Important Considerations for eDNA Sample Collection

Prior to outlining sampling guidance, it is necessary to emphasize a number of important considerations for eDNA sample collection for the GEN-FISH '500-lake' survey:

1. If eDNA sample collection is physically paired with conventional survey efforts (e.g., eDNA samples are collected in close proximity to where survey nets are placed, or eDNA will be sampled from a stream reach that is being electrofished) it is ***imperative*** that eDNA sample collection occur prior to conventional survey efforts. Survey equipment (e.g. gill nets, waders, electrofishers, seines) are often used on numerous water bodies and come into direct contact with fish. As a result, sampling equipment is a potent source of eDNA contamination from residual fish scales, mucus, blood, etc. This equipment can also transfer residual DNA from one ecosystem to another – eDNA methods can be extremely sensitive and can reliably detect as low as 2 copies of a gene per reaction. Introducing *allochthonous* (i.e., external to the ecosystem) DNA from organisms that do not naturally inhabit a surveyed ecosystem can compromise eDNA survey results.
2. Environmental DNA samples should be kept out of the sun after collection. UV light degrades DNA, and as a result water samples should be stored in a cool shaded location and, if processed on-site, should be filtered under a clean tarp (which also protects samples from rain).
3. Environmental DNA samples must be transported in a clean, contaminant-free, and sealed storage device (e.g. a bleached cooler). Under no circumstances should eDNA samples be transported or stored in close proximity to conventional survey gear.
4. To slow degradation, eDNA samples (either water samples or filters) should be kept cool after collection and during transportation (e.g. placed on clean, bleached freezer gel packs). Environmental DNA will eventually degrade at room temperature even when stored with silica beads, ethanol, etc. (Allison et al. 2021). Sample filters should therefore be placed in a freezer (minimum -20C) as soon as logistically possible after returning from field collection.

Guide Overview

Applications of eDNA for conservation management broadly encompass a number of objectives. Presence/absence applications of eDNA include broad assessments of species composition or more targeted, species-specific approaches that attempt to detect specific species of conservation concern (e.g. rare or invasive species). Emergent research has highlighted additional applications beyond species detection including biomonitoring, assessing abundance based on the concentration of eDNA observed in an ecosystem or based on the number/frequency of alleles collected in a sample, and deriving population-level genetic information (e.g. allele frequencies).

This sampling guide will outline general sampling recommendations for three objectives that are the primary focus of the '500-waterbody' GEN-FISH survey: (i) targeted species detection; (ii) community biodiversity assessments; and, (iii) applications quantifying eDNA concentrations. This guide will also further highlight sampling design considerations for two ecosystem types: (i) small-moderate lotic systems; and (ii) large lotic and lentic systems. A major objective of the GEN-FISH '500' waterbody survey is to compare the efficacy of eDNA relative to conventional survey methods for a number of applications; at a minimum, eDNA sampling efforts should be equal to conventional survey efforts, regardless of study objectives (eDNA sampling sites should at least be numerically/spatially paired with netting sites, electrofishing survey reaches, etc.). For many applications, effective eDNA sampling may require the collection of samples from more locations within a water body relative to conventional sampling efforts. However, it is important to note that eDNA sample collection typically requires less effort than comparable surveys employing conventional physical collection methods, and the marginal cost of adding additional eDNA sampling sites within a waterbody generally decreases substantially with the number of samples collected.

Sampling design: the importance of study objectives

Sampling design and effort will depend on a combination of project objectives, the type of ecosystem surveyed (e.g., lentic or lotic), ecosystem size, and available project resources. However, some general principles can guide sampling design foreach of the three primary applications listed above, regardless of the surveyed ecosystem. Additionally, a major strength of eDNA is that a number of objectives can

simultaneously be accomplished from sampling eDNA by adaptively modifying samplingdesign – broad community biodiversity assessments, targeted high-sensitivity species- specific detections (e.g., invasive or rare species detections), and the estimation of species-specific eDNA concentrations (e.g. to infer abundance) can be accomplished from the same study. For example, a project could track abundance trends of a socio- economically important game fish from species-specific eDNA concentrations by extensively spatially sampling an ecosystem while simultaneously assaying community biodiversity by subsampling eDNA collected from representative microhabitats to broadly assess species presence/absence. If the project had a further objective of detecting a rare or invasive species in the same ecosystem, supplemental sampling efforts targeting microhabitats likely to harbour those species could be conducted. As a general rule, though, the larger the number of project objectives, the more extensive thesampling effort (and its complexity) will be required to effectively accomplish all objectives.

Targeted Species Detections:

Targeted species detection applications refer to projects applying eDNA to detect the presence/absence of a specific species, and often target species that are of conservation concern but are challenging to detect (i.e. occur at low densities and/or at high densities in a limited number of areas). Typical applications often target rare/threatened/endangered species or invasive species at their invasion front, for which detection probabilities in a given individual sample are low (Jerde 2019). These applications can benefit from allocating disproportionate sampling effort to preferred microhabitats of the target species within an ecosystem to increase detection probability (Jerde et al. 2013), although it is important to note that such sampling strategies can limit or bias inferences regarding population trends at broader spatial scales (Erickson et al. 2019). Targeted species detection applications can also, however, be used to broadly characterize the spatial distribution of a targeted species (Eichmiller et al. 2014; Goldberg et al. 2018; Wilcox et al. 2018). If detection probabilities are expected to be high, then it may be more advantageous to spatially standardize sampling locations throughout the surveyed area. Whether to allocate sampling effort towards ‘preferred’ microhabitats or to spatially standardize sampling depends on a combination of trade-

offs between project resources (i.e., the number of samples that can be collected), project goals, and expected detection probabilities.

The number of samples needed to adequately detect a rare species is determined by a combination of expected probability of detection, the size of the area surveyed, the resources available to survey efforts (i.e., how many samples can realistically be collected), and the level of 'certainty' managers are comfortable with regarding the likelihood of false negatives. The number of samples required to detect an organism at a pre-determined level of certainty can be determined from theoretical formulas based on the assumption that sampling distributions should approximate the Poisson distribution for rare species (Green and Young 1993). However, Green & Young (1993) demonstrate that the effort required to reliably detect rare species tends towards infinity at very low densities/abundance, and this pattern similarly applies to eDNA sampling efforts. Detection probabilities can also be influenced by a number of biological and technical issues (inhibition, assay specificity/efficiency, species-specific eDNA production rates, organism life-history stages, etc.) that can affect sample sizes needed to confidently infer the presence/absence of species (Goldberg et al. 2016; Erickson et al. 2019). As a result, if rare species detection is the primary focus of the project the number of samples that *should* be collected likely boils down to 'as many as project resources can realistically facilitate'.

Surveys to quantify the spatial distribution of targeted species should ensure adequate coverage of the surveyed ecosystem, and the number of samples adequate to do so depends on the size of the area surveyed – the larger the area, the more samples required. At the very least, if eDNA sampling is being paired with conventional methods the same number of locations should be surveyed using both methods. However, the relative ease with which eDNA can be collected and filtered should facilitate the collection of eDNA from a larger number of locations relative to conventional surveys.

Community Biodiversity Assessments (presence/absence):

Community Biodiversity assessments apply eDNA to quantify species assemblages within a surveyed area/ecosystem. Variation in microhabitat conditions (e.g. sediment, vegetation, oxygen levels, temperature, etc.) within an ecosystem determine what species are capable of persisting at a given spatial location. Whereas targeted species detection efforts can benefit from focusing sampling efforts on

preferred microhabitats of targeted species to improve detection probability (Jerde et al. 2013), the goal of biodiversity surveys is to recover as many species as possible present in an ecosystem. As a result, biodiversity surveys can benefit from broad 'stratified' sampling over a range of microhabitats (e.g. rocky vs sandy vs. vegetated littoral shorelines, pelagic zones, riffles/runs/pools, etc.), with extensive coverage of the surveyed area (Evans et al. 2017; Lawson Handley et al. 2019; Carraro et al. 2020). However, as with targeted species detections, non-random and/or non-standardized spatial distribution of sampling effort across the surveyed area could also limit the capacity to infer trends in relative or species-specific abundance from eDNA (Erickson et al. 2019; Lawson Handley et al. 2019), although it may be possible to account for sampling stratification through weighting (by area, volume, etc.) (G.Cochran 1977).

Sampling effort considerations (i.e., the number of samples to collect) for biodiversity surveys face the same general issues as species-specific surveys targeting rare species. Common species that occur at high densities can usually be easily and reliably detected from eDNA using targeted species-specific approaches (Wilcox et al. 2016). Sampling effort decisions should therefore account for the difficulties associated with the detection of rare species and, as a result, the number of samples that should 'ideally' be collected is still probably 'as many as possible given project resources' (see previous discussion). The sampling effort needed to effectively quantify biodiversity will also likely increase with habitat heterogeneity and the size of the surveyed area (Blabolil et al. 2020). However, rarefaction methods can be used *post-hoc* to evaluate the sampling effort necessary to adequately represent species diversity in a given surveyed ecosystem to inform future sampling efforts (Hanfling et al. 2016; Bylemans et al. 2019; Lawson Handley et al. 2019). Similarly, it may be possible to *post-hoc* estimate the number of 'missing'/undetected (presumably rare) species by using nonparametric species richness estimators (e.g. Chao II species richness estimator) (Colwell and Coddington 1995; Olds et al. 2016; Evans et al. 2017; Bylemans et al. 2019; Sard et al. 2019).

Applications quantifying eDNA concentrations:

In addition to providing data on species presence/absence, qPCR assays (such as those employed by GEN-FISH) can provide information on the concentration of a

species' eDNA present in an environment. This information can inform a number of different applications, but a primary emergent application of eDNA has been to monitor population trends in abundance. A number of studies have accumulated demonstrating a consistent and positive correlation between the concentration of eDNA in an environment and organism abundance (Yates et al. 2019; Rourke et al. 2021).

How abundance is estimated is an important consideration for studies evaluating correlations between eDNA concentrations and organism abundance. Corresponding abundance estimates can either be relative (from catch-per-unit-effort (CPUE)/biomass-per-unit-effort (BPUE) data [e.g. Lacoursière-Roussel, Côté, Leclerc, Bernatchez, & Cadotte, 2016; Wu et al., 2018], telemetry data [e.g. Eichmiller, Bajer, & Sorensen, 2014; Ghosal, Eichmiller, Witthuhn, & Sorensen, 2018; Littlefair, Hrenchuk, Blanchfield, Rennie, & Cristescu, 2020], etc.) or absolute (from depletion curve estimates [e.g., Wilcox et al., 2016], capture-mark-recapture (CMR) studies [e.g. M. J. Spear, Embke, Krysan, & Vander, 2020; M. Yates et al., 2020], etc.). However, it is important to note that relative abundance estimates (e.g., CPUE/BPUE) can sometimes be a poor proxy for absolute abundance (Rose and Kulka 1999; Hubert et al. 2012; Yates et al. 2020); there is a particular need for eDNA studies that compare absolute organism abundance. Abundance estimates can also apply to different spatial scales. 'Local' estimates of abundance can correspond to a specific spatial location (e.g., CPUE for a specific net in a specific location, or a depletion curve estimate for a specific stream section). Abundance estimates can also correspond to broad 'ecosystem-level' spatial scales (e.g. the number of fish inhabiting a lake determined from a CMR study, or average CPUE from nets distributed throughout a waterbody).

