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eDNA as a tool for identifying freshwater species in sustainable forestry: a critical review and potential future applications

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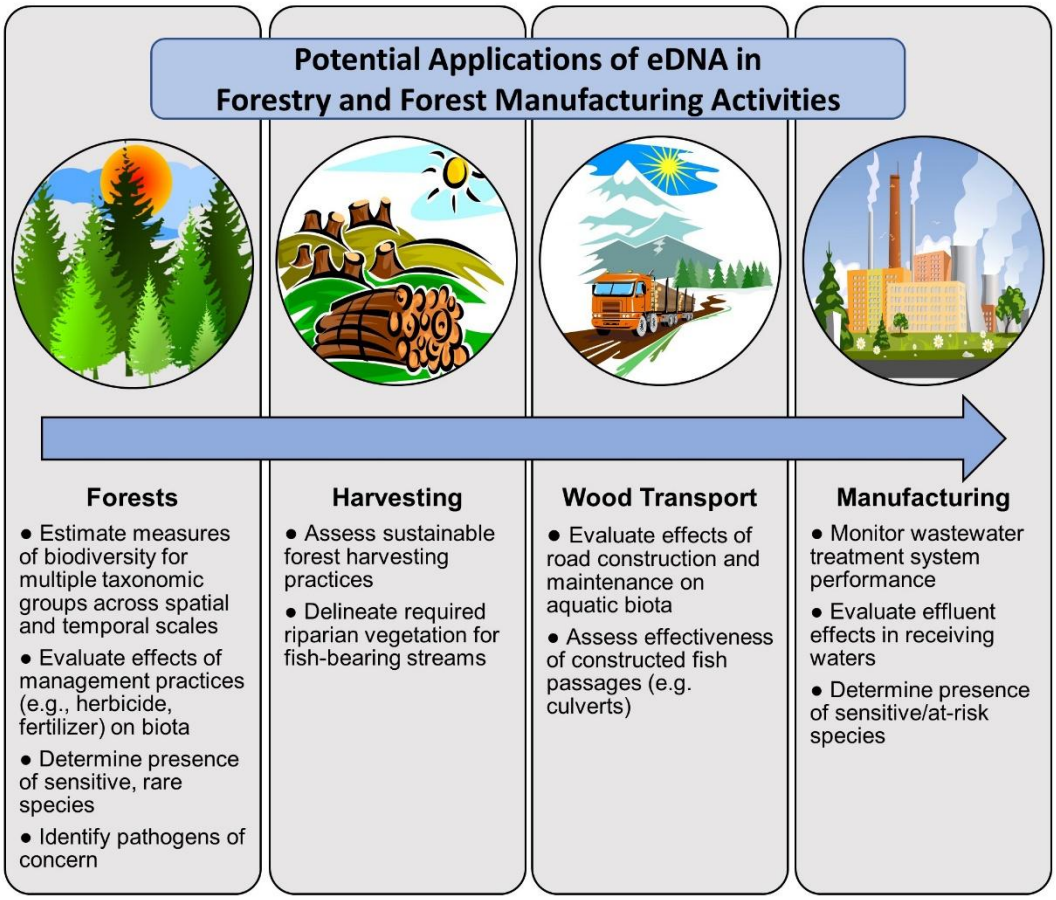
Abstract

Environmental DNA (eDNA) is an emerging biological monitoring tool that can aid in assessing the effects of forestry and forest manufacturing activities on biota. Monitoring taxa across broad spatial and temporal scales is necessary to ensure forest management and forest manufacturing activities meet their environmental goals of maintaining biodiversity. Our objectives are to describe potential applications of eDNA across the wood products supply chain extending from regenerating forests, harvesting, and wood transport, to manufacturing facilities, and to review the current state of the science in this context. To meet our second objective, we summarize the taxa examined with targeted (PCR, qPCR or ddPCR) or metagenomic eDNA

methods (eDNA metabarcoding), evaluate how estimated species richness compares between traditional field sampling and eDNA metabarcoding approaches, and compare the geographical representation of prior eDNA studies in freshwater ecosystems to global wood baskets. Potential applications of eDNA include evaluating the effects of forestry and forest manufacturing activities on aquatic biota, delineating fish-bearing versus non fish-bearing reaches, evaluating effectiveness of constructed road crossings for freshwater organism passage, and determining the presence of at-risk species. Studies using targeted eDNA approaches focused on fish, amphibians, and invertebrates, while metagenomic studies focused on fish, invertebrates, and microorganisms. Rare, threatened, or endangered species received the least attention in targeted eDNA research, but are arguably of greatest interest to sustainable forestry and forest manufacturing that seek to preserve freshwater biodiversity. Ultimately, using eDNA methods will enable forestry and forest manufacturing managers to have data-driven prioritization for conservation actions for all freshwater species.



Highlights

- eDNA can evaluate management effects on biota or delineate fish-bearing streams
- eDNA can monitor wastewater treatment performance and evaluate effluent effects
- Fish and invertebrates are well-represented by targeted and metagenomic eDNA studies
- Sensitive species are least studied with eDNA, but are important to forestry

Keywords: environmental DNA, metagenomics, biodiversity, pulp and paper

Introduction

Environmental DNA (eDNA) has been shown to be effective for identifying organisms from fresh water ecosystems, and shows promise for forestry and forest manufacturing managers to identify the presence of sensitive species, invasive species, pathogens, or to quantify biodiversity in natural or effluent waters. eDNA refers to any DNA that is collected from an environmental sample rather than directly from an organism, originating in cells from the body or waste products (saliva, urine, feces) of organisms (Taberlet et al., 2012). Estimating the presence of single-species using eDNA has been well-vetted in research (Bohmann et al. 2014; Deiner et al. 2017; Doi et al., 2017; Keck et al. 2017; Thomsen and Willerslev, 2015), and has many potential benefits including: achieving high detection probabilities for low abundance species, non-invasive sampling that may be particularly important for threatened or endangered species, reduced permitting requirements because organisms are not handled, sampling of locations that are unsafe or difficult to access with traditional methods, and identification of target organisms using uniform, reproducible criteria that are accurate over different life stages.

Despite the rapid expansion of techniques for identifying and quantifying eDNA in recent years (e.g., Deiner et al., 2017; Doi et al., 2017; Keck et al. 2017), limitations and challenges remain in field sampling, lab processing, and analyzing and interpreting results (Thomsen and Willerslev, 2015; Trebitz et al., 2017). These challenges include potential contamination of samples in the field or lab leading to false positive results, false negative results (e.g., inhibition of DNA amplification, field detection; Jane et al. 2015), occurrence of "zombie" DNA (detection of eDNA from dead, rather than live individuals), and difficulty in estimating species abundance or biomass (Thomsen and Willerslev, 2015; Trebitz et al., 2017). Currently, eDNA is used for identifying the presence of taxa over space and time, estimating species assemblages of a

specific environment, and estimating relative abundance of taxa. However, eDNA has not yet been broadly used as a management tool for industrial applications. To incorporate eDNA as an applied tool to address the environmental needs of the forest industry, forestry and forest manufacturing managers need access to the current state of the science for this rapidly-evolving technique and refined knowledge of the circumstances when eDNA can complement or replace traditional sampling approaches, evaluate logistics of obtaining eDNA results, and understand the limits of eDNA sampling.

Forests supply ecosystem services by protecting water supplies, providing erosion control, flood mitigation, and habitat conditions suitable for freshwater species (FAO, 2015). Freshwater biodiversity hotspots also are centered on regions with high forest cover (Abell et al., 2008; FAO, 2015; Mittermeier et al., 2015), yet freshwater biodiversity is declining globally mainly due to habitat degradation and declines in water quality (Hoffmann et al., 2010; Reid et al., 2013; Stuart et al., 2004). In the forest industry, each step along the supply chain from active land management, harvesting, and wood transport, to manufacturing, can potentially affect freshwater habitat and biodiversity. Primary concerns for freshwater habitat and biota due to forestry and forest manufacturing activities include the alteration of light, temperature, sediment, organic matter, flow regimes, aquatic organism passage, or water chemistry (e.g., effluent discharges, fertilizer, herbicide, or fire retardant; Cristan et al. 2016; Kovacs et al. 2005; Warrington et al. 2017). For example, pulp and paper mill wastewater discharged into natural waters, can increase organic matter (color) and conductivity (Hall et al, 2009), affect macroinvertebrate biomass and assemblages (Culp et al., 2000; Culp et al., 2003), or alter fish physiology (Hewitt et al., 2008) while harvesting and associated road building can increase water temperature (Brown and Krygier 1971), discharge (Bosch and Hewlett 1982), or sediment

92 delivery to streams (Croke and Hairsine 2006). Contemporary forest practices and water
93 treatment technologies are effective in reducing or eliminating many of these adverse effects
94 (Cristan et al., 2016; Flinders et al. 2009a-c; Martel et al., 2008; Warrington et al., 2017).
95 Nevertheless, cost-effective monitoring of species responses across space and time remains
96 essential to meet voluntary certification goals and environmental regulations that seek to
97 preserve biodiversity and freshwater resources.

