA (or eDNA) includes extracting cellular-bound or exogenous DNA from a water or tissue sample as way of capturing information about resident organisms or processes occurring in a wetland.

The integration of NGS, eDNA, and metasystematics for wetland assessment we term "***wetland ecogenomics***." Wetland ecogenomics is already opening new avenues for wetland assessment by allowing for efficient assessment of multiple trophic levels, functional genes, and taxonomically comprehensive community composition as measures of wetland function. Example applications of wetland ecogenomics for wetland assessment include the following (Table 2):

- Evaluation of trophic complexity and food web energetics through reconstruction and observation of community composition across multiple trophic levels from primary producers to top consumers (Peralta et al. 2010).
- Overall biodiversity assessment (from microbes to mammals) through cataloguing richness and phylogenetic diversity in multiple taxa and functional guilds simultaneously.
- Development of advanced bioassessment tools through improved taxonomic resolution that allows identification of taxa sensitive to specific environmental stressors (e.g., Dafforn et al. 2014). This may also facilitate use of trait-based assessment through either species identification or direct measure of functional genes.
- Rapid detection of target species of interest, such as invasive species, species of primary management interest, or sensitive, rare, threatened, or endangered species (Zhan et al. 2013).
- Direct evaluation of the effects of changes in water quality. Microbial communities may be highly sensitive to even small fluxes of contaminants in the environment (Sims et al. 2013; Gardham et al. 2014).
- Measurement of the putatively active community (via RNA) and/or the ratio of relative abundance of RNA to DNA. Capture of the active microbial community using a 16S rRNA approach provides significant information about the potential activity of the microbial community in glacier-fed streams (Wilhelm et al. 2014). Such approaches could also be applied in other wetland communities, including phytoplankton and zooplankton.
- Measurement of ecosystem functions and biogeochemical process, such as denitrification, sulfate reduction, or methanogenesis and methanotropy. This may occur through either measurement of microbial communities responsible for these processes or through detection of functional genes that indicate the level to which these processes are occurring (Eaton et al. 2011, 2012).
- Linkage of high-resolution biodiversity information with stable isotope and observational data to delineate food web structure (e.g., Gray et al. 2014).

Figure 1: Wetland ecogenomics workflow for biodiversity and functional assessment



Future Prospects

Wetland ecogenomics provides a potential new frontier in assessment of wetland functions and biodiversity. A number of stages are necessary for the successful implementation of an ecogenomic approach to wetland research (Figure 1; Box 2). Further operational implementation of ecogenomic approaches will require additional development and testing of each of these stages. Based on Bohmann et al. 2014 and Rees et al. 2014, some specific technical issues that must be addressed include:

- Refining wetland sampling methods to be both field-efficient and DNA-friendly.
- Automating the bioinformatics process with tools and methods for consistent and easily applied data processing.
- Improving methods to minimize false positives and pseudo-absences.
- Developing approaches to recover biomass and/or abundance information from bulk DNA samples.
- Cataloguing and understanding the processes and variables that affect eDNA half-life and persistence in the environment.
- Improving our understanding of the dispersive properties of eDNA in various environments and partitioning between different environmental compartments (e.g., soil vs. water).
- Building and populating reference libraries to support assignment of taxonomic names to DNA sequences.

- Revising the conceptual approach to ecological reference definitions and index construction to incorporate the use of operational taxonomic units as opposed to (or in addition to) Linnaean taxonomy.

Wetlands have been consistently undervalued by society, and, arguably, this indicates a failure on the part of advocates of wetland conservation to present a convincing policy case for their protection, coupled with the reluctance of governments to implement international commitments for wetland conservation and wise use (Finlayson 2012). Part of the challenge in developing compelling policy arguments to support wetland protection is the sheer diversity of reasons motivating conservation practitioners, which are often in conflict. For example, wetlands are unique reservoirs of biodiversity, yet they are also valued for their ability to filter or otherwise retain contaminants in situ - yet it is unclear how these two conflicting goals can be easily reconciled (Baird et al. 1995). Clearly, we need to greatly improve our understanding both of the capacities of wetlands to regulate and support societal conservation and environmental management goals, while also protecting their ability to provide ecosystem services without significant degradation of their key ecological functions, and supporting biodiversity structure. The Ramsar Convention on Wetlands has developed structures and guidance for the wise use of wetlands in support of these goals (Finlayson et al. 2011), but the success of implementation measures at a national level is questionable (Finlayson 2012). Wetland ecogenomics can provide an appropriate set of tools to establish a new, systematic approach to wetlands functional assessment. This, in turn, offers the possibility to develop a more focused wetlands conservation research paradigm, where specific wetland management options can be evaluated using a knowledge-based framework, constructed on a more holistic understanding of how the various biodiversity components and their functions support key wetland functions and service. High-throughput genomics can provide access to rapid, dynamic information on the deep structure of wetland communities - particularly those areas of ‘dark diversity’ that until now have proved resistant to practical observation. What is even more exciting is the prospect of linking these structural observations to broad-scale observations of function at the assemblage level - made possible by functional transcriptomics. While these methods are currently in their infancy, their potential to revolutionize wetlands observation, and to support science-based policy for wetlands management is clear.

Box 2

Continuing the Wetland Ecogenomics Discussion at SWS 2015 in Providence

A series of events will be included in the program of the SWS Annual meeting to be held in Providence, RI, May 31-June 4, 2015. Please make plans to join us for both to continue our shared progress on the development of these technologies.

“Incorporating Genomic Technologies into Wetland Research”
Sunday, May 31st, 1-5pm
This workshop, conducted by the authors and other collaborators, will introduce participants to the essential components of a genomics-based wetlands research and monitoring program. The goal of the workshop will be to assist participants in operationalizing their own plans for genomic research and monitoring of wetlands. Please see the meeting website (swsannualmeeting.org) for registration and location details. Pre-registration will be required for this event.

“Monitoring and assessing changing wetlands using new genomic tools”
Date and time TBA
This symposium, co-chaired by the authors, will cover ongoing uses of genomic technologies to investigate changing wetland conditions. The talks included in this symposium will present results from research in wetland genomics, as conducted in wetland systems from the Arctic to the tropics. Participation in this event is included in your meeting registration. Please come to hear these fascinating talks and join in the discussion.

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