When comparing eDNA to organism abundance, estimated eDNA concentrations must be representative of the corresponding unit of abundance (Chambert et al. 2018; Erickson et al. 2019). Environmental DNA concentration estimated for a specific sample location should only be directly compared with spatially paired estimates of 'local' abundance. When using paired local eDNA/abundance estimates to monitor broad population trends, it is important to avoid targeting specific microhabitats; sample site locations should proportionately reflect ecosystem habitat heterogeneity. Sampling effort should therefore be extensive and either spatially standardized or randomly distributed throughout a surveyed area (Krebs 2014; Erickson et al. 2019), although

stratified sampling with weighting (e.g. by strata area) can also be used to monitor population trends (G.Cochran 1977).

However, obtaining spatially localized estimates of absolute abundance can often be difficult due to individual movement within a system; absolute abundance estimates derived from CMR studies, for example, are often limited to the scale at which organisms' movement 'outside' of the study area is limited (Schwarz and Seber 1999). As a result, abundance estimates may often only apply to broader 'ecosystem-level' spatial scales over which movement into and out of the system is restricted (e.g. abundance within an entire lake or in a large stream section); 'ecosystem-level' abundance estimates must also be spatially standardized (density/ha in a lake, biomass/km in a stream, density/volume in a pond, etc.) to facilitate abundance/eDNA comparisons across ecosystems (Klobucar et al. 2017; Spear et al. 2020; Yates et al. 2020). Estimates of abundance over broad 'ecosystem-level' spatial scales must therefore be compared to the *average* eDNA concentrations observed across a similar spatial scale. It is important to note that sampling efforts that disproportionately target micro-habitats within an ecosystem likely to harbour targeted species can introduce bias into the calculation of an 'ecosystem-level' average eDNA concentration (Erickson et al. 2019), particularly due to the heterogenous spatial distribution of eDNA that likely reflects the heterogenous spatial distribution of study organisms (Eichmiller et al. 2014; Ghosal et al. 2018; Goldberg et al. 2018; Lawson Handley et al. 2019; Littlefair et al. 2020).

To estimate 'ecosystem-level' mean eDNA concentrations, eDNA sample locations should therefore be randomly or systematically distributed throughout a surveyed area. This could be accomplished by comprehensively sampling eDNA in a standardized manner across the whole ecosystem (e.g., using a 'grid' system in lentic environments (Ghosal et al. 2018; Brys et al. 2020) or 'even-interval' sampling in lotic environments (Wood et al. 2021). However, many studies may also have 'detection' applications as a primary focus (e.g., biodiversity surveys), with correlations between eDNA and abundance a secondary objective. Researchers or managers sampling eDNA for detection applications may often (justifiably) engage in stratified subsampling of microhabitats, putting effort into sampling different zones or microhabitats within an ecosystem disproportionate to the fraction of the total ecosystem area or volume

represented by each zone/microhabitat. 'Ecosystem-level' eDNA averages, however, can still be calculated from stratified designs by weighing the relative contribution of samples from each ecological zone by the fraction of the total ecosystem represented by that ecological zone (G.Cochran 1977; Yates et al. 2020). Determining relevant ecological zones to sample, and how weighting should be distributed, must be done on a study- and species-specific basis with careful consideration.

Environmental DNA production rates can also vary significantly with size and life-history stage (Maruyama et al. 2014; Takeuchi et al. 2019; Yates et al. 2020). It is therefore important to account for size-structure when pairing eDNA concentrations with conventional abundance estimates (Yates et al. 2020). At a minimum, individual body mass (and length) should be collected for all individuals captured during paired conventional surveys, and the collection of any additional biological information would be beneficial (e.g. sex, life-history stage, maturity status, etc.).

The timing of sample collection is also important, at both seasonal and diurnal scales. Temperature fluctuates diurnally, as does fish activity and movement. Environmental DNA concentrations, for example, can also peak during seasonal reproductive events (Spear et al. 2015; Curtis et al. 2020). Given that eDNA production/degradation can vary significantly with temperature, metabolic rates, feeding, and activity (Klymus et al. 2015; Thalinger et al. 2021b), eDNA samples should be collected at similar diurnal phases during the day to control for differences in fish movement, activity rate, temperature, etc. At broader time scales, comparisons in eDNA concentrations across waterbodies should be limited to seasonal comparisons, and likely even to time-scales within several weeks to avoid major changes in weather patterns. Patterns of fish movement, for example, can fluctuate with temperature/season/lake stratification status, with significant resulting effects on the distribution of eDNA within an ecosystem (Littlefair et al. 2020).

For studies validating relationships between eDNA concentration and organism abundance, efforts are typically limited by the capacity to conduct traditional assessments. Conventional survey methods such as capture-mark-recapture (CMR) or depletion estimates can be costly, time-consuming, and labor intensive to obtain relative to the ease with which eDNA samples can be collected and filtered. Broad 'ecosystem-level' estimates of absolute abundance (e.g. the number of fish in a lake) can be

particularly difficult and costly to obtain, often limiting sample sizes for comparisons with eDNA. Nevertheless, enough sites must be sampled using conventional methods to facilitate, at the very least, a bivariate regression between organism abundance and eDNA concentrations. A minimum of 8-10 datapoints would be adequate to model a bivariate correlation, bearing in mind that a study with such a limited sample size could not integrate the effect of other independent variables beyond abundance (e.g. temperature) on eDNA concentrations; larger samples sizes are recommended, when possible.

Surveys in Small- to Moderate-Sized Lotic Systems

For the purposes of this guide, we define small- to moderate-sized lotic systems as flowing stream/river systems in which eDNA water samples can be collected without the assistance of watercraft. This includes shoreline sampling, wading, back-pack samplers (Smith root, OSMOS, etc.), pole-sampling, and/or multiple combinations of these methods. When collecting eDNA samples in a lotic system, ***always*** collect eDNA samples beginning downstream and working in an upstream direction. Sample collection at upstream locations can introduce eDNA contamination that can subsequently be carried downstream and collected during sampling at downstream locations.

Directional water-flow in lotic systems ensures a relatively unidirectional transportation of eDNA, small quantities of which can be carried for remarkably long distances (e.g. up to ~100 km) (Pont et al. 2018). Hydrological conditions tend to homogenize midstream and bankside eDNA concentrations in a 'plume' after a (relatively) short distance from its point of origin (Wood et al. 2020, 2021; Thalinger et al. 2021a) (Figure A2.2.1). As a result, we recommend sampling several 10s-to- 100 m below conventional survey points or at the downstream terminus of surveyed stream sections ('reaches). We also recommend, when possible, either midstream sampling or, more optimally, eDNA samples collected from midstream and both banks across a transverse stream section (Wood et al. 2021) (Figure A2.2.2). Environmental DNA sampling equipment does not need to be decontaminated between midstream/bank samples originating from the same transverse section. In large and/ordeep streams mid-stream samples can be collected using pole-samplers or devices with extendable arms. If only a single mid-stream sample is collected, it should be collected furtherdownstream (e.g. 40-100 m) of targeted habitat,as it takes some distance from point of origin for eDNA to 'homogenize' laterally

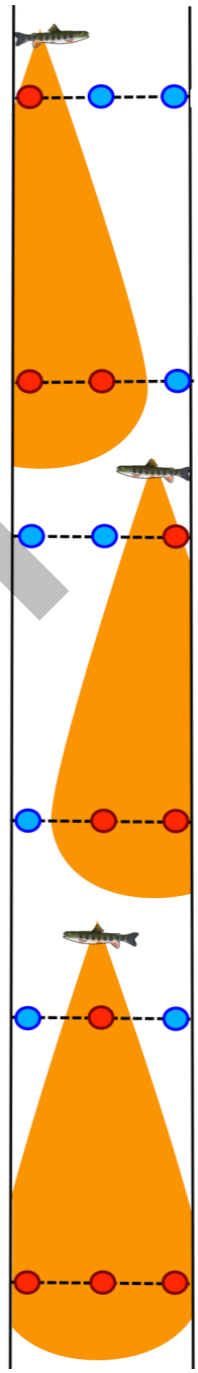

Figure 1: Sampling downstream of eDNA point-of-origin can increase detection probabilities, as can mid-stream sampling. Collecting multiple samples along a horizontal transect can also improve detections – for comprehensive surveys, we suggest collecting a sample on both banks and from midstream. Hypothetical eDNA distribution is represented by orange plume with red and blue circles indicating 'sample sites' with positive and negative detections, respectively

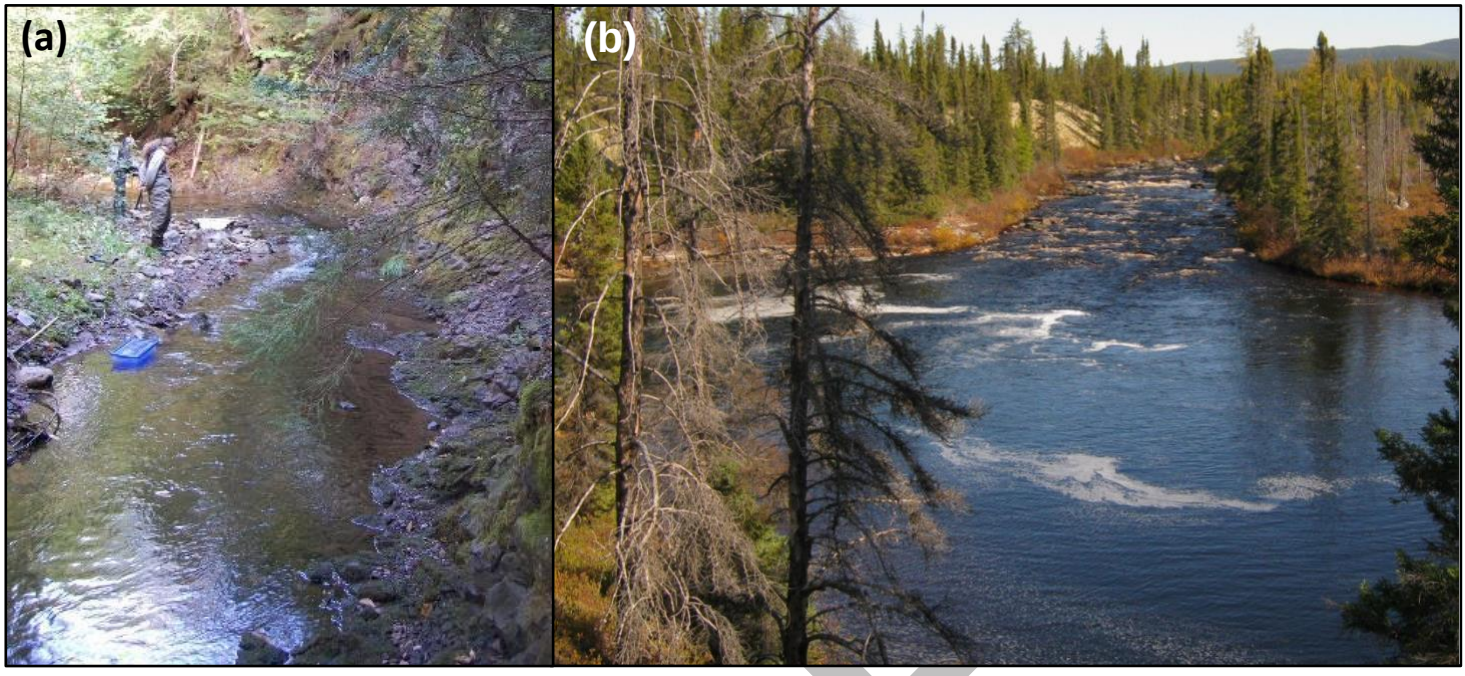

Figure 2: Examples of discrete 'microhabitats' in lotic systems. Picture (a) shows a riffle/run segment in a small stream dominated by Atlantic Salmon that leads into a deeper pool inhabited by larger brook trout. Picture (b) shows a riffle/rapids section inhabited by brook trout in a moderately sized river that flows into a deep pool section dominated by pike.