98 Biotic monitoring priorities for forestry and forest manufacturing managers include at-
99 risk species (declining, threatened, or endangered), as well as fish and macroinvertebrate
100 assemblages because well-established biocriteria methods focus on these taxonomic groups
101 (Barbour et al. 1999; Karr 1981; Kerans and Karr 1994; Ziglio et al. 2006). Adherence to
102 biocriteria standards, whether voluntary or regulatory, includes the conservation of at-risk
103 species in forested streams or receiving waters (U.S. EPA 2010), and monitoring of
104 macroinvertebrate or fish assemblages as indicators of water quality (Environmental Canada
105 2010; Fortino et al. 2004; Walker et al. 2002). Furthermore, regulatory or voluntary best
106 management practices (BMPs) often rely on whether fish are present or absent in streams to
107 determine riparian management practices (e.g., how close harvest can occur to a stream; Cristan
108 et al. 2016; Warrington et al. 2017), and greater forest harvest restrictions can occur when at-risk
109 species are present (e.g., salmon, Steelhead, and Bull Trout streams in Oregon; Oregon
110 Department of Forestry 2018). Current field methods to monitor biota are often time-consuming
111 and labor-intensive, and their application can be limited by resources (in the collection and/or
112 analysis of samples), accessibility and permitting for sampling locations, and ability to
113 capture/quantify target organisms. As such, eDNA may be a useful tool for these and other
114 applications for forestry and forest manufacturing activities.

In this review, our primary objectives are to: 1) describe potential applications of eDNA as a tool for managers in forestry and wood product manufacturing and 2) review the current state of the science in this context. For objective 2, we also present a systematic review of studies that used eDNA from freshwater ecosystems to: identify the geographical representation of freshwater eDNA studies in the literature, summarize eDNA species targets using different analysis techniques (i.e. polymerase chain reaction (PCR), quantitative PCR (qPCR), or digital droplet PCR (ddPCR) (targeted eDNA methods), and evaluate how estimated taxa richness compares between traditional field approaches and eDNA techniques using metagenomic methods. Finally, given the rapid development and adoption of eDNA approaches, we summarize the geographic extent of prior eDNA sampling to aid managers in assessing whether eDNA methods have been developed for the geographic range of interest and to identify where gaps may overlap with forested landscapes

Potential applications for forest management and considerations for study designs

Biodiversity and biological monitoring of silvicultural and forest management activities

The conservation of biological diversity across landscapes is a central tenet of sustainable forest management, and developing effective and efficient tools to estimate species presence and species richness is critical for assessing whether forest practices achieve this goal. Within managed forest landscapes, freshwater systems (streams, rivers, wetlands) often serve as centers of biodiversity, yet many knowledge gaps remain regarding the effects of forest management on presence, distribution, and abundance of freshwater species. eDNA may be a useful tool to address a broad range of potential applications across forestry and manufacturing activities, although the limitations of this approach warrant consideration (Table 1).

Environmental effects of forestry activities and BMPs often are examined at small watershed scales (e.g., headwaters) where watersheds can be controlled and experimentally manipulated (Bateman et al., 2018; Gravelle et al., 2009; Stednick, 2008), but these scales may not be representative of the broader river network that is also influenced by upstream activities. Key species of concern, such as Salmonids, freshwater turtles, or aquatic salamanders, may occur downstream of forest management activities in larger streams or rivers. Monitoring biotic responses across broad areas and along longitudinal river networks, however, is often limited by sampling time, effort, and cost affiliated with traditional field techniques. For example, electrofishing or kicknetting to monitor fish and macroinvertebrates are feasible for small, shallow streams, but may be unsafe, difficult, or expensive in larger, non-wadeable, or remote rivers, which limits large-scale replication. Thus, sampling for eDNA may be particularly useful for estimating biodiversity of multiple taxonomic groups across spatial and temporal scales that are not feasible with traditional techniques, and facilitate increased spatial replication and sub-sampling. Further, developing accurate and contemporary geographic distributions for at-risk freshwater species ensures that policy decisions on conservation status are based on the best available science. As a complementary approach, eDNA may enhance the understanding of species distribution, but estimates of species presence do not provide other information that can be measured with an organism in hand (e.g., abundance, size, reproductive status, health assessments).

Sustainably managed forests provide a wide range of habitat conditions to support freshwater biodiversity (Johnson et al., 2016; Jones et al., 2010; O'Bryan et al., 2016; Richman et al., 2015) and protect water quality, but a direct link of species richness or persistence to implementation of forestry BMPs is lacking. For example, the southeastern United States is a

161 global biodiversity hotspot for fish, crayfish, amphibians, and reptiles, and this region coincides
162 with one of the largest wood baskets in the world (Jenkins et al., 2015). Sustainable forestry
163 certification programs, which cover 440.3 million hectares globally and have broad participation
164 in North America (51% of total certified forest area by regional share) (Kraxner et al., 2017),
165 include objectives to maintain and/or enhance biological diversity. However, data demonstrating
166 a positive influence of these objectives on biodiversity is lacking, due in part to high costs of
167 monitoring and small unrepresentative sampling sizes (Sheil et al., 2010). Increased
168 understanding of the hypothesized positive impact of voluntary, third-party sustainability
169 certification on freshwater biodiversity on managed forest land is critical to continual
170 improvement in standards and forest practices, and informing policy. Biodiversity objectives in
171 certification programs are adaptive and integrate new science. Thus, incorporating multispecies
172 eDNA approaches could provide essential data to assess effects of sustainable forest
173 management practices on freshwater biodiversity, advance knowledge of freshwater community
174 responses to sustainability certification, and improve management practices to achieve
175 biodiversity goals.

176 Evaluating biological responses to forest management using eDNA could focus on
177 diverse activities: forest harvest, herbicide application, fertilizer application, manipulation of
178 riparian vegetation, or road building and maintenance. An important consideration when using
179 eDNA in an experimental framework to evaluate large-scale manipulation responses must
180 consider how other environmental characteristics may be altered by forest management and how
181 these changes may influence eDNA results. For example, forest harvest has been shown to alter
182 discharge (Bosch and Hewlett, 1982), temperature (Brown and Krygier, 1971), light availability
183 (Kaylor et al., 2016), organic matter concentration (Cawley et al., 2014), and substrate (Scrivener

and Brownlee, 1989). In turn, these changes could affect the shedding or degradation rates of eDNA (Robson et al., 2016; Strickler et al., 2015) or longitudinal transport of eDNA (Jane et al., 2015; Wilcox et al. 2016). Additionally, the feasibility of eDNA as a tool to monitor biodiversity hotspots (e.g. southeastern U.S.) requires a clear understanding of eDNA's ability to classify resident taxonomic groups that include diverse taxa such as amphibians, reptiles, fish, and macroinvertebrates at multiple life stages. Further, much of the current eDNA research has been conducted in low-turbidity headwater streams or lakes. Slow-moving, high turbidity waters from riverine systems in the Gulf Coastal Plain of the southeastern U.S. present sampling challenges from long filtering times, and interactions between DNA, sediment, and filter media (Hinlo et al., 2017b; Williams et al., 2017).

Beyond conventional freshwater organisms, eDNA may also provide an effective means to identify the presence of plant or animal pathogens of concern (Catalá et al., 2015; Mohiuddin and Schellhorn, 2015). For *Phytophthora* species, a fungal pathogen of concern to forest industry and public forest lands, greater species diversity was identified with eDNA collected from streams and rivers (35 species) than from soil (13 species) (Catalá et al, 2015). Identifying pathogens in freshwater samples is beneficial due to the reduction in pre-processing procedure times as compared to soil samples (Catalá et al., 2015). In addition, multiplexed metabarcoding approaches can include screens for pathogen DNA as part of routine eDNA monitoring programs for fish, amphibians, or invertebrates. Other tree pathogens of concern, including foliar diseases (e.g., *Phaeocryptopus gaeumanni*), blister rust (e.g., *Cronartium ribicolais*) or root rots (e.g., *Phellinus pini*), can also be detected with eDNA methods. Likewise, pathogens that affect amphibians (Hall et al., 2015; Hartikainen et al., 2016; Huver et al., 2015; Mohiuddin and Schellhorn, 2015), reptiles, or fish (Carraro et al., 2017; Hartikainen et al., 2016; Mohiuddin and

Schellhorn, 2015) such as chrytrid fungus, ranavirus, snake fungal disease, or myoxozoans may all be detected using eDNA methods. Because early and widespread detection of pathogen presence can aid in minimizing their future impact, the use of eDNA to monitor the increasing threat of emerging infectious diseases affecting vegetation and wildlife is likely to expand significantly in the future.

eDNA as a tool for assessing riparian management

In some jurisdictions, the distance from a stream that forest management activities occur differs based on whether the stream is fish bearing or non-fish bearing. Similarly, BMPs and some regulations (e.g., Road Maintenance and Abandonment Plan; Washington, U.S.A) ensure improved road construction and maintenance on forested lands allow fish passage across forest roads via culverts, bridges, or other crossings. Accessible fish passage is particularly important for anadromous fish that migrate from freshwater streams to marine environments and then return to spawn. Several anadromous fish are federally listed under the US Endangered Species Act or the Committee on the Status of Endangered Wildlife in Canada (e.g., Coho Salmon or Chinook Salmon). Passage is also important for freshwater taxa of concern, including mussels with fish hosts, aquatic amphibians, or darters.

Currently, many forest managers rely on habitat-based delineations of fish habitat (e.g., presence of a fish-blocking waterfall, steep gradient) or field verification of fish presence with electro-shocking. Here, eDNA may also provide a powerful tool to document occupancy of fish species, to delineate the boundary between fish bearing and non-fish bearing reaches of a stream network, or to evaluate the effectiveness of upstream passage. eDNA techniques may be particularly effective for identifying the seasonal presence of spawning anadromous fish, which

may have the added benefit of informing protection and rehabilitation efforts for endangered anadramous species (e.g., Laramie et al. 2015). Others have shown that eDNA can be used to identify spawning sites for Mekong Giant Catfish (Eva et al., 2016), Bigheaded Carp (Erickson et al., 2016), Macquarie Perch (Bylemans et al., 2017), and to identify which salmon species constructed a given redd (Strobel et al., 2017). However, challenges in using eDNA approaches to determine anadromous fish passage may include differentiating eDNA between adults and young of the year residing in the stream, or the location of sampling. For example, sampling in the water column versus in interstitial spaces in sediment may be important in identifying spawning species (Strobel et al., 2017). Detecting the presence of fish in a water sample indicates that fish are present somewhere upstream of the collection point. However, because downstream distance traveled and eDNA detection can vary with discharge (Jane et al., 2015) and organism density (Pilliod et al., 2014), seasonal conditions in the stream system may be an important factor in interpreting eDNA results. Despite potential challenges in using eDNA approaches in forestry applications, a careful study design that considers the current state of knowledge of eDNA benefits and limitations will allow for achievement of management and research goals.

Potential applications for forest products manufacturers

Dischargers to natural waters

As dischargers of industrial wastewaters, eDNA approaches may be a valuable tool to augment or improve biomonitoring data collected by forest products manufacturers to comply with their discharge permit (regulated in the US through the National Pollutant Discharge Elimination System, NPDES). For example, water bodies that receive mill effluent are monitored

253 for changes in species assemblages as a response to treatment system upgrades (Kovacs et al.,
254 2003, 2010), studied to understand potential discharge-related effects to aquatic biota (Flinders et
255 al., 2009a-c), and evaluated to measure the response of process modifications on freshwater
256 assemblages (Burgess, 2015). Similarly, mills with temperature-related conditions in their
257 discharge permits (i.e. Section 316(a) variances) may also be required to confirm "balanced,
258 indigenous" biological populations associated with thermal discharges as mandated by the U.S.
259 Clean Water Act (e.g., Peredo-Alvarez et al., 2016). The ability of eDNA to detect numerous
260 species with a single sample may reduce the resources necessary to gather these data, which
261 often include multiple taxa groups. Additionally, mills may be required to demonstrate that no
262 sensitive species or vulnerable life stages occur near water intake structures or effluent
263 discharges. This may include freshwater mussels (which are the most endangered animals in the
264 US; Williams et al., 1993), and threatened/endangered fish that have specific thermal
265 requirements during early life stages (e.g., salmonids, sturgeon; Chapman and Carr, 1995; Sauter
266 et al., 2001). As a noninvasive method to document presence of rare and cryptic species, eDNA
267 may be a particularly valuable tool.

268 Because most U.S. pulp and paper mills discharge into large rivers (Strahler Stream
269 Order ≥6; NCASI data, unpublished) or impoundments, eDNA methods may be effective for
270 sampling water bodies where traditional techniques are logistically difficult or ineffective. For
271 example, bioassessment programs used by state and other agencies often evaluate fish,
272 macroinvertebrates, and/or periphyton (e.g., U.S. E.P.A.) using sampling protocols designed for
273 shallow streams (Barbour et al., 1999). Although agencies and researchers have developed
274 modified sampling protocols to address the logistic, safety, and data quality concerns associated
275 with sampling biota in deeper rivers (e.g., Di Sabatino et al., 2015; Flotemersch et al., 2006a, b;

Ultrup and Fisher, 2006), eDNA may be a more effective tool for obtaining these data. However, replacing traditional techniques with eDNA may not be feasible for dischargers requiring information on population structure such as biomass or relative abundance, which is a current limitation of eDNA (Table 1).

Monitoring efficiency and effectiveness of wastewater treatment

Mill personnel also may use eDNA to assess and monitor the efficiency and effectiveness of wastewater treatment in manufacturing operations. The treatment of wastewater produced by mills is an integral component for meeting water quality targets mandated by the Clean Water Act. A variety of engineering designs have been developed (aerated stabilization basins; activated sludge) to treat organic materials and other contaminants used in the manufacturing process. Regardless of process type, wastewater treatment relies on the biochemical activity of bacterial assemblages to reduce, remove, or transform suspended solids, and toxic compounds through oxidation or uptake for cellular process (e.g., growth, reproduction), all of which reduce biological oxygen demand (BOD). Historically, bacterial species comprising treatment systems assemblages were largely unknown, but increasing use of molecular techniques to identify bacterial assemblages may have applications as a monitoring, assessment, and diagnostic tool within the wastewater treatment systems.

The composition of bacterial assemblages in treatment systems and, by extension, system performance, is influenced by environmental conditions such as temperature, pH, dissolved oxygen, and nutrient concentrations, as well as the type and concentration of organic and inorganic compounds. Forest manufacturing managers often use metrics such as ammonia concentrations, BOD, and suspended solids to monitor performance, and deviation from metric targets may indicate system upset and reduced treatment efficiency. Troubleshooting the cause(s)

of treatment system underperformance in any wastewater treatment system can be challenging, and often relies on microscopic examination of treatment system water samples. Although this method can be informative, microbe identification is typically limited to those that are culturable on traditional media or have unique morphology, and this typically represents a fraction of bacteria present (Gilbride et al., 2006). Molecular techniques to characterize bacterial assemblage diversity, temporal variation, and functional roles and relationships to environmental conditions have improved wastewater treatment processes and optimization of system operations (e.g., Cydzik-Kwiatkowska and Zielińska, 2016; Forster et al., 2003; Moura et al., 2009). At present, comparatively little is known about microbial assemblages from pulp and paper mill treatment systems. Pulp and paper mill treatment systems have been examined using traditional microscopy (e.g., Fulthorpe et al., 1993; Liss and Allen, 1992). Molecular assessments derive from 'pre-genomics era' evaluations (Gillbride and Fulthorpe, 2004), and these show relatively consistent bacterial assemblages over time under normal operating conditions, with similarities in a fraction of the assemblage across mills even though treatment systems and processes differ.

The advancement of metagenomic eDNA analyses to develop baseline databases of treatment system bacteria and assemblage-condition relationships may offer a powerful approach for addressing treatment system challenges in wood products manufacturing facilities. For example, documenting treatment system bacterial assemblages under baseline and upset conditions (e.g., following an unintended release of spent pulping chemicals) may provide an early indication of a decrease in treatment system efficiency, and identify the source of treatment system upsets (e.g., presence of certain type of indicator bacteria for specific effluent constituents). This approach has been described for municipal wastewater treatment plant effluents to diagnose the source of a common treatment system upset (Rosso et al. 2018) and

could be expanded to develop operational decision trees for managing treatment system performance. While prior research focused on a single problem common to activated sludge aeration basins (foaming), the framework is applicable to other treatment system operation issues and has the potential to be tailored to address site-specific concerns. Examples of this include identifying the presence of organisms that may contribute to adverse outcomes in regulatory whole effluent toxicity assays (e.g., cyanobacteria), and validating the presence and/or tracking the source of positive enterococci indicator tests in treatment systems (e.g., Silva and Domingues, 2015).

Current state of the science in the context of forestry and forest manufacturing applications

Systematic review methods

We identified peer-reviewed publications for our review with Web of Science (https://login/webofknowledge.com; Clarivate Analytics, Philadelphia, PA, USA) and searched for "eDNA" and either: 1) "stream" 2) "river" 3) "wetland" 4) "pond" 5) "lake" 6) "freshwater" 7) "aquatic" in the topic field, which searches within the title, abstract, author keywords, and keywords plus. We supplemented our search by examining bibliographies of selected publications and citations of those with Google Scholar (https://scholar.google.com). For our analysis, we only included data from publications focused on eDNA collected from surface water in freshwater ecosystems, or on eDNA from a freshwater organism in an experimental system (e.g., mesocosm studies). We excluded eDNA studies from marine ecosystems and from sediment in freshwater, marine, or terrestrial ecosystems. The literature search was completed on

November 17, 2017 with the oldest citation being from 2005 and data were extracted from previously published manuscripts.

We categorized articles based on study design (literature review, laboratory experiment, field study, or mesocosm). Our synthesis focuses on using eDNA to understand biological and ecological responses and does not synthesize laboratory procedures and methodology, which have been the subject of previous reviews (e.g., Creer et al., 2016; Diaz-Ferguson and Moyer, 2014; Goldberg et al., 2015; Goldberg et al., 2016). Thus, we excluded studies that solely examined laboratory methods, and only included publications that incorporated environmental sampling (lab + environment). We included field studies that sampled freshwater systems across time, space, location, or compared eDNA methods to traditional sampling techniques to gain knowledge about species in natural habitat types. Mesocosm studies included experiments conducted in containers to simulate lentic or lotic freshwater environments.