(Thalinger et al. 2021a; Wood et al. 2021). If mid-stream and bankside samples are collected, samples can be collected closer (e.g., 10 - 40 m) downstream of targeted habitat sections. Notably, single-bankside eDNA sample collection is not recommended because the transverse distribution of eDNA in a stream can be affected by the position of its point-of-origin and hydrological conditions (Thalinger et al. 2021a; Wood et al. 2021). For rare species at low densities (and low likely eDNA concentrations) this could result in false negatives.

Targeted single-species detection applications

For applications in which detection probabilities are assumed to be low (e.g., rarespecies), the specific locations within a surveyed area from which eDNA is collected should be selected based on the microhabitat preferences of targeted species (riffles, runs, pools, vegetated areas, etc.). Riffle sections, for example, should be sampled when attempting to detect species that preferentially inhabit fast-moving water, pools when targeting species that prefer slow-moving water, etc. (Figure A2.2.2). Sampling several 10s-to-100s of meters downstream of microhabitat likely to harbour the speciesof interest (e.g., at the terminus of riffle stream sections for species that prefer riffle habitat) or downstream of paired conventional sampling efforts may

improve eDNA detection probabilities and their potential correspondence with conventional surveys.

It is important to note that preferentially targeting specific microhabitats can limit inferences regarding population abundance trends and distribution at larger spatial scales (Erickson et al. 2019). 'Even-interval' sampling in lotic systems (i.e. collecting samples at intervals with standardized lengths) represent potential alternatives that do not introduce sampling bias originating from microhabitat selection, although this may come with a trade-off of lower detection likelihood for a given sample and greater sampling effort required to adequately cover survey areas. However, with efficient qPCR assays, sample replication, and adequate water sample volume (e.g. ≥ 0.5 - 1 L) sampling mid-stream every 100 m can be sufficient to detect the presence of a single individual with approximately 90-95% probability in small streams under some conditions (Wilcox et al. 2016; Wood et al. 2021); this interval can increase to 400 m under low-velocity conditions (Wood et al. 2021), and also increases with organism density (Wilcox et al. 2016; Wood et al. 2021).

At the watershed-level, the optimal selection of survey areas for rare species detection applications requires some *a-priori* knowledge of likely patterns of species distribution within that watershed. Stream sections likely to harbour targeted species can be identified based on habitat characteristics and/or conventional occupancy modelling (e.g. Wilcox et al., 2018). At a broad scale, however, distributing sample sites throughout the watershed is required to document general patterns of species occupancy. When the number of samples collected is low relative to sample area and target species are concentrated in a small number of areas with high densities, it may

be beneficial to disproportionately allocate sampling effort to stream sites with a higher Strahler order value (Carraro et al. 2021). For species with more even watershed distributions or when sampling effort is high, balanced sampling among Strahler order sections or sampling lower Strahler-order streams may be more optimal (Carraro et al. 2021).

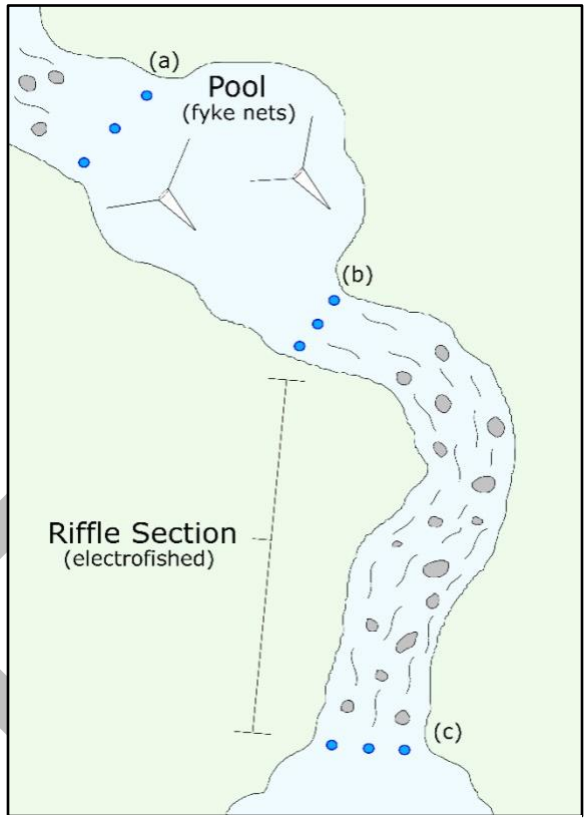

Figure 3: eDNA samples should be collected downstream of conventional surveys. eDNA samples (blue dots) collected at site (b) can be paired with upstream fyke net surveys conducted in a slow-moving pool section. eDNA samples collected at point (c) correspond to short 'riffle' section that could be sampled using an electrofisher. eDNA sample points (a) and (b) can be used to estimate 'upstream' eDNA inputs for sections corresponding to sample points (b) and (c), respectively.

Biodiversity survey applications

While targeted species detections can benefit from targeting specific microhabitats preferred by the targeted species, community biodiversity surveys can benefit from allocating resources to sampling a range of microhabitats while also broadly distributing sampling efforts over study catchments. Sampling discrete lotic habitat types (e.g., 'riffles', 'runs', and 'pools'), for example, could improve the diversity of species recovered from eDNA samples.

When pairing eDNA biodiversity sampling with conventional sampling efforts, eDNA survey design depends on the spatial scale of the conventional sampling. For point-sampling efforts targeting a small area (e.g., fyke nets or a seine in a large pool) eDNA samples should be collected several tens of meters (e.g. ~30-50 m) downstream of conventional sampling points (Figure A2.2.3). Similarly, if conventional surveys cover a short stream reach (e.g. electrofishing a reach < 200 m) then samples should be collected at the downstream terminus of the reach (Figure A2.2.3). We also recommend mid-stream sampling combined with transverse banksideeDNA collections; otherwise, we recommend prioritizing mid-stream sampling if sampling effort/resources are limited (see above for discussion).

Determining whether collected eDNA originates from organisms within surveyed areas or from progenitor organisms upstream of the survey area can also be challenging but important for biological inference and comparisons with conventional

surveys. While much of any 'upstream' eDNA input is likely to degrade and/or deposit out of the water column during its transit through a survey site, some proportion would be expected to remain in the water column at the downstream terminus of the survey site (Wilcox et al. 2016). We therefore also recommend eDNA sample collection immediately upstream of surveyed sites. These samples can be used to estimate upstream 'eDNA inputs' into the survey area and facilitate the quantification of eDNA produced by organisms directly within the survey area, potentially improving resulting comparisons with conventional surveys (Figure A2.2.3).

Alternatively, if conventional surveys cover a large spatial area (e.g., electrofishing over stream sections > 200 m), then eDNA samples should be collected at standardized intervals (i.e., 'even-interval' sampling) throughout the survey site. Most eDNA generally deposits out of the water column on a scale of hundreds of meters (Jane et al. 2015; Shogren et al. 2017). We recommend standardizing interval length to a consistent value between 100 and 200 m, given that sampling approximately every 100 m can be sufficient to detect a single individual with high probability using species-specific qPCR approaches (Wilcox et al. 2016; Wood et al. 2021). When sampling in continuous intervals, samples collected at upstream points can also be used to quantifyupstream eDNA 'inputs' for downstream intervals. When pairing even-interval samplingwith conventional surveys, ensure that data from conventional surveys also correspond to eDNA sampling intervals (e.g., assign captured fish to specific intervals).

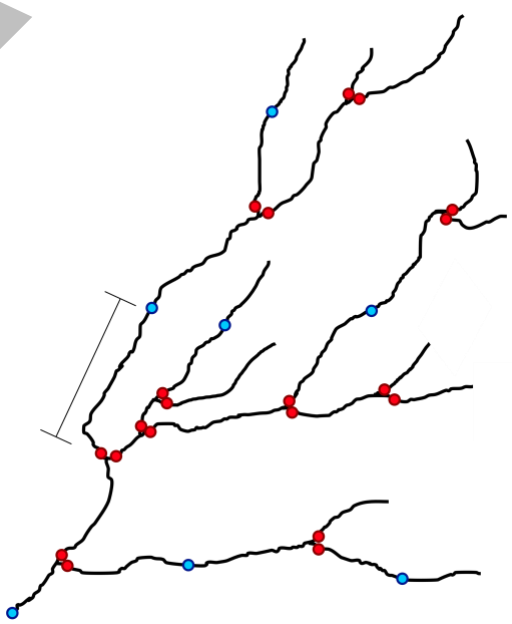

Figure 4: Broad sampling design focused on sampling above tributary confluence points combined with 'even-interval' sampling. Red circles indicate sample sites associated with tributary confluences; blue sites indicate 'even-interval' sites on stretches lacking tributary confluences. Bar indicates maximum interval length.

At the watershed-scale, sampling efforts for biodiversity surveys should be broadly distributed throughout the study catchment and sample multiple orders of tributaries (Carraro et al. 2020). An important point to note, however, is that the optimal distribution of sampling sites for eDNA surveys may not correspond to the optimal distribution of sites for conventional sampling. For example, tributary confluence points can represent areas of particular importance for eDNA surveys. Due to

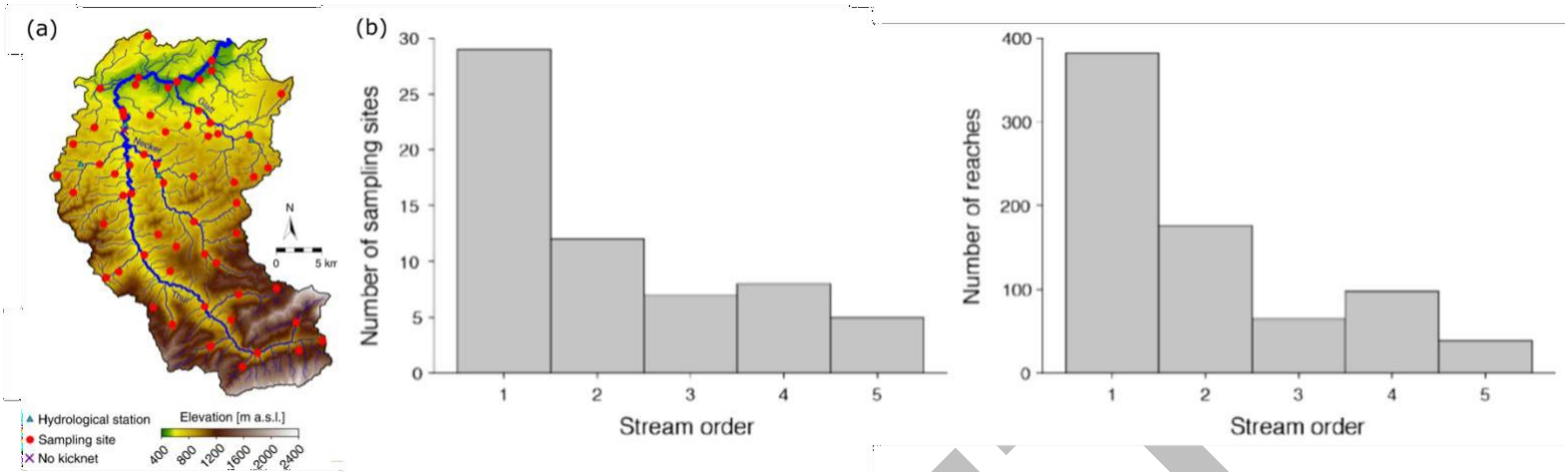

Figure 5: Example sample design in which sampling effort is distributed throughout the study catchment but also allocated proportionately to the distribution of stream order in the river network. Figure **(a)** displays the geographic location of sampling sites in the Thur river network from Carrero et al. 2020 (*Nature Commun*); figure **(b)** shows the distribution of Strahler stream order values across the 61 eDNA sampling sites and across the 760 reaches constituting the Thur river network. Figure reproduced with permission from authors.