Freshwater eDNA generally is analyzed by collecting water samples (usually 500mL to 5L), filtering samples to capture fine particles and cells (pore sizes of 0.45µm to 5 µm), extracting DNA from the captured material, and testing the DNA for the presence of one or a few species of interest (targeted eDNA) or for all representatives of broad taxonomic or functional groups (e.g., teleost fish, Chironomidae, zooplankton) using eDNA metabarcoding. There are multiple eDNA methods, each with varying taxonomic resolution, that can be used to address a variety of management objectives including: qPCR, ddPCR, metabarcoding, multiplex metabarcoding, and shotgun sequencing (Table 2). qPCR and ddPCR methods amplify a region of DNA from a target species (or group of closely related species) and measure the amount of amplified DNA produced, usually through the use of a fluorescent reporting molecule. Metabarcoding methods amplify an informative region of DNA from a target taxonomic group,

and the amplified fragments are then sequenced. Based on its sequence, each fragment is classified against a reference database to determine which member of the taxonomic group it came from. Multiplex metabarcoding methods allow for the simultaneous measurement of multiple DNA targets and multiple samples. Shotgun sequencing attempts to directly sequence the DNA fragments obtained from the environmental sample, which in most environments will be dominated by bacterial and viral genomes. Multiplex metabarcoding and shotgun sequencing for macrofauna are still in early stages of development.

To quantify which species were the focus of targeted eDNA approaches, we categorized species into one of three groups: (1) invasive or nonnative, (2) rare but not invasive or nonnative, threatened or endangered, or (3) common, native but not rare or invasive, or unspecified based on descriptions and location of the study. Species were not dually classified. Finally, we examined the subset of literature using metagenomic techniques, and quantified the number of studies focused on taxonomic groups. To compare estimated taxa richness between traditional field approaches and eDNA techniques using metagenomic methods, we extracted data from 8 studies for fish and 6 studies for invertebrates (Supplemental Table 1). To determine the difference between taxa richness we subtracted taxa richness of traditional field methods (single year) from taxa richness determined from eDNA methods (single year) for each site. Then the difference across sites was determined to examine the overall effect sizes. Similarly, historical taxa richness (multiple years) was subtracted from eDNA richness or traditional field method richness (single year) for each site.

We included all studies (except for review articles) with geographical locations to determine the global representation of eDNA research and how they relate to global wood baskets. Global production of forest products in 2016 were obtained from the United Nations'

Food and Agriculture Organization (FAO) (http://www.fao.org/forestry/statistics/80938/en/) and were displayed as a percentage of global production by country. Production was separated into two groups with wood representing the sum of production of roundwood, sawnwood, and wood based panels, and pulp and paper representing the sum of pulp, paper, and pellet products.

Review of the current state of the science

Prior to implementing eDNA into applications for forestry and forest manufacturing, managers must understand how species ecology and environmental factors may affect interpretation and detection of eDNA and utilize prior information to develop study designs that meet monitoring objectives. In particular, understanding the interplay among forestry activities and environmental conditions that affect eDNA detection, transport, or degradation is critical. Here, we review the literature in this context to aid managers in designing robust studies based on the current state of knowledge of eDNA detection and we integrate the results of our critical review into this discussion. Based on our review criteria, we identified 214 peer-reviewed publications focused on freshwater eDNA, including 21 review articles (Figure 1) and 193 studies; an additional 10 opinion articles or replies to editors were identified, but excluded (Supplemental Table 1; Supplemental Figure 1).

Our review of 163 studies using targeted eDNA approaches demonstrates that rare, threatened, or endangered species have received the least research focus overall (Figure 2a), but are likely of greater interest for forestry and manufacturing professionals because management activities seek to provide adequate protections for species of greatest conservation concern. In contrast, invasive and nonnative species of fish, invertebrates, reptiles, and aquatic vegetation received the most attention (Figure 2a). Collectively, these publications targeted 157 species with

the primary focus on fish (46%), invertebrates (19%), and amphibians (14%) (Figure 2a; Supplemental Figure 2). Seven species (6 fish species, 1 amphibian species) had >1 classification status. For example, depending on the location of the study, Brown Trout (*Salmo trutta*) was either classified as an invasive species (Carim et al., 2016c; Clusa et al., 2017) or native, but not rare or invasive (Gustavson et al., 2015). However, within a single study an organism was not given dual classification (e.g., threatened species were not also included as native). Thus, a total of 157 species were identified, while dual classification allows for Figure 2a to depict 164 species (Supplemental Table 2). Only 40 species were targeted in more than one study and the remaining 117 species were limited to individual studies.

eDNA persistence and water temperature

eDNA from lentic and lotic ecosystems show a wide range of degradation rates that can vary with temperature, UV exposure, pH, microbial communities, or trophic state (Barnes et al., 2014; Eichmiller et al., 2016; Lance et al., 2017; Maruyama et al., 2014; Strickler et al., 2015; Tsuji et al., 2017). The range of eDNA half-lives reported in prior studies extend from as short as 2.8 hours (0.12 days) for Ayu Sweetfish (*Plecoglossus altivelis altivelis*) and Common Carp (*Cyprinus carpio*) when incubated at 30°C (Tsuji et al., 2017) to 48.7 to 332.6 hours (6.8 to 46 days) for American Bullfrog (*Lithobates catesbeianus*) incubated at a range from 5 to 35°C (Strickler et al., 2015). A wide range in degradation rates have also been reported for a single species. For Common Carp, eDNA half-lives ranged from 2.8 hours to 20.5 hours when exposed to different environmental conditions, but at temperatures of 20 or 25°C half-lives were restricted to ~5 and 7 hours across studies (Eichmiller et al., 2016; Strickler et al., 2015; Tsuji et al., 2017).

In lentic ecosystems, eDNA detection is considered to reflect relatively current species assemblages because of the short persistence of eDNA typically lasting from 4 days to a month

436 (Barnes et al., 2014; Dejean et al., 2011; Huver et al., 2015; Piaggio et al., 2014; Thomsen et al.,
437 2012). eDNA was detectable for as few as 4 days for Burmese Python (*Python bivittatus*) and
438 Common Carp, 1 to 2 weeks for amphibians, 3 weeks for the trematode *Ribeiroia ondatrae*, and
439 up to one month for freshwater vertebrates (Barnes et al. 2014; Dejean et al., 2011; Huver et al.,
440 2015; Piaggio et al., 2014; Thomsen et al., 2012). Studies of fish carcasses have found eDNA
441 was detectable > 1 month for Bigheaded Carp (*Hypophthalmichthys molitrix* and *H. nobilis*)
442 (Merkes et al., 2014) and > 35 days but <70 days for Northern Pike (*Esox lucius*) (Dunker et al.,
443 2016). Given the range in the persistence in eDNA, study designs that incorporate temporal
444 eDNA sampling from manufacturing holding ponds or from natural ponds or lakes should
445 carefully consider the sampling intervals and inferences regarding species presence in relation to
446 eDNA degradation.

447 Interpreting patterns of eDNA in lotic systems necessitates an understanding of factors
448 affecting eDNA transport in flowing water. The downstream distance that eDNA is detected
449 varies with flow (Jane et al., 2015) and substrate (Shogren et al., 2017), and may also vary by
450 species (Jerde et al., 2016; Shogren et al., 2017) and density (Pilliod et al., 2014). However, the
451 upper limit of transport distance is likely on the order of kilometers (Civade et al., 2016; Deiner
452 and Altermatt, 2014; Jane et al., 2015; Sansom and Sassoubre, 2017). For example, eDNA was
453 detected at 0.24 km (greatest distance sampled) for Brook Trout (Jane et al., 2015), 0.96 km for
454 Atlantic Salmon (Balasingham et al., 2016), and 2 -3 km for various freshwater fish (Civade et
455 al., 2016). Transport distance of freshwater mussel eDNA was even greater with up to 10 km for
456 *Unio tumidus* (Deiner and Altermatt, 2014), and 4.3-36.7 km for *Lampsilis siliquoidea* (Sansom
457 and Sassoubre, 2017). In additional to abiotic factors, species density may also affect

downstream transport of eDNA, with higher densities of species leading to detections further from the source (Pilliod et al. 2014).

Both flow and substrate have been shown to influence the distance downstream that eDNA is detected. eDNA counts monitored downstream from caged fish declined with increasing distance at the lowest flows, yet remained elevated under high flow conditions (Jane et al., 2015) suggesting that eDNA travels greater distances under elevated discharge. Using Common Carp eDNA in a series of experiments designed to quantify transport, retention, and resuspension rates and distances, Shogren et al. (2017) found that a finer, homogenous substrate removed eDNA more quickly, resulting in shorter transport distances than cobble. However, a similar experiment using Largemouth Bass (*Micropterus salmoides*) and Bluegill (*Lepomis macrochirus*) eDNA showed no difference in eDNA transport with substrate type (Jerde et al., 2016). Increased runoff and stream discharge (Andreassian, 2004; Abdelnour et al., 2011; Bosch and Hewlett, 1982; Surfleet and Skaugset, 2013) and changes in substrate composition (Scrivener and Brownlee, 1989) may occur following forest harvest or other management activities. Limited information exists on the scope or magnitude of forest management activities necessary to affect eDNA transport, but improved understanding of the potential for these variables to affect downstream transport of specific species will be important when interpreting differences in eDNA due to forestry activities.

Water temperature may affect the shedding of eDNA from organisms (Robson et al., 2016), and thus the availability of eDNA for detection (Strickler et al., 2014). This may be relevant to consider in forestry applications because stream temperatures may exhibit a small short-term increase after forest harvest, but these are typically minimized by incorporating riparian buffers of unharvested trees next to streams (Brown and Krygier, 1971). In a study of

Mozambique Tilapia with three temperature regimes (23, 29, and 35°C), more DNA was shed into the environment at 35°C than the lower temperatures, and resulted in a longer duration of eDNA detection (Robson et al., 2016). The authors suggested that the higher shedding rate at 35°C may be due to increased metabolism or thermal stress. However, studies examining similar temperature ranges for Bigheaded (Klymus et al., 2015) and Common Carp (Takahara et al., 2012), and a much narrower temperature range (<2°C) for a multi-species assemblage (Seymour et al., 2018), did not find a temperature-related difference in eDNA shedding. Although it is unlikely finer scale differences in temperature, such as that expected from an adjacent forest harvest, might influence eDNA shedding and subsequent detectability, more information is needed.

Temperature can also affect degradation rates of eDNA with greater rates observed at warmer temperatures (Eichmiller et al., 2016; Tsuji et al., 2017 but see Robson et al., 2016). At 5°C, degradation rates of bullfrog and common carp eDNA were significantly lower than at temperatures of 20°C and 35°C (Strickler et al., 2015) or 15°C, 25°C, or 35°C (Eichmiller et al., 2016). These studies suggest that slight increases in temperature due to forest harvest may have minimal effect on eDNA degradation rates, but that larger seasonal changes between winter and summer temperatures could have a pronounced effect. Forest harvest increases light availability onto surface waters, and this could increase eDNA degradation rates due to increased exposure to ultraviolet radiation (e.g., Strickler et al., 2015).

eDNA and trophic state, microbial communities and organic matter

Trophic state and microbial community composition can influence eDNA degradation rates. Bacteria use DNA as a food source, enhancing its degradation (Finkel and Kolter, 2001), and dissolved organic matter (DOM) can bind DNA protecting it from degradation (Saunders et

al., 2009; Stotzky, 2000). Because the microbes responsible are often nutrient limited, the nutrient status of an ecosystem can influence the breakdown of DOM. Increases in microbial load or changes in microbial assemblage can increase eDNA degradation rates (Lance et al., 2017) and may explain why eDNA has been observed to breakdown more rapidly in natural systems than in mesocosms, or when natural pond water is added to mesocosms (Dejean et al., 2011; Lance et al., 2017). eDNA decay rates measured across different lake trophic states were greatest in oligotrophic (low nutrient availability; eDNA half-life = 7.1 hours) and eutrophic (high nutrient availability; eDNA half-life = 9.8 hours) lakes, and lowest in dystrophic (high DOC concentration; eDNA half-life = 25.2 hours) lakes and well water (eDNA half-life = 20.0 hours; Eichmiller et al., 2016). In another study, relatively small variations in nitrogen concentration were not significantly related to eDNA degradation rates (Seymour et al., 2018). Collectively, these studies suggest that the quantity of DOM rather than the quantity of nutrients may influence eDNA degradation.

Additionally, eDNA degradation rates and PCR inhibition can be greater in the presence of organic matter (Jane et al., 2015) or under acidic environments (Strickler et al., 2015; Seymour et al., 2018), although there are mixed results in the literature on the effect of pH on eDNA degradation (Lance et al., 2017; Seymour et al., 2018; Strickler et al., 2015). Strickler et al. (2015) found that pH was most influential on eDNA decay via interactions with other environmental variables such as temperature and ultraviolet radiation. Lance et al. (2017) noted that pH had a relatively minor effect on eDNA degradation rates in their study, but reported less eDNA degradation at low (pH = 6.5; eDNA half-life = 96 hours) than at high pH (pH = 8; eDNA half-life = 62 hours). In contrast, Seymour et al. (2018) found that acidic environments increased eDNA degradation.

DOM concentrations and composition in surface waters can change with forestry activities (Cawley et al., 2014; Eckley et al., 2018; Lee and Lajtha, 2016; Yamashita et al., 2011) or as a result of DOM or pH changes following treatment in mills. Although effluent is treated to meet specific water quality targets (e.g., color) prior to release in natural waters, changes in DOM concentration may still be an important consideration for monitoring with eDNA. Nutrient concentrations, particularly nitrate, may increase following forest harvesting (Gravelle et al., 2009), but nutrients do not appear to have a major impact on eDNA degradation rates (Eichmiller et al., 2016; Seymour et al., 2018). The interactions of other environmental factors including DOC concentration, pH, microbial load, or temperature can clearly influence eDNA degradation rates. Incorporating eDNA methods into environments with high concentrations of organic matter (i.e. in wetlands, fluvial systems in the southeastern US, during leaf fall, or in mill effluent) should consider the potential impacts on the residence time of eDNA in the system, and account for these environmental changes in study designs.

Comparisons of eDNA and traditional field sampling techniques

Most studies have found that eDNA approaches are comparable to, or more effective than, traditional techniques in determining presence or absence of targeted species, particularly when species are present in low abundances (e.g., Biggs et al., 2015; Boothyroyd et al., 2016; Dejean et al., 2012; Doi et al., 2017; Mächler et al., 2014; Matuhashi et al., 2016; McKelvey et al., 2016; Pierson et al., 2016; Pilliod et al., 2013; Smart et al., 2015; Smart et al., 2016; Wilcox et al., 2016). Traditional survey methods led to greater detection rates than eDNA methods for Gizzard Shad (*Dorosoma cepedianum*), Largemouth Bass, and Bluehead Suckers (*Catostomus discobolus* and *C. discobolus yarrow*) (Perez et al., 2017; Ulibarri et al., 2017), but eDNA and traditional methods led to divergent results for Redswamp Crayfish (*Procambarus clarkii*)

(Tréguier et al., 2014). While eDNA is generally comparable to traditional techniques for species detection, some researchers recommend eDNA as a complementary sampling approach to expand the spatial distribution of surveys (Hinlo et al., 2017a; Lim et al., 2016; Machler et al., 2014).

Metabarcoding may be particularly useful for understanding the effects of forest practices on freshwater biodiversity because of its potential to provide estimates of taxa richness from a single sampling technique. However, few studies have compared metabarcoding eDNA approaches with traditional methods relative to targeted eDNA approaches, which have been well vetted. We found that metabarcoding approaches, where high-throughput DNA sequencing occurs simultaneously for multiple taxa, were applied in 34 publications (4 of these studies also used targeted approaches; Supplemental Table 1), with nearly half of those studies published in 2017. Most metabarcoding studies examined microorganisms (n=12), invertebrates (n=12), fish (n=11), or amphibians (n=6) (Figure 2b). Mammals (n=2), reptiles (n=1), and birds (n=1) were also identified using metabarcoding approaches. Twenty-three metabarcoding studies included samples collected from lotic ecosystems and 18 included samples from lentic ecosystems.

In our review of metabarcoding approaches, estimates of taxa richness (categorized to lowest taxonomic level - species, genera, or family) within a single year were qualitatively greater for fish (8 studies, 67 sites) using eDNA methods than traditional methods (gillnetting, beach seining, or electrofishing) but not for invertebrates (6 studies, 88 sites) (Figure 3a; Supplemental Table 1). Taxa richness based on comprehensive historical species lists were greater than single-year datasets regardless of sampling method (Figure 3b). However, single-year eDNA techniques performed better than single-year traditional field sampling methods for fish but not invertebrate taxa richness, with eDNA typically identifying 10 fewer fish taxa than

historical records compared to 15 fewer using traditional methods (Figure 3b). The number of study sites for comprehensive historical species record comparisons for invertebrates (n =6) was much lower than for fish (n = 16), and may bias the observed differences. While eDNA and traditional sampling methods were generally comparable in estimating taxa richness in freshwater ecosystems, ultimately the estimation of taxa richness by metagenomic techniques is limited by the reference database, because taxa not represented in the database cannot be identified to species using operational taxonomic units (OTU) (Elbrecht et al., 2017a, Yang et al., 2017). The paucity of studies on amphibians and reptiles prevented us from evaluating the effectiveness of eDNA metabarcoding with traditional methods, although others have found eDNA detection was effective for amphibians and reptiles (Lacoursiere-Roussel, 2016a; Valentini et al. 2016). eDNA metabarcoding is a promising tool for estimating freshwater biodiversity responses to forest practices and release of mill effluent into natural receiving waters, particularly as reference databases expand and methods are refined.

Estimation of species abundance and biomass using eDNA

While eDNA has been shown to be particularly effective in estimating presence or absence, there is great interest in using eDNA to estimate relative abundance or biomass. Numerous studies across a range of taxa have found positive correlations between eDNA concentration and species abundance (Baldigo et al., 2017; Doi et al., 2015; Doi et al., 2017; Goldberg et al., 2013; Pilliod et al., 2013; Sansom and Sassoubre, 2017; Secondi et al., 2016; Thomsen et al., 2012; Wilcox et al., 2016; Baldigo et al., 2017; Doi et al., 2017; Sansom and Sassoubre, 2017) or biomass (Baldigo et al., 2017; Doi et al., 2015; Doi et al., 2017; Jane et al., 2015; Lacoursiere-Roussel et al., 2016a, b; Matuhashi et al., 2016; Piggot et al., 2016; Pilliod et al., 2013; Takahara et al., 2012). Most previous studies used qPCR approaches, but in a method