the downstream transportation of eDNA in lotic systems, sampling tributaries and higher Strahler-order streams above confluence points could be particularly effective to model species' spatial distributions, facilitating biodiversity estimates that correspond to particular tributaries (Carraro et al. 2021) (Figure A2.2.4). For long stream sections that lack significant tributary confluence points (or when tributaries are not of interest and/or too numerousto sample), samples could be collected at evenly spaced intervals (with maximum interval length dependent on catchment size and resources) to ensure adequate coverage of the entire watershed (Figure A2.2.4).

Even-interval/confluence point sampling strategies require significant sampling effort, however, and may be prohibitive at large spatial scales. An alternative strategy for distributing sampling effort throughout large catchments while also broadly sampling representative habitats could be to distribute survey areas throughout the river network while simultaneously stratifying sampling effort in a manner that proportionately represents the distribution of stream orders within that watershed (Carraro et al. 2020) (Figure A2.2.5). An additional advantage of this strategy is that it standardizes sampling effort proportionate to habitat heterogeneity; if sampling is extensive, data obtained could also potentially be used to monitor population trends.

Quantitative estimates of eDNA in lotic systems

The majority of eDNA in small-moderate lotic systems likely degrades and/or deposits out of the water column on scales of hundreds of meters (Jane et al. 2015;

Shogren et al. 2017). For applications seeking to quantify eDNA concentrations to pair with abundance estimates, we suggest establishing conventional stream surveys of reaches approximately 100-200 m in length to pair with eDNA samples collected at the downstream terminus of each surveyed reach (see Figure A2.2.6).

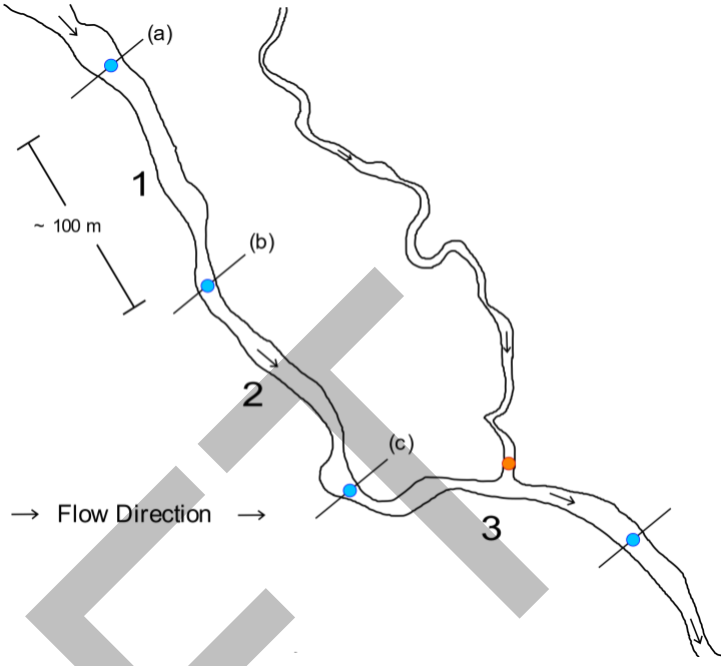

Figure 6: Paired eDNA samples (blue) with stream survey reaches. Contiguous survey reaches can increase 'efficiency' of sampling – eDNA sample sites 'a' and 'b' correspond to the upstream and downstream (respectively) sites for survey reach 1, eDNA samples sites 'b' and 'c' correspond to the upstream and downstream (respectively) sites for survey reach 2, etc. Note the location of an additional potential eDNA sampling site on the secondary tributary – sampling just above the confluence with the main stem would help quantify contribution of eDNA inputs from the secondary tributary to downstream concentrations estimated at terminus of reach 3.

Quantifying 'upstream' eDNA inputs into survey reaches is particularly critical when attempting to correlate eDNA with fish abundance (Wilcox et al. 2016). Estimating upstream 'inputs' to survey reaches, in conjunction with the collection of additional environmental parameters that affect particle deposition (e.g. stream depth, velocity, etc.), could enable the estimation of the proportion of eDNA collected at the downstream terminus that was produced by organisms upstream of the surveyed reach, enabling the estimation of eDNA produced solely by organisms in the intervening reach (Wilcox et al. 2016; Sepulveda et al. 2020). We recommend collecting eDNA samples at both the upstream origin and downstream terminus of survey reaches, as well as on any lower Strahler-order tributaries above their confluence with surveyed reaches (see Figure A2.2.6). Note that sampling contiguous 'even-interval' survey reaches can improve the 'efficiency' of eDNA sampling; eDNA samples collected at the terminus of upstream reaches can be used to estimate 'upstream' eDNA inputs for reaches immediately downstream (Figure A2.2.6). It is, however, important to note that systematic sampling has the potential to introduce bias into estimates if there is underlying periodic variation in an ecosystem (e.g. clusters of fish occur every 100 m) (G.Cochran 1977; Krebs 2014). However, we suspect that in most lotic systems this is unlikely to occur, and 'even-interval' systemic sampling is likely a reasonable approach.

For applications obtaining quantitative estimates of eDNA it is also critical to estimate discharge by quantifying stream depth and velocity. Discharge has important dilutive effects on eDNA; assuming two streams with identical study populations (e.g. identical absolute abundance and size structure) and environmental conditions (e.g. pH, temperature) but varying discharge rates, expected eDNA concentrations from equal-volume water samples would be lower in the stream with a higher discharge. The appropriate estimate of eDNA particle number relevant to lotic systems is not the concentration of eDNA estimated from a water sample (e.g. eDNA copies/L filtered), it is instead the absolute number of eDNA particles moving downstream (i.e. 'flow-corrected' eDNA rate) (Levi et al. 2019). This can be calculated by multiplying estimated eDNA particle concentration for a standardized sample volume by stream discharge (Levi et al. 2019), e.g.:

*Flow corrected eDNA rate = eDNA copies/time = eDNA copies/L * L/sec*

Stream discharge rates can be highly variable both temporally within and spatially across sites, with documented effects on eDNA detection probabilities and concentrations (Jane et al. 2015; Levi et al. 2019; Curtis et al. 2020). A failure to account for the dilutive effects of discharge on observed eDNA concentrations will introduce additional unexplained variation in eDNA flow rates.

This comparison, however, is complicated by the fact that high discharge events likely reduce eDNA deposition, resulting in further transport downstream and more stable spatial concentrations (Jane et al. 2015; Pont et al. 2018; Curtis et al. 2020). Additionally, eDNA can accumulate in benthic sediment (Barnes et al. 2014; Turner et al. 2015; Nevers et al. 2020); increased turbulence during extreme discharge events could resuspend FPOM (including eDNA), obscuring the relationship between organism abundance and eDNA (Jane et al. 2015; Shogren et al. 2017). High discharge events could either dilute eDNA produced by study organisms (thus decreasing eDNA concentration) or reduce deposition of particles produced upstream and resuspend particles built up in sediment (thus increasing eDNA concentration); both opposing effects have been observed even within a single study (Jane et al. 2015). Experimental observations from periods of abnormally high discharge should therefore be interpreted with caution when attempting to quantify eDNA concentrations; sampling during base-

flow conditions is preferred, and ideally sample collection should not occur during or immediately following heavy precipitation.

Although it is important to account for the 'scale' at which abundance and eDNA are compared, in most small-moderate sized lotic environments it is often relatively straightforward to pair site-specific eDNA samples with local estimates of abundance because eDNA samples can be paired directly with abundance estimates immediately upstream of sample sites (Pilliod et al. 2013; Wilcox et al. 2016; Baldigo et al. 2017; Sepulveda et al. 2020). As discussed previously, a minimum sample size of 8-10 stream reaches with paired conventional surveys and eDNA samples would be necessary to model bivariate relationships between organism abundance and eDNA concentrations. Based on the sample sizes commonly observed in published studies on eDNA/abundance relationships in lotic systems, 15-25 stream reaches would be considered a good sample size; any study examining more than 25 reaches would be considered excellent. Stream reaches should also represent a gradient of organism density (i.e., from low to high density), with some stratified replication across the gradient(e.g., a minimum of X samples each from low, medium, and high density environments) to avoid large low- or high-density outliers. As a result, some *a-priori* knowledge of the distribution/abundance of targeted species would be useful when designating sampling sites/reaches.

If abundance estimates cannot be spatially paired with eDNA samples (e.g., a conventional mark-recapture survey estimated an absolute population abundance of 600 fish distributed throughout a small 2 km stream) then abundance should be compared to average eDNA concentrations estimated from samples distributed throughout the surveyed stream area. To characterize the average concentration of eDNA in a surveyed stream we would recommend estimating a mean 'ecosystem-level' (flow-corrected) eDNA concentration from a *minimum* of 8-10 randomly chosen locations or using standardized 'even-interval' sampling. Comparisons with other streams can be facilitated by spatially standardizing density/flow-corrected eDNA estimates to a standardized stream length (e.g., 'X' fish per 500 m corresponds to a mean flow-corrected eDNA rate of 'Y').

Surveys in lentic systems and large lotic systems

For the purposes of this guide, we define large lotic systems as river systems in which eDNA water samples must be collected with the assistance of watercraft. Also note that the sampling of pelagic/offshore zones in lentic systems also requires the use of watercraft to collect samples. Water craft must be decontaminated (if possible) when moving between waterbodies. Residual DNA on canoes, kayaks, paddles, life-jackets, etc. can contaminate water collected during surveys, leading to erroneous species detections due to the collection of allochthonous eDNA and resulting false-positive eDNA tests (Darling et al. 2021). If using an inflatable watercraft to collect samples (e.g., an 'Alpaca') we recommend soaking watercraft in a 2-3% household bleach solution for 20 minutes and drying in sunlight for 48 hours. If the watercraft is too large to soak, we recommend allowing the watercraft to dry in sunlight and then wipe it down with a 10- 20% bleach solution.