596 comparison Doi et al. (2015) found that ddPCR provided better estimates for abundance and
597 biomass than qPCR. A few studies found poor relationships between eDNA concentration and
598 abundance or biomass, including for Eastern Hellbender, Great Crested Newt, Rusty Crayfish,
599 Gizzard Shad, and Largemouth Bass using qPCR (Biggs et al., 2015; Dougherty et al., 2016;
600 Perez et al., 2017; Spear et al., 2015) and no correlation was found for the Round Goby using a
601 PCR assay approach (Adrian-Kalchhauser and Burkhardt-Hom, 2016). With targeted eDNA
602 approaches (qPCR or ddPCR), site-specific relationships need to be established to estimate how
603 eDNA concentration relates to abundance or biomass for taxa of interest. Understanding the age
604 structure of a population is also important to ensure biomass is not overestimated because eDNA
605 release rate standardized to fish body weight was greater for juveniles than adults (Maruyama et
606 al., 2014). For studies that require the abundance or biomass of a specific organism, traditional
607 techniques need to complement eDNA approaches, and may be useful in establishing site-
608 specific relationships between eDNA and population biomass or density.
609 Metabarcoding read counts have also been examined for relationships with species
610 abundance or biomass with some finding poor or modest positive relationships based on read
611 counts (Bista et al., 2017; Elbrecht et al., 2017a; Evans et al., 2016; Lim et al., 2016; Yang et al.,
612 2017) or ranked read count (Hanfling et al., 2016). However, authors are cautious in their
613 interpretation of these data because, in addition to the considerations listed above for targeted
614 eDNA approaches, multiple quantitative biases in metabarcoding data limit its ability to quantify
615 taxon abundance. A primary concern is primer bias, which is differential amplification of a locus
616 among species targeted by the same primer pair (Elbrecht and Leese, 2015; Leray and Knowlton,
617 2015; Piñol et al., 2015). Sequence abundance may also be related to the biomass of different
618 taxa (Elbrecht et al., 2017b) further complicating interpretation of relationships between

sequence abundance and species abundance or biomass. Additionally, eDNA from different taxa may behave differently at any point in the process from its release into the environment until it is finally sequenced (e.g., differing rates of release, degradation, or capture by and extraction from filters), so that each taxon has a unique relationship between sequence abundance and species abundance or biomass. These relationships may also vary by site or by season.

Conclusions for incorporating eDNA into forestry and forest manufacturing

Given the important role of prior development of primers and bioinformatics for a given ecoregion in facilitating use of eDNA methods by managers, it is essential to understand the geographic scope of prior eDNA studies, and how these relate to the geographic distribution of global wood baskets. We found that the global distribution of eDNA studies focused on freshwater ecosystems (n=188) were conducted primarily in North America (51% of studies), Europe (25%), and Asia (15%) with less representation in Australia (6.4%), South America (1.6%), Africa or Antarctica (0.5% each) (Figure 4). By country, most freshwater research using eDNA methods occurred in the USA (44% of studies), followed by Japan (12%), Canada (7.4%), Australia (6.4%), and the UK (6.4%) (Figure 4). We found there was considerable overlap in countries that are major wood-commodity producers with countries focused on eDNA development including the USA, Canada, and Japan (Figure 4). Implementation of eDNA methods in other major wood-commodity producing countries (e.g., Brazil, India, Russia, South Korea, Congo, Ethiopia, and Nigeria) is currently limited (Figure 4). Where overlap exists, forestry and forest manufacturing managers can utilize existing primer development and eDNA methods to integrate eDNA methods into monitoring and research studies, but elsewhere use of these methods may be more limited.

642 Method cost comparisons are an important consideration for long-term monitoring of any
643 study. Previous cost comparisons focused on targeted eDNA approaches suggest that eDNA can
644 be more cost effective than triple pass electrofishing for a single species of fish (Evans et al.,
645 2017), and vastly less expensive than traditional techniques for species of turtles, fish, and
646 parasites (Davy et al., 2015; Huver et al., 2015; Sigsgaard et al., 2015). However, eDNA is not
647 always the most cost effective, and the costs will depend on the initial effort required to establish
648 a genetic database and resources (primers or probe development, specimen collection,
649 vouchering), sample processing, the method used for eDNA analysis (e.g., single target qPCR or
650 multitarget metabarcoding), and the intensity and type of traditional field sampling technique
651 used (e.g., triple pass vs. single pass electrofishing for fish) (Evans et al., 2017; Smart et al.,
652 2016). Metabarcoding and other multi-species eDNA methods are relatively new techniques, and
653 while their per-sample costs are less well-defined, they are expected to be considerably higher
654 than traditional quantitative PCR methods (qPCR, ddPCR) due to the higher per-sample cost of
655 DNA sequencing. In some cases, this drawback will be outweighed by the large number of
656 target species that can be simultaneously evaluated, as the cost per target taxon will be
657 significantly lower with metabarcoding methods. Few studies have provided a detailed cost
658 analysis of multi-species eDNA approaches, but Elbrecht et al. (2017a) reported that the cost of
659 eDNA metabarcoding was comparable to morphology-based monitoring for macroinvertebrates.
660 Currently, incorporating eDNA techniques into monitoring, experimental studies, or other
661 applications requires collaboration with researchers that have laboratories to develop primers and
662 process eDNA samples, access to expensive instrumentation (e.g., qPCR machines or massively-
663 parallel sequencers), and a computational infrastructure capable of modern bioinformatics
664 analysis (in the case of multi-species approaches). Such collaborations are typically developed

665 with researchers at academic institutions or government agencies (e.g., US Forest Service, US
666 Geological Survey, state natural resource agencies), and can involve varying levels of
667 complexity (Table 2). Selection of the type of eDNA method depends upon the number of
668 species to identify (one versus many), and whether quantitative data (to estimate abundance) or
669 genetic diversity estimates are a goal for forestry and forest manufacturing managers (Table 2).
670 As demand for eDNA monitoring increases, commercial genotyping and genome sequencing
671 laboratories are likely to develop eDNA services, but the ability to completely outsource this
672 work depends on the eDNA method selected (Table 2).

673 In the future, two developments are likely to make eDNA studies more flexible,
674 affordable, and powerful:

675 (1) First, *miniaturization* has resulted in the development of portable field instruments
676 that can amplify, screen, and even sequence eDNA in remote settings (Russell et al.,
677 2018). Handheld qPCR devices like the 'Biomeme two3' (Biomeme, Inc.,
678 Philadelphia, PA, USA) have already been developed to detect the presence of up to 3
679 target species in the field. While target species are currently limited (Coho Salmon,
680 Atlantic Salmon, Brook Trout, etc.; Biomeme eDNA test kits, Smith-root Inc.,
681 Vancouver, WA, USA), further development of this technology could produce a
682 powerful tool for real-time detection of select species in forestry applications.
683 Portable PCR machines can be combined with newly-developed nanopore DNA
684 sequencers, such as the 'MinION' (Oxford Nanopore Technologies, Oxford, England;
685 Loman and Watson, 2015), to provide rapid detection of a broad spectrum of DNA
686 sequences. These cell phone-sized devices have the capacity to serve as rapid-

687 detection devices *and* fully-functional sequencers, giving them extra capabilities of de
688 novo sequence discovery and database improvement.
689 (2) Second, *data accumulation* from metabarcoding studies will make it possible to
690 identify and screen diagnostic sequences using genetic assays that are simpler to
691 execute and interpret. Assays used for routine genetic analysis of cattle breeds or crop
692 plant management (such as mass spectroscopy-based methods; Ragoussis, 2009) are
693 flexible, accurate and easily outsourced to commercial facilities. Adapting these
694 methods to eDNA applications would do much to 'democratize eDNA', making it
695 possible for end-users with diverse interests to adapt the power of genomics to their
696 own interests and applications.
697 Linking these new technologies with traditional field methods used to estimate population
698 structure, abundance, biomass, or condition of individuals will do much to enhance the
699 usefulness of eDNA as a tool for numerous forestry and forest manufacturing applications that
700 seek to better understand and predict their impacts on the environment.
701
702 **Competing interests**
703 We have no competing interests to declare.

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List of Figures

are shown. Production is separated into two groups: Wood represents the sum of production of roundwood, sawnwood, and wood based panels (green) and Pulp and paper represents the sum of pulp, paper, and pellet products (red). Both are displayed as a percentage of global production.

Table 1. Potential benefits and limitations of using environmental DNA techniques in forestry and forest manufacturing research and monitoring of freshwater systems.

Potential benefits	**Potential limitations**
Sampling numerous species with a single technique	Initial costs and time to develop primers and genomic library
Increased sample sizes and geographic breadth of sampling with single approach	Does not provide information on population structure (biomass, abundance, reproductive status, or health)
Field sampling requires limited training, no animal handling permits, and single set of equipment. Ease of sampling could allow for increased public engagement via community science campaigns that facilitate sampling of broad spatial areas.	Potential for field and lab contamination or zombie DNA leading to false positives, or misinterpretation of data.
	Limited information on how environmental metrics that may vary with forestry or manufacturing activities (e.g., temperature, UV radiation, streamflow, trophic state) affect DNA persistence and detectability
Noninvasive method to document presence, abundance, and genetic diversity of common, rare, and cryptic species	Positive control tissue samples may be difficult to obtain for rare species and obtain limited information on reproductive status, health, morphology, or age of individuals
Genomic library builds upon itself and may reduce long-term costs	Meta-barcoding approach requires developing data pipeline and bioinformatics
Well-suited to occupancy analysis framework	

Table 2. Summary of typical analyses and expected taxonomic resolutions for different eDNA methods, and potential for addressing different management objectives. Methods run from lowest complexity (qPCR) on the left to highest complexity (shotgun DNA sequencing) on the right, and include considerations for the number of species resolved, requirements for the assay, and potential for outsourcing. An assay is characterized as quantitative if it has the potential of correlating signal strength with the abundance of target molecules in the sample provided for the assay; see the text for discussion of why the target DNA abundance in the assay may be decoupled from the abundance of the target organism in the environment. (qPCR = quantitative PCR, ddPCR = digital droplet PCR)

Target species quantity	1	2 - several		Many (10s-100s)	
Methods available	qPCR	ddPCR	metabarcoding	Multiplex metabarcoding	Shotgun sequencing
Detection method	DNA fluorescence	DNA fluorescence + flow cytometry	DNA sequencing	DNA sequencing	DNA sequencing
Quantitative?	Y	Y	Y	Y	?
Genetic diversity?	N	N	Y	Y	Y
PCR-bias?	Low-high	Low	Low-high	Low-high	None
Information required to design assay?	High	High	Medium	Medium	Low
Complexity bioinformatics?	Low	Low	Medium	Medium	High
Complexity - methodological?	Low	Low	Medium	High	Medium
Possible to outsource?	Y	?	N	N	Y

Figure 1

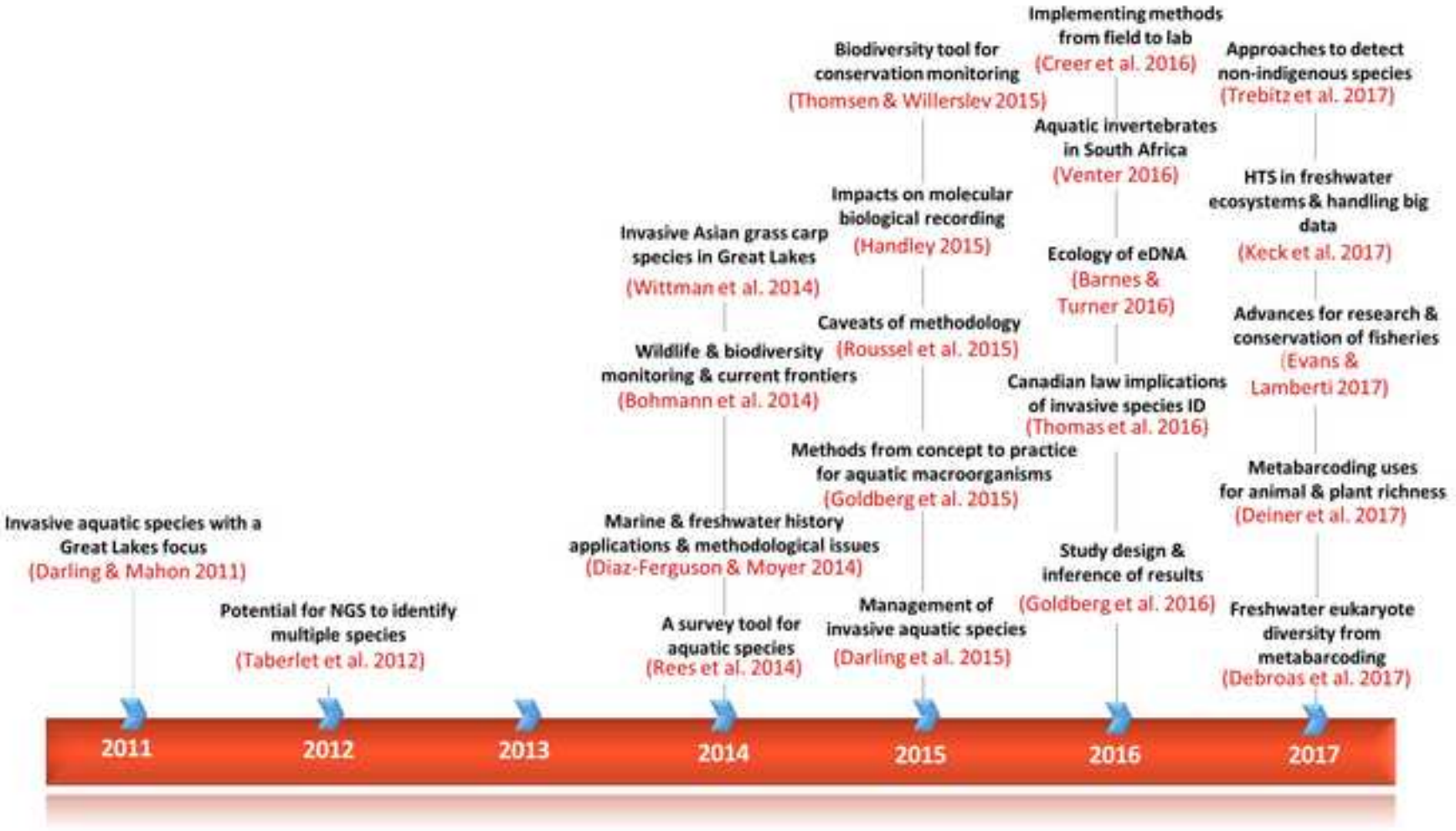

Invasive aquatic species with a Great Lakes focus (Darling & Mahon 2011)
Potential for NGS to identify multiple species (Taberlet et al. 2012)
Invasive Asian grass carp species in Great Lakes (Wittman et al. 2014)
Wildlife & biodiversity monitoring & current frontiers (Bohmann et al. 2014)
Marine & freshwater history applications & methodological issues (Diaz-Ferguson & Moyer 2014)
A survey tool for aquatic species (Rees et al. 2014)
Biodiversity tool for conservation monitoring (Thomsen & Willerslev 2015)
Impacts on molecular biological recording (Handley 2015)
Caveats of methodology (Roussel et al. 2015)
Methods from concept to practice for aquatic macroorganisms (Goldberg et al. 2015)
Management of invasive aquatic species (Darling et al. 2015)
Implementing methods from field to lab (Creer et al. 2016)
Aquatic invertebrates in South Africa (Venter 2016)
Ecology of eDNA (Barnes & Turner 2016)
Canadian law implications of invasive species ID (Thomas et al. 2016)
Study design & inference of results (Goldberg et al. 2016)
Approaches to detect non-indigenous species (Trebitz et al. 2017)
HTS in freshwater ecosystems & handling big data (Keck et al. 2017)
Advances for research & conservation of fisheries (Evans & Lamberti 2017)
Metabarcoding uses for animal & plant richness (Deiner et al. 2017)
Freshwater eukaryote diversity from metabarcoding (Debroas et al. 2017)
2011
2012
2013
2014
2015
2016
2017

Figure 2

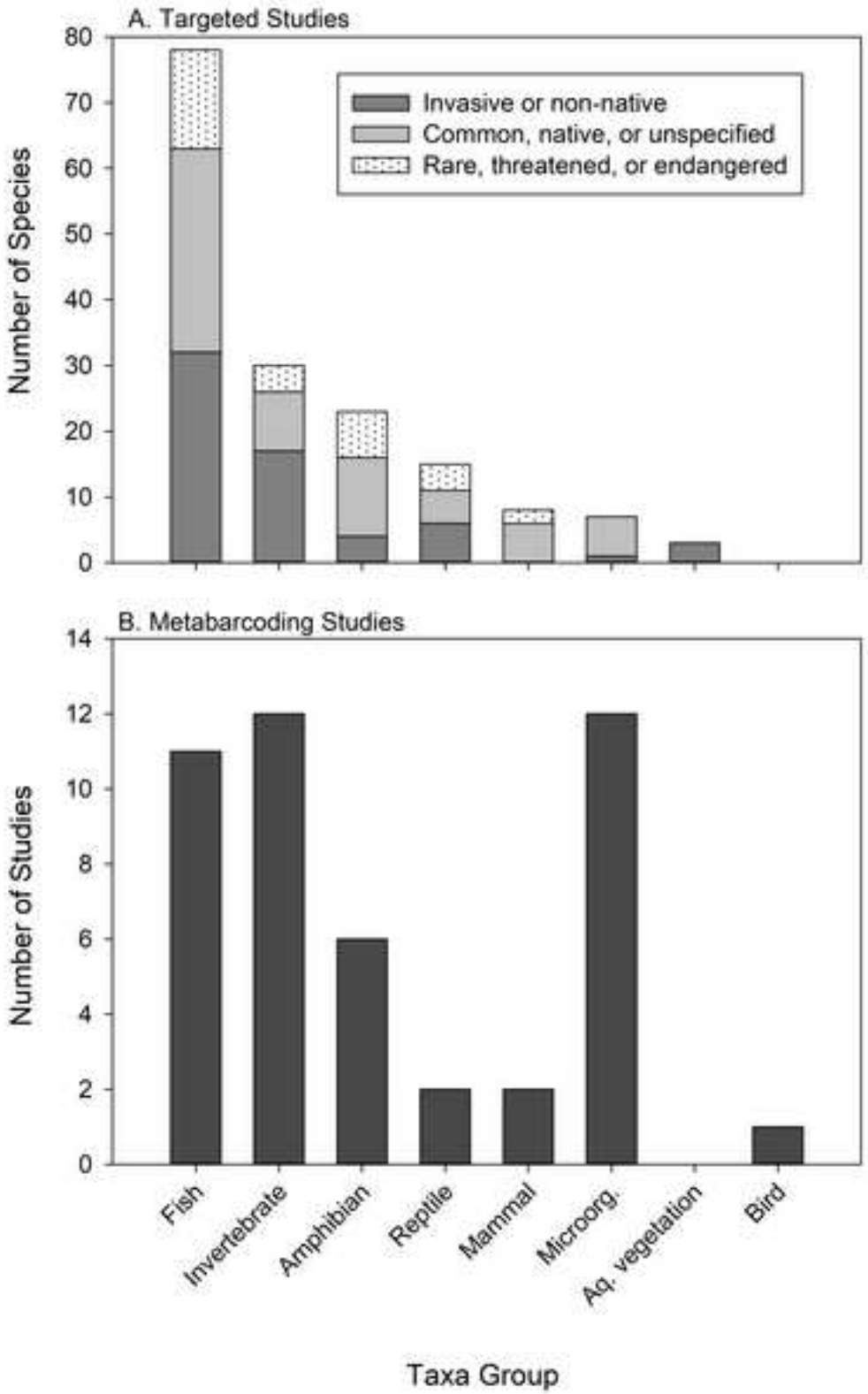

A. Targeted Studies
Number of Species
80
70
60
50
40
30
20
10
0
Invasive or non-native
Common, native, or unspecified
Rare, threatened, or endangered
B. Metabarcoding Studies
Number of Studies
14
12
10
8
6
4
2
0
Fish
Invertebrate
Amphibian
Reptile
Mammal
Microorg.
Aq. vegetation
Bird
Taxa Group