In large lotic systems, sampling efforts must still account for the downstream transportation of eDNA. However, in lentic systems, the horizontal movement of eDNA is likely limited, given that it tends to be localized to areas of high organism density/occupancy (Eichmiller et al. 2014; Ghosal et al. 2018; Goldberg et al. 2018; Brys et al. 2020); notable exceptions to this may include 'mixing' events in temperate lakes pre- or post- formation of a thermocline (Hanfling et al. 2016; Harrison et al. 2019; Littlefair et al. 2020). Generally, however, eDNA concentrations decline significantly outside of areas of high density/occupancy at a scale of tens of meters (Eichmiller et al. 2014; Ghosal et al. 2018); this rate of decline is hypothesized to be exponential (Goldberg et al. 2018). In larger lentic systems, eDNA sample points are likely to be far enough away from each other that detected concentrations will likely not be conditionally dependent on each other. However, in small lentic systems (e.g., <1 ha) samples may be close enough that organisms from one sample point can affect eDNA concentrations at adjacent points. We therefore recommend a minimum sampling distance of at least 15-25 m between samples to help reduce this conditional dependence. Due to the limited horizontal transportation of eDNA in lentic systems, samples should also be collected directly within targeted habitat sections (as opposed to downstream of targeted habitat sections, as in lotic systems). Similarly, the spacing of samples required to reduce spatial autocorrelation in detected eDNA (and its

concentrations) in lentic systems is not well understood, although community composition and abundance estimates derived from eDNA undoubtedly exhibit autocorrelation dependent on the scale and heterogeneity of the surveyed system. Spatial autocorrelation, for example, was detected for samples within 250 m and 2 km for a medium and large lentic system (Zhang et al. 2020).

When pairing eDNA surveys with conventional sampling, the low rate of horizontal transportation of eDNA in lentic systems also means that samples should be collected in close proximity to conventional sampling locations – we recommend that samples be collected within 5 m of conventionalsampling stations. However, it should again be emphasized that eDNA samples should always be collected before conventional sampling gearis introduce to the waterbody.

Further complicating sampling in lentic and large lotic waterbodies is the need to account for the vertical water column. Surface vs. sub-surface sampling (e.g. 0.5 m depth) may have little effect on observed eDNA concentrations (Eichmiller et al. 2014). However, the movement of eDNA across thermoclines, for example, appears to be very limited during thermal stratification in lentic systems (Littlefair et al. 2020); at the very least, gravitational settling is likely to result in a general unidirectional movement of particles from surface waters to lower depths outside of lake mixing events. Detecting benthic deepwater communities may therefore require collecting samples at lower depth profiles, depending on season/stratification status (Lawson Handley et al. 2019; Littlefair et al. 2020). The optimal timing and location in the vertical water column for the collection of eDNA samples will depend on a combination of project objectives and the ecology of the species targeted

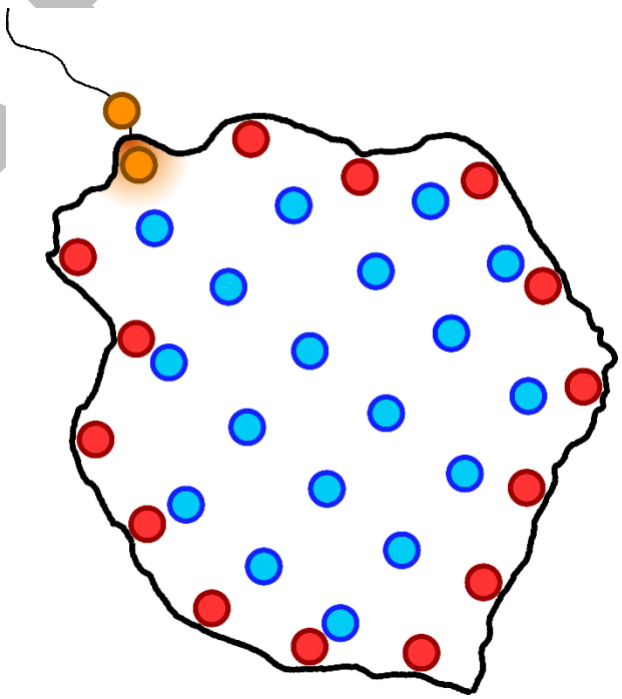

Figure 8: Example eDNA sampling design to characterize spatial distribution of species in a lentic system. Pelagic (offshore) samples [blue] are collected in a grid, with littoral (nearshore) samples [red] collected at standardized intervals. Note the inflow stream at the top of the lake – the orange littoral sample may contain eDNA carried downstream from the inflow stream, which may affect the eDNA composition of the area in close proximity to the stream inflow (depicted by the orange hue). A sample should be collected from the tributary immediately above the inflow to quantify any 'upstream inputs'.

during surveys. It is important to note, however, that care should be taken to avoid disturbing/resuspending benthic sediment, which can act as a reservoir for eDNA (Barnes et al. 2014); if suspended sediment is collected by accident from a deepwater sample, move to an adjacent area and collect a new sample.

Finally, careful consideration is required when sampling tributary inflow points in lentic systems. Tributaries can carry low levels of eDNA remarkable distances downstream (Pont et al. 2018), and can introduce eDNA into lentic/large lotic water bodies from upstream organisms that are not actually present within thesystem (Evans et al. 2017; Blabolil et al. 2020). Sampling efforts may therefore mischaracterize the community composition of a lentic system when sampling in close proximity to a stream inflow or channel (Evans et al. 2017; Blabolil et al. 2020), although these areas can also represent locations where lotic and lentic communities mix (Blabolil et al. 2020). The interpretation of samples from areas in close proximity to inflows can be challenging; if eDNA samples must be collected from areas close to inflows, we also recommend collecting a sample directly from the tributary itself to quantify upstream 'eDNA inputs'(see above for sampling recommendations for small-moderate lotic systems) (Figure A2.2.8).

Targeted single-species detection applications

As with smaller lotic systems, targeted species detection applications focused on rare species that are difficult to detect can benefit from allocating sampling effort to preferred microhabitats of the targeted species (e.g., vegetated littoral shorelines, rocky littoral shorelines, deep benthic zones, pelagic zones, etc.), although it is worth reiterating that this can limit inferences regarding population trends at broader spatial scales. To maximize the likelihood of detection, samples should be collected directly within targeted microhabitats; this includes the location of sample collection within the vertical water column. When targeting benthic species, for example, samples should be collected lower in the water column closer to the lake floor. This is particularly critical during thermal stratification, which can limit mixing across thermal layers - accounting for the temporal/seasonal occupancy of the vertical water column for targeted species is therefore crucial during sampling design (Figure A2.2.9) (Lawson Handley et al. 2019; Littlefair et al. 2020).

Efforts to characterize the spatial distribution of a targeted species in a lentic system or large lotic system must distribute sampling effort over a broad spatial scale. While sampling efforts can still focus on general zones of high likelihood of occupancy (e.g., you may not need to sample pelagic zones for a species with strong littoral preferences), we recommend systematically standardizing sampling efforts such that samples are collected in a grid (e.g. offshore zones) (Ghosal et al. 2018; Brys et al.2020; Zhang et al. 2020) or at even intervals throughout a zone (e.g. nearshore zones) (Yates et al. 2020; Zhang et al. 2020) (Figure A2.2.8). The size of the grid network or length of the sampling interval will be dependent on project resources and desired spatial resolution. However, it is important to note that the limited horizontal distribution of eDNA in lentic systems can make it particularly challenging to detect rare species at low densities, especially in large lentic systems; as a result, systematic sampling designs targeting rare species may be prohibitive in large systems due to the effort/resources necessary to ensure adequate spatial coverage.

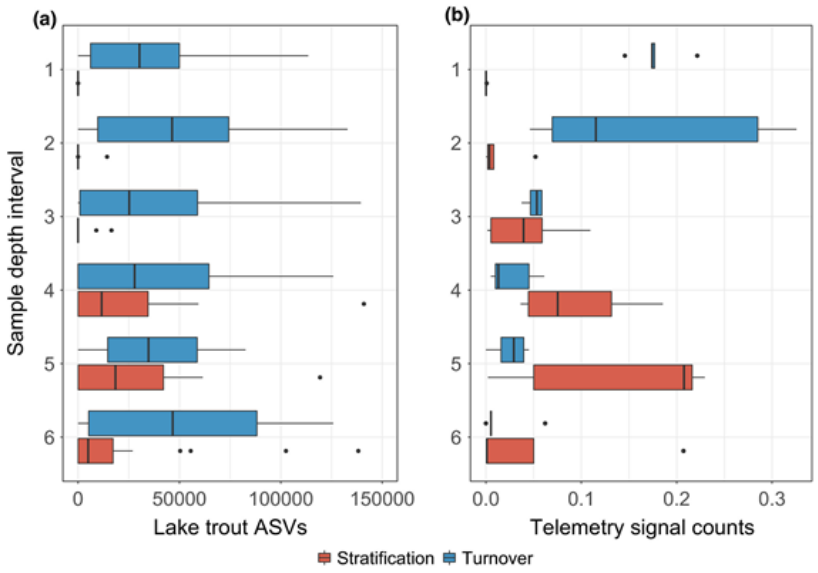

Figure 9: (a) Lake trout Amplicon Sequence Variants (ASVs) from eDNA and (b) Lake trout telemetry signals recovered at six different depths during lake turnover (red) and stratification (blue). The distribution of eDNA with fish occupancy and thermal stratification highlights the importance of species ecology and the position of eDNA sample collection in the vertical water column. Figure reproduced from Littlefair et al. (2020) *Mol. Ecol* with permission from authors; for further details, see manuscript.

Biodiversity survey applications

Applications quantifying community biodiversity can benefit from targeting a variety of microhabitats and/or ecological zones. In lentic systems and large lotic systems, microhabitat heterogeneity can be particularly pronounced in littoral zones/shorelines (rocky littoral habitat, sandy littoral habitat, silty littoral habitat, vegetated, woody debris, etc.). A key structural feature in lentic systems that affects community composition and species detection from eDNA is the proximity of a sample

to shoreline; a number of studies have consistently found that preferentially sampling from shoreline adjacent sites recovered greater biodiversity relative to offshore sites (Hanfling et al. 2016; Evans et al. 2017; Lawson Handley et al. 2019; Blabolil et al. 2020; Zhang et al. 2020). Stratified sampling strategies allocating disproportionate effort to nearshore zones and subdividing nearshore sampling based on microhabitat variation while simultaneously distributing sampling sites spatially throughout the system may therefore be a particularly effective strategy to quantify biodiversity in lentic systems (Blabolil et al. 2020). Pelagic/offshore/benthic zones would still need to be sampled, however, to detect primarily pelagic and benthic species, particularly when a system exhibits stratification (Lawson Handley et al. 2019). Nevertheless, preferentially allocating sampling effort to shoreline areas may help maximise numerical species recovery and sampling efficiency.

The number of samples needed to effectively quantify overall community composition in a given water body/location likely depends on waterbody size, environmental heterogeneity, and the expected number of species present in the ecosystem. A number of eDNA metabarcoding studies have estimated sampling effort necessary to quantify community biodiversity in lentic systems based on species accumulation curves (see Table A2.2.1). It should be noted, however, that these studies employed metabarcoding to quantify community composition. GEN-FISH utilizes qPCR, a technology with a putatively higher detection sensitivity relative to metabarcoding (Harper et al. 2018; Bylemans et al. 2019) and less dependent on community composition; the number of samples needed to adequately quantify community composition using the GEN-FISH chip may well be lower.

Nevertheless, based on these studies we recommend varying sampling effort with lake size and nearshore/offshore zone; our recommendations are also relatively conservative, given that underestimating species richness/composition can have significant management implications. To quantify overall community composition, we recommend preferentially allocating sampling effort to littoral zones, with supplemental efforts targeting offshore zones and deepwater zones to ensure detection of primarily pelagic and deepwater species. Samples should also be distributed throughout the system as much as possible, sampling different microhabitats and potentially including deepwater benthic samples that may often be necessary to detect

Table A2.2.1. Environmental DNA metabarcoding studies reporting species accumulation curve data and sampling effort needed to pass pre-defined community detection thresholds (e.g. % of species detection).

Study	Lake size (ha)	Nearshore, offshore, or combined sampling	Sample Size	Species accumulation 'threshold' %	Threshold based on % of species detections, known a-priori composition, or species richness estimator (SRE, e.g. 'Chao')	Number of samples to pass 'threshold'
Zhang et al. 2020	3	Nearshore	6	>95%	Total Species	5
Zhang et al. 2020	3	Offshore	9	>95%	Total Species	7
Zhang et al. 2020	122	Nearshore	17	>95%	Total Species	12
Zhang et al. 2020	122	Offshore	14	>95%	Total Species	13
Zhang et al. 2020	4343	Nearshore	18	>95%	Total Species	12
Zhang et al. 2020	4343	Offshore	36	>95%	Total Species	27
Evans et al. 2017	2.2	Combined	31	>85%	SRE	8*
Di Muri et al. 2020	0.3	Nearshore (too small for offshore)	8	100%	A-priori	6
Gehri et al. 2021	140	Nearshore	30	>90%	Total Species	~17
Hanfling et al. 2016	1480	Offshore	60	>85%	Total Species	10
Handley et al. 2019	1480	Nearshore (Winter)	40	>85%	Total Species	~10
Handley et al. 2019	1480	Offshore (Winter)	47	>85%	Total Species	~10
Handley et al. 2019	1480	Nearshore (Summer)	40	>85%	Total Species	6
Handley et al. 2019	1480	Offshore (Summer)	67	>85%	Total Species	15-16
Sard et al. 2019	13 – 1728	Combined	34-57	>95%	SRE	2-62
Sato et al. 2017	8.4	Combined	9	>90%	Total Species	~5-6
Sato et al. 2017	21.6	Combined	9	>90%	Total Species	~5-6
Sato et al. 2017	49.0	Combined	9	>90%	Total Species	~5-6
Sato et al. 2017	221.9	Combined	17	>90%	Total Species	~11-12

*Under moderately stringent bioinformatics criteria

~Visually estimated from species accumulation curve graph

deepwater species (Lawson Handley et al. 2019; Sard et al. 2019). Also note that the ratio of nearshore and offshore samples may need to vary with lake size; larger lakes have disproportionately larger pelagic zones (Figure A2.2.10), potentially necessitating greater sampling efforts to ensure adequate spatial coverage. In extremely small lentic systems (ponds < 1 ha), it may not be necessary to stratify the pond into nearshore/offshore zones; systematically distributing 6-15 (depending on size) samples throughout the pond (e.g. in a grid) can likely comprehensively sample the entire waterbody while proportionately representing habitat heterogeneity. For lakes between 1 and 10 hectares, we tentatively recommend a minimum sample size of 10 and 6 samples from nearshore and offshore zones, respectively. For lakes between 10 and 100 ha, we recommend a minimum sample size of 15 and 12 nearshore and offshore samples. For lakes between 100 ha and 1000 ha, we recommend collecting at least 20 nearshore and 20 offshore samples, and for lakes between 1000 and 5000 ha we recommend collecting a minimum of 25 nearshore and 30 offshore samples. For extremely large lakes (> 5000 ha) we recommend subdividing the lake into relevant regions of interest and following sampling guidelines described above. We also recommend, when possible, collecting at least two (and preferably at least three) independent samples from each stratified microhabitat zone identified and targeted beyond simple nearshore/offshore strata (rocky littoral shorelines, vegetated littoral shorelines, etc.) within a sampled lentic system.

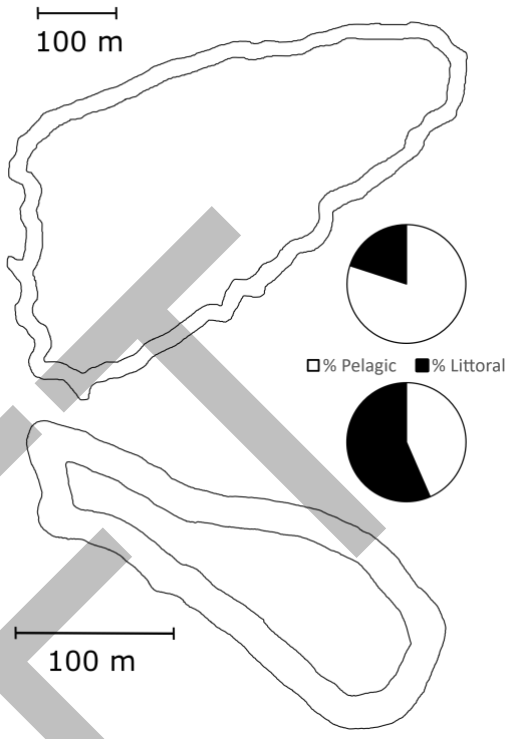

Figure 10: As lake size increases, the fraction of total lake area represented by the pelagic/offshore zone generally increases relative to the littoral/nearshore zone. In this illustrative example, any point within 20 m of the shoreline is considered littoral/nearshore.

Systematic sampling at defined intervals and/or in a grid can also be effective at quantifying spatial community composition (Zhang et al. 2020), but can require significant resources and effort to effectively cover an entire water body, particularly when it is large (e.g. > 100 ha). The allocation of relative sampling effort to offshore zones can be particularly problematicfor larger lentic systems, given the non-linear

relationship between therelative proportion of total surface area represented by nearshore andoffshore zones as lake size increases. Total nearshore/littoral zone area is often largely a function of the perimeter of a lake, whereas total offshore/pelagic area is largely a function of total surface area. As lake size increases, the relative proportion of total area represented by offshore zones generally increases (Figure A2.2.10). Although lake shape/contour can affect relative offshore/nearshore area, in large lentic waterbodies the sampling effort needed to comprehensively sample pelagic/offshore zones in a grid may be prohibitive because,as discussed previously, the limited horizontal dispersion of eDNA in lentic systems can also pose a challenge for detecting rare species at low densities.

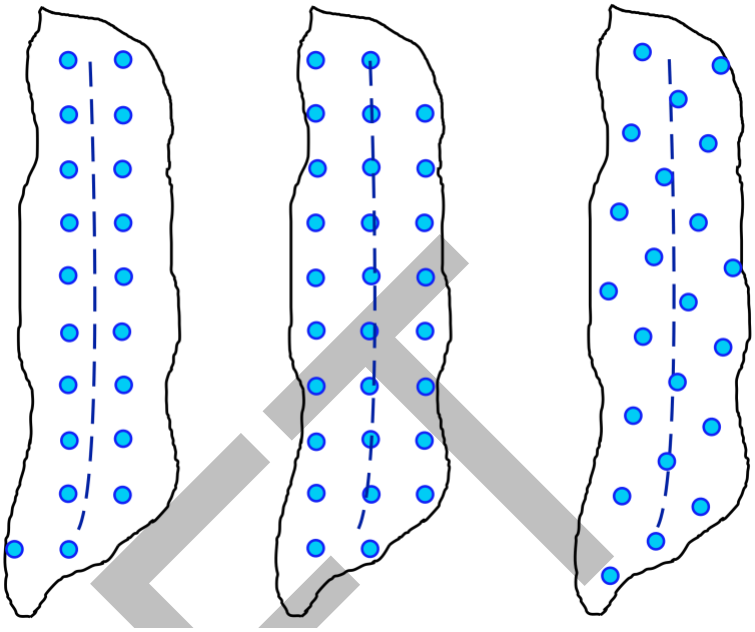

Figure 11: Systematic grid-based sampling might bias estimates in the presence of periodic and/or ecologically relevant spatial variation. In this hypothetical example, sampling in a grid pattern might completely miss (left) or oversample (middle) water above the deepest area of the lake (represented by the dashed blue line) due to grid orientation/placement. Note that altering grid orientation may help partially alleviate this problem (right).

Nevertheless, to quantify spatial community composition in lentic systems and large lotic systems we recommend stratified systematic sampling. Sampling should be stratified between nearshore and offshore zones, with systematic even-interval sampling in littoral/nearshore zones (e.g., every XX m at a standardized distance). Due to the horizontal dispersion range of eDNA, we recommend collecting samples approximately 2-5 m from the shore (keep the distance consistent across sample sites). In offshore zones, we recommend grid-based sampling designs (Figure A2.2.8) or sampling along the mid-line of the pelagic zone (Lawson Handley et al. 2019; Yates et al. 2020; Zhang et al. 2020), which may be more tractable in smaller lentic systems (e.g., < 2 ha) (Zhang et al. 2020). Determining sampling effort to quantify spatial community composition is more challenging, and depends on the desired spatial resolution (with higher resolution requiring increased sampling effort). The relative division of samplingeffort between nearshore and offshore zones, the depth in the

vertical water column where samples are collected, and the size of the standardized intervals/grid units will depend on projected community composition, project objectives, and resources.

It is important to note, however, that systematic sampling has the potential to introduce bias when underlying periodic spatial variation in the sampled strata occurs (Krebs 2014); there may be circumstances where ecologically relevant spatial variation could bias systematic sampling in lentic systems. As an example, imagine a survey for abenthic species inhabiting a long but narrow lake in which the deepest habitat in the lake runs along its approximate mid-line; a grid-based sampling scheme sampling multiple points in the vertical water column may miss sampling relevant ecological variation if a grid-lines are oriented in such a manner that they completely miss sampling the lake mid-line or, conversely, over-represent that habitat if they completely cover the mid-line (Figure A2.2.11). Systemic sampling with a grid or even-interval sampling is probably *generally* fine in most lentic systems and/or for most species, but its use does require careful consideration of the spatial structure of the system, the ecology of relevant species, and the orientation of the grid. If grid-based sampling may bias results, random sampling (or grid-based sampling at a much higher resolution) maybe necessary.

Quantitative estimates of eDNA in lentic systems

Spatially pairing eDNA samples with relative estimates of abundance in lentic systems is relatively straightforward – eDNA samples can be collected in close proximity to conventional sampling gear locations, after which CPUE/BPUE estimates from conventional sampling can be directly paired with eDNA concentrations. However, as discussed in previous sections, estimates of CPUE/BPUE can often exhibit a poor relationship with absolute organism abundance (Yates et al. 2020), for a number of potential reasons. Conventional sampling gear can often be size- and species- selective (Sard et al. 2019); if species or different life-history stages within a species exhibit different spatial preferences and/or movement patterns, relative estimates of abundance may not accurately capture total population abundance. Yates et al. 2020 also found that CPUE/BPUE exhibited a poor correlation with brook trout abundance across nine study lakes, primarily due to differences in ‘catchability’ across populations (unpublished

data). Absolute abundance estimates (e.g. obtained through CMR methods) may be particularly important for correlating eDNA and abundance in lentic systems.