Figure 3

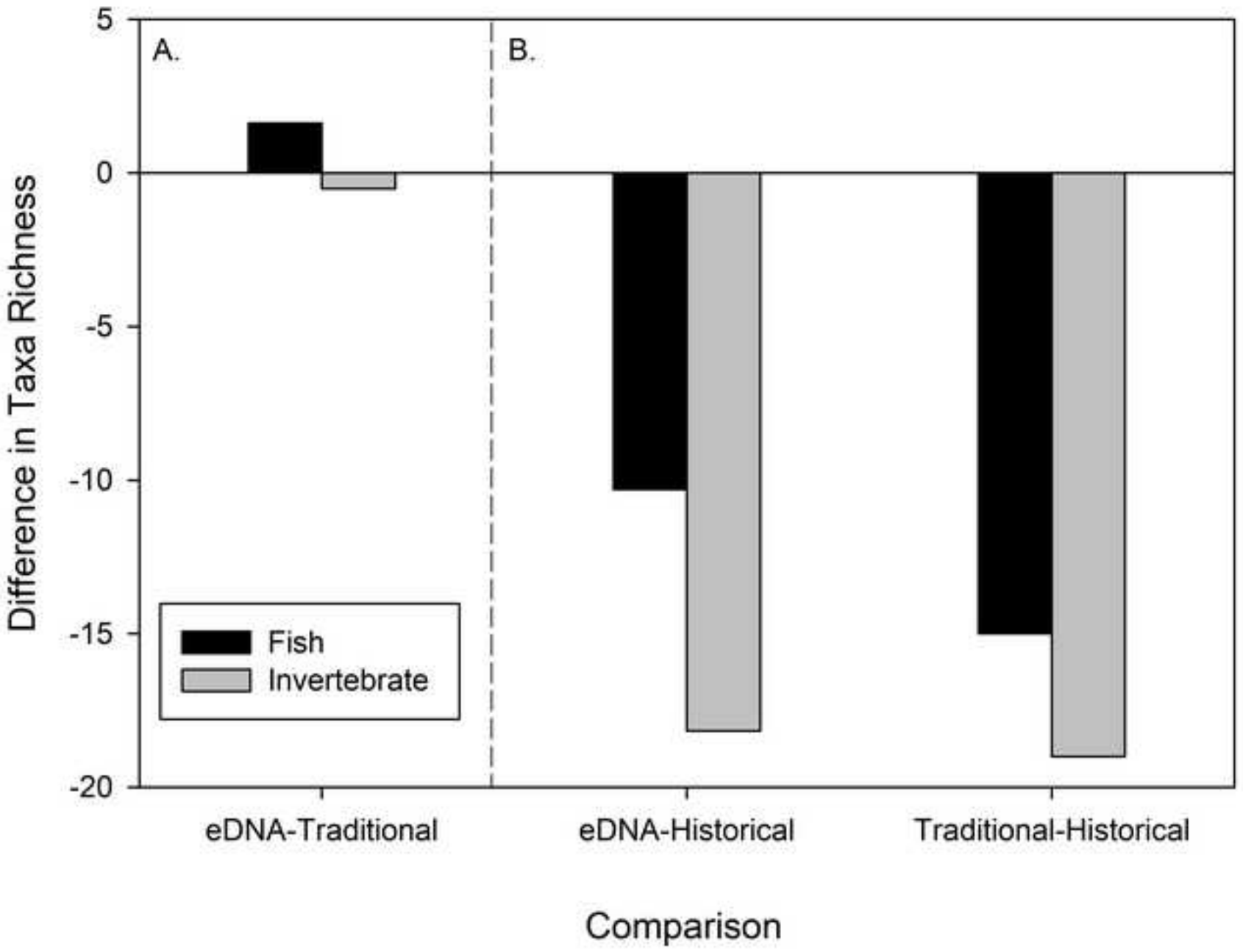
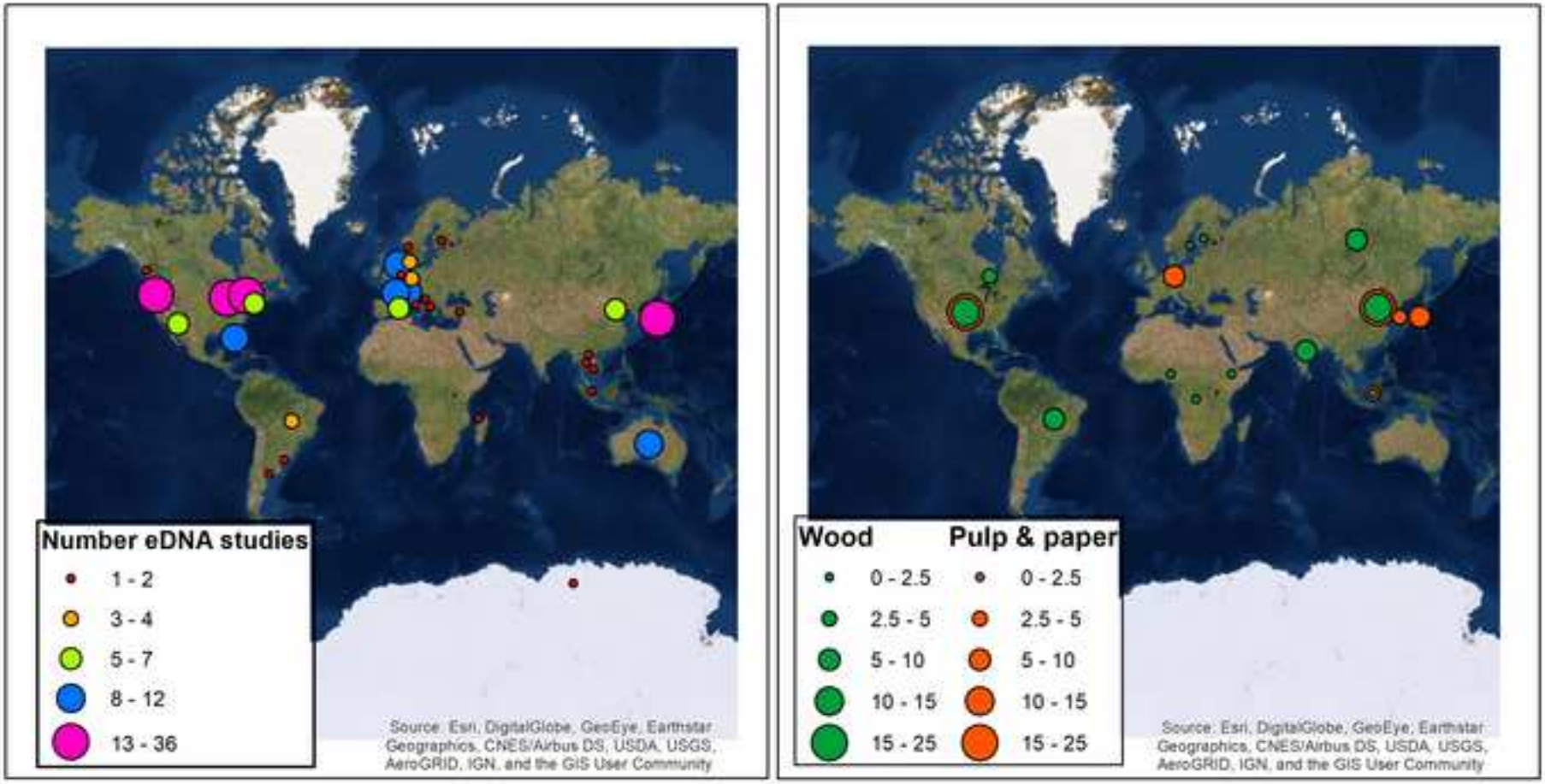

A.
B.
5
0
-5
-10
-15
-20
Difference in Taxa Richness
Fish
Invertebrate
eDNA-Traditional
eDNA-Historical
Traditional-Historical
Comparison

Figure 4


Number eDNA studies
1 - 2
3 - 4
5 - 7
8 - 12
13 - 36
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Wood
0 - 2.5
2.5 - 5
5 - 10
10 - 15
15 - 25
Pulp & paper
0 - 2.5
2.5 - 5
5 - 10
10 - 15
15 - 25
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community