Obtaining absolute estimates of abundance in lentic systems and pairing them with eDNA concentrations estimated at the appropriate comparable 'scale', however, can be challenging. CMR estimates are limited to the general scale of movement of organisms into and out of an area (Schwarz and Seber 1999). For some species that exhibit relatively high site-specific fidelity, CMR estimates can be spatially paired with local estimated of eDNA concentration (e.g Nevers et al. 2018). However, for many species it may only be possible to obtain 'ecosystem-level' estimates of abundance from CMR that correspond to the number of fish present within the entire lentic system (i.e. 'whole-lake' estimates of abundance). To facilitate comparisons with other lentic systems, such estimates of abundance must be further standardized by spatial metrics (e.g., surface area, volume, etc.). The suitability of the spatial metric used should depend on the ecology of the focal species combined with seasonal changes in thermal stratification (Littlefair et al. 2020). During mixing phases prior to and after thermal stratification, for example, focusing on total lake volume could potentially improve modelling efforts. When lakes are stratified, abundance estimates for benthic or littoral/pelagic species could be standardized by volumes in the hypolimnion and epilimnion, respectively. Simplifying assumptions, however, could potentially be made in the absence of detailed bathymetric data needed to quantify relevant layer volumes. If, for example, relationships between organism abundance and eDNA are being examined across a number of stratified lakes for species that inhabit the epilimnion, standardizing density metrics by surface area could be appropriate if thermocline depth was relatively similar across study systems. Several studies, for example, have found strong correlations between eDNA concentrations and surface-area standardized metrics of abundance in lentic systems (Klobucar et al. 2017; Spear et al. 2020; Yates et al. 2020).

'Ecosystem-level' estimates of abundance (standardized by water volume or surface area) must be compared to eDNA concentrations estimated at similar 'ecosystem-level' scales (Chambert et al. 2018). However, many studies may also have 'detection' applications as a primary focus (either species-specific or community-level), and may often put effort into sampling different zones or microhabitats within an

ecosystem disproportionate to the fraction of the total ecosystem area or volume represented by each zone/microhabitat. There are several potential solutions to overcoming this problem, the most obvious being to not introduce spatial bias in sampling regimes. This could be done by collecting eDNA samples at randomly determined locations (G.Cochran 1977; Krebs 2014), but systematic spatial sampling represents a potential alternative strategy in which eDNA samples are collected in a comprehensive manner across the whole ecosystem (e.g. using a 'grid' system) (Krebs 2014; Ghosal et al. 2018; Brys et al. 2020).

Box I: Spatial sampling regimes can introduce bias in average 'ecosystem-level' estimates of eDNA. Reproduced with permission from authors from Yates et al. (*submitted*)

In Yates et al. 2020a, equal sampling effort was given to littoral and pelagic zones - four samples were collected from each zone in each lake. However, the fraction of the total area represented by each zone differed significantly with lake size, because the littoral area of a lake is largely a function of its perimeter whereas the pelagic zone is largely a function of its area (Figure 9). Calculating a 'lake-wide' eDNA average by averaging eDNA concentrations across all collected samples would bias estimates from larger lakes, where the pelagic zone tends to represent a larger fraction of total lake area; resulting lake-wide averages would be increasingly biased towards littoral concentrations as lake size increased. Study organisms (Brook Trout) exhibited strong littoral preferences (Tiberti et al. 2017) and, as a result, eDNA concentrations were correspondingly higher in littoral samples across all study lakes. Corresponding surface-area standardized 'ecosystem-level' abundance estimates (e.g. fish/ha) in Yates et al. 2020a were calculated from total lake area and included low-density pelagic zones. Averaging eDNA concentrations across all littoral and pelagic samples would have thus biased mean 'lake-wide' eDNA estimates higher in large lakes with proportionately larger pelagic zones, obscuring the relationship between mean lake-wide eDNA concentration and organism abundance. As an alternative, the contribution of littoral and pelagic eDNA concentrations to the estimation of a 'lake-wide' mean eDNA concentration were weighted by the fraction of total lake area represented by each zone (Figure 12, Table 2).

However, random sampling can be logistically complex and prohibitive from an effort perspective, as the number of samples needed to comprehensively spatially sample an entire lentic system can be substantial. Many eDNA surveys in lentic systems are also often a part of comprehensive projects with a multitude of research goals; random or grid-based sampling designs may not be possible or desirable, depending on study system and objectives. There may also be relevant ecological reasons to preferentially sample different habitats—fish species, for example, may exhibit strongpreferences for nearshore, offshore, or benthiczones.

Stratified sampling represents an alternative sampling strategy that can

simultaneously reduce the sampling effort required to sample the entire ecosystem and increase the precision of 'overall' mean eDNA estimates (Krebs 2014). If sampling is conducted in a manner that disproportionately biases sampling effort in different ecological zones/microhabitats, the relative contribution of samples from each ecological zone to the calculation of a 'whole-ecosystem' eDNA average could alternatively be weighted by the fraction of the total ecosystem represented by that ecological zone (G.Cochran 1977; Krebs 2014; Yates et al. 2020) (see Box I). Determining relevant ecological zones to sample, and how weighting should be distributed, must be done on a study- and species-specific basis with careful consideration. Strata for species with clear littoral or pelagic preferences are relatively simple to demarcate and weigh. As described above for spatial biodiversity surveys, eDNA samples could be collected systematically in a standardized grid in pelagic zones (or along the pelagic mid-line in small systems) and at even-intervals in the littoral zones (Figure A2.2.12). Averages from each zone could then be calculated and their contribution to a 'whole-lake' eDNA average weighed by the fraction of total lake area represented by each zone (Table A2.2.2). However, species can have more complex preferences based on aquatic macrophyte cover, substrate, depth, season etc.Sampling regimes for species with benthic habitat preferences (e.g. lake trout), for example, would need to ensure adequate sampling of the vertical water column while also accounting for seasonal effects on habitat preferences and thermal mixing (Littlefairet al. 2020).

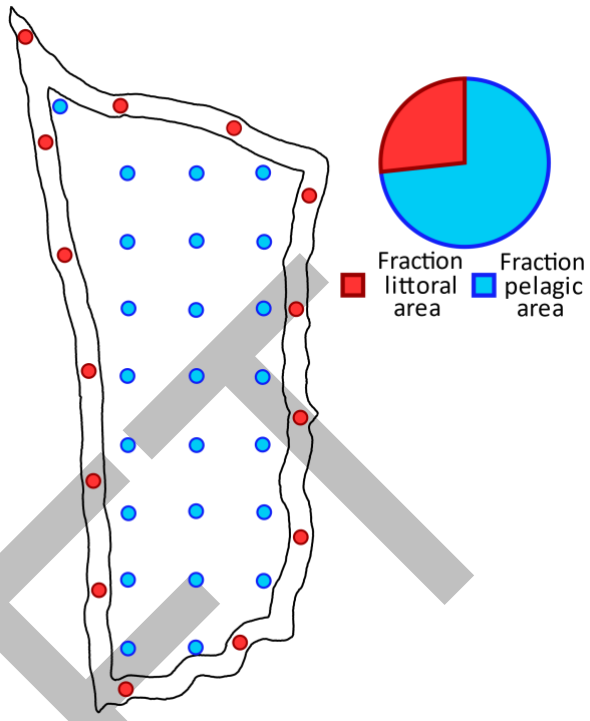

Figure 12: Sample design to quantify average lake eDNA concentration for a species with strong pelagic/littoral preferences, with offshore (pelagic) samples collected using a grid system and nearshore (littoral) samples collected at even-intervals. Mean pelagic (blue) and littoral (red) eDNA concentrations could be weighted by fraction of total area represented by each zone (pie graph) when calculating 'whole-lake' eDNA average.

Whether sampling in stratified zones should be collected randomly or systematically largely depends on whether systematic sampling might introduce bias due to underlying periodic spatial variation in the waterbody. Once again, careful

consideration of the spatial structure of the sampled ecosystem, the ecology of targeted species, and the orientation of a grid are necessary to prevent the introduction of bias into sampling efforts. If there is potential for systematic sampling to introduce bias into estimates, sampling should be conducted randomly instead.

No study to date has published on the sampling effort necessary to adequately estimate the mean eDNA concentration of a lentic system. Environmental DNA concentrations are likely to approximate a negative binomial distribution (Chambert et al. 2018), with occasional samples containing relatively high concentrations. When the mean of a sample is expected to be a large value (likely the case for eDNA concentrations/L for all but the rarest species), the sampling effort to accurately estimate that mean under the negative binomial distribution is largely a function of the dispersion parameter (θ), as can be seen from the below equation (Krebs 2014):

$$n = (100t_{\alpha})^2/r^2 * (1/\mu + 1/\theta)$$

Where:

n = sample size
t = student's t-value for desired n-1 at α level of confidence
r = desired level of error in estimate of the mean (percent)
u = expected sample mean
θ = expected dispersion parameter

θ also describes sample variance under the negative binomial distribution (Krebs2014):

$$s^2 = \mu + \mu^2/\theta$$

Therefore, as the value of θ generally decreases (and thus sample variance increases) the sampling effort necessary to accurately quantify the mean of a negative binomial distribution increases. In simple terms, the higher the expected variance of eDNA concentrations, the more samples will be needed to accurately quantify the mean. Careful consideration of how the ecology of a species affects the distribution of eDNA within an ecosystem will help inform necessary sampling effort. Yates et al. (2020), for example, found that eDNA concentrations for brook trout in littoral zones (their preferred habitat) tended to exhibit higher mean estimates but also higher variability than pelagic zones (Table A2.2.2). Fewer samples might be needed to accurately quantify the mean pelagic brook trout eDNA concentration in a lake, despite the fact that the pelagic zone often represents a larger fraction of surface area of

moderate-large lentic systems. Generally, we would predict that higher habitat heterogeneity in nearshore zones will likely lead to larger variation in nearshore eDNA concentrations for most species, necessitating greater sampling effort to accurately quantify concentrations in nearshore strata. Without any a-priori knowledge or expectations regarding expected variance in eDNA (and thus potential for low values of θ), we would generally recommend collecting at least a minimum of 15 samples in lenticsystems larger than 3 ha to accurately quantify the concentration of eDNA in a given 'zone'/strata, with 20-25 samples representing a more ideal sample size (particularly fornearshore zones). This level of sampling may not be possible in smaller lentic systems (e.g. between ~1-3 ha) without violating conditional independence of samples; it is probably adequate to take 8-12 samples distributed throughout the nearshore zone and4-8 in 'offshore' zones, depending on pond morphology. In extremely small lentic systems (ponds < 1 ha), systematically distributing 6-15 (depending on size) samples throughout the pond (e.g. in a grid) can likely comprehensively sample the entire waterbody without the need for stratified sampling between nearshore and offshore zones.

Samples collected during initial and/or pilot sampling efforts can be used to perform *post-hoc* power analyses to determine required future sampling effort and its allocation across zones using the theoretical formula described above or through empirical estimation using bootstrapping. Alternatively, values published from previous studies (assuming similar collection, extraction, and analysis protocols) can be used to parameterize values for power analyses.

Table A2.2.2. Lake zone area and corresponding eDNA concentrations (minimum and maximum observed eDNA concentrations per lake zone included in parentheses). Reproduced from Yates et al. (2020) with permission from authors.

Site	Pelagic area (ha)	Littoral area (ha)	Mean Pelagic eDNA (copies/L)	Mean Littoral eDNA (Copies/L)	Weighted Mean eDNA (Copies/L)
Cobb	1.0	1.3	253.8 (39.6 - 557.9)	854.6 (35.9 - 2650.4)	592.2
Dog	8.5	3.1	3447.1 (683.8 - 9148.1)	9796.7 (3705.6 - 16839.3)	5131.1
Helen	1.2	1.3	1342.4 (854.3 - 1586.9)	3514.4 (2083.2 - 5060.5)	2445.9
Margaret	14.4	3.6	791.9 (706.8 - 968.1)	3034.1 (814.3 – 5689.7)	1240.4
McNair	0.7	1.0	2395.4 (2214.9 - 2495.9)	3505.0 (3181.1 - 4886.4)	3050.5
Mud	4.7	2.6	399.3 (261.7 - 580.7)	1550.6 (628.3 - 3833.3)	797.5
Olive	0.5	1.2	8084.6 (5115.9 - 11758.9)	7684.7 (1839.6 - 11829.1)	7805.1
Ross	4.6	2.0	790.5 (439.7 - 1101.9)	1209.8 (3763 - 2576.0)	917.4
Temple	1.6	1.7	1180.1 (854.3 - 1685.7)	1850.3 (1133.6 - 3887.0)	1530.6
Hidden	11.8	2.6	342.0 (149.3 - 472.4)	2652.9 (1277.2 – 5758.1)	847.2

References

Allison, M.J., Round, J.M., Bergman, L.C., Mirabzadeh, A., Allen, H., Weir, A., and Helbing, C.C. 2021. The effect of silica desiccation under different storage conditions on filter - immobilized environmental DNA. BMC Res. Notes: 1–6. BioMed Central. doi:10.1186/s13104-021-05530-x.

Baldigo, B.P., Sporn, L.A., George, S.D., and Ball, J.A. 2017. Efficacy of Environmental DNA to Detect and Quantify Brook Trout Populations in Headwater Streams of the Adirondack Mountains, New York. Trans. Am. Fish. Soc. **146**(1): 99–111. Taylor & Francis. doi:10.1080/00028487.2016.1243578.

Barnes, M.A., Turner, C.R., Jerde, C.L., Renshaw, M.A., Chadderton, W.L., and Lodge, D.M. 2014. Environmental Conditions Influence eDNA Persistence in Aquatic Systems. doi:10.1021/es404734p.

Blabolil, P., Harper, L., Ricanova, S., Sellers, G., Muri, C. Di, Můza, T., Vašek, M., Sajdlová, Z., Rychtecký, P., Znachor, P., Hejzlar, J., Peterka, J., and Hanfling, B. 2020. Environmental DNA metabarcoding uncovers environmental correlates of fish communities in spatially heterogeneous freshwater habitats. Ecol. Indic. **126**: 107698. doi:10.1016/j.ecolind.2021.107698.

Brys, R., Haegeman, A., Halfmaerten, D., Neyrinck, S., Staelens, A., Auwerx, J., and Ruttink, T. 2020. Monitoring of spatiotemporal occupancy patterns of fish and amphibian species in a lentic aquatic system using environmental DNA. Mol. Ecol. (April): 1–14. doi:10.1111/mec.15742.

Bylemans, J., Gleeson, D.M., Duncan, R.P., Hardy, C.M., and Furlan, E.M. 2019. A performance evaluation of targeted eDNA and eDNA metabarcoding analyses for freshwater fishes. Environ. DNA **1**(4): 402–414. doi:10.1002/edn3.41.

Carraro, L., Mächler, E., Wüthrich, R., and Altermatt, F. 2020. Environmental DNA allows upscaling spatial patterns of biodiversity in freshwater ecosystems. Nat. Commun. **11**(1): 1–12. Springer US. doi:10.1038/s41467-020-17337-8.

Carraro, L., Stauffer, J.B., and Altermatt, F. 2021. How to design optimal eDNA sampling strategies for biomonitoring in river networks. Environ. DNA **3**(1): 157–172. doi:10.1002/edn3.137.

Chambert, T., Takahara, T., Pilliod, D.S., Goldberg, C.S., and Doi, H. 2018. An analytical framework for estimating aquatic species density from environmental DNA. Ecol. Evol. **8**: 3468–3477. doi:10.1002/ece3.3764.

Colwell, R.K., and Coddington, J.A. 1995. Estimating terrestrial biodiversity through extrapolation. Biodivers. Meas. Estim. **345**(1311): 101–118. doi:10.2307/3544596.

Curtis, A.N., Tiemann, J.S., Douglass, S.A., Davis, M.A., and Larson, E.R. 2020. High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. Divers. Distrib. (July): 1–14. doi:10.1111/ddi.13196.

Darling, J.A., Jerde, C.L., and Sepulveda, A.J. 2021. What Do You Mean by false positive? Environ. DNA (March): Early view. doi:10.1007/3-540-45323-7_66.

Eichmiller, J.J., Bajer, P.G., and Sorensen, P.W. 2014. The relationship between the distribution of common carp and their environmental DNA in a small lake. PLoS One **9**(11): 1–8. doi:10.1371/journal.pone.0112611.

Erickson, R.A., Merkes, C.M., and Mize, E.L. 2019. Sampling Designs for Landscape-level eDNA Monitoring Programs. Integr. Environ. Assess. Manag. **15**(5): 760–771. John Wiley & Sons, Ltd. doi:10.1002/ieam.4155.

Evans, N.T., Li, Y., Renshaw, M.A., Olds, B.P., Deiner, K., Turner, C.R., Jerde, C.L., Lodge, D.M., Lamberti, G.A., and Pfrender, M.E. 2017. Fish community assessment with eDNA metabarcoding: effects of sampling design and bioinformatic filtering. CJFAS (January): 1362–1374.

G.Cochran, W. 1977. Sampling Techniques: third edition.

Ghosal, R., Eichmiller, J.J., Witthuhn, B.A., and Sorensen, P.W. 2018. Attracting Common Carp to a bait site with food reveals strong positive relationships between fish density, feeding activity, environmental DNA, and sex pheromone release that could be used in invasive fish management. Ecol. Evol. **8**(March): 6714–6727. doi:10.1002/ece3.4169.

Goldberg, C.S., Strickler, K.M., and Fremier, A.K. 2018. Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: Increasing efficacy of sampling designs. Sci. Total Environ. **633**: 695–703. Elsevier B.V. doi:10.1016/j.scitotenv.2018.02.295.

Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., Spear, S.F., McKee, A., Oyler-McCance, S.J., Cornman, R.S., Laramie, M.B., Mahon, A.R., Lance, R.F., Pilliod, D.S., Strickler, K.M., Waits, L.P., Fremier, A.K., Takahara, T., Herder, J.E., and Taberlet, P. 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol. Evol. **7**(11): 1299–1307. doi:10.1111/2041-210X.12595.

Green, R.H., and Young, R.C. 1993. Sampling to Detect Rare Species. Ecol. Appl. **3**(2): 351–356.

Hanfling, B., Handley, L.L., Read, D.S., Hahn, C., Li, J., Nichols, P., Blackman, R.C., Oliver, A., and Winfield, I.J. 2016. Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. Mol. Ecol. **25**: 3101–3119. doi:10.1111/mec.13660.

Harper, L.R., Lawson Handley, L., Hahn, C., Boonham, N., Rees, H.C., Gough, K.C., Lewis, E., Adams, I.P., Brotherton, P., Phillips, S., and Hänfling, B. 2018. Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great crested newt (Triturus cristatus). Ecol. Evol. **8**(12): 6330–6341. doi:10.1002/ece3.4013.

Harrison, J.B., Sunday, J.M., and Rogers, S.M. 2019. Predicting the fate of eDNA in the environment and implications for studying biodiversity. Proc. R. Soc. B **286**.

Hubert, W.., Pope, K.L., and Dettmers, J.M. 2012. Passive Capture Techniques. *In* Fisheries Techniques, 3rd edition. *Edited by* A.V. Zale, D.L. Parrish, and T.. Sutton. American Fisheries Society, Bethesda, Maryland.

Jane, S.F., Wilcox, T.M., Mckelvey, K.S., Young, M.K., Schwartz, K., Lowe, W.H., and Letcher, B.H. 2015. Distance , flow and PCR inhibition : eDNA dynamics in two headwater streams. : 216–227. doi:10.1111/1755-0998.12285.

Jerde, C.L. 2019. Can we manage fisheries with the inherent uncertainty from eDNA? J. Fish Biol. (November): 1–13. doi:10.1111/jfb.14218.

Jerde, C.L., Chadderton, W.L., Mahon, A.R., Renshaw, M.A., Corush, J., Budny, M.L., Mysorekar, S., and Lodge, D.M. 2013. Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program. Can. J. Fish. Aquat. Sci. **70**(4): 522–526. doi:10.1139/cjfas-2012-0478.

Klobucar, S.L., Rodgers, T.W., and Budy, P. 2017. At the forefront: evidence of the applicability of using environmental DNA to quantify the abundance of fish populations in natural lentic waters with additional sampling considerations. Can. J. Fish. Aquat. Sci. **74**: 2030–2034.

Klymus, K.E., Richter, C.A., Chapman, D.C., and Paukert, C. 2015. Quantification of eDNA shedding rates from invasive bighead carp Hypophthalmichthys nobilis and silver carp Hypophthalmichthys molitrix q. Biol. Conserv. **183**: 77–84. Elsevier Ltd. doi:10.1016/j.biocon.2014.11.020.

Krebs, C.J. 2014. Ecological Methodology. *In* 3rd edition. Online.

Lacoursière-Roussel, A., Côté, G., Leclerc, V., Bernatchez, L., and Cadotte, M. 2016. Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. J. Appl. Ecol. **53**(4): 1148–1157. doi:10.1111/1365-2664.12598.

Lawson Handley, L., Read, D.S., Winfield, I.J., Kimbell, H., Johnson, H., Li, J., Hahn, C., Blackman, R., Wilcox, R., Donnelly, R., Szitenberg, A., and Hänfling, B. 2019. Temporal and spatial variation in distribution of fish environmental DNA in England's largest lake. Environ. DNA **1**(1): 26–39. doi:10.1002/edn3.5.

Levi, T., Allen, J.M., Bell, D., Joyce, J., Russell, J.R., David, A., Vulstek, S.C., Yang, C., and Yu, D.W. 2019. Environmental DNA for the enumeration and management of Pacific salmon. Mol. Ecol. Resour. **19**: 597–608.

Littlefair, J.E., Hrenchuk, L.E., Blanchfield, P.J., Rennie, M.D., and Cristescu, M.E. 2020. Thermal stratification and fish thermal preference explain vertical eDNA distributions in lakes. Mol. Ecol. (July): 1–14. doi:10.1111/mec.15623.

Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M., and Minamoto, T. 2014. The release rate of environmental DNA from juvenile and adult fish. PLoS One **9**(12): 1–

13. doi:10.1371/journal.pone.0114639.

Nevers, M.B., Byappanahalli, M.N., Morris, C.C., Shively, D., Przybyla-kelly, K., Spoljaric, A.M., Dickey, J., and Roseman, E.F. 2018. Environmental DNA (eDNA): A tool for quantifying the abundant but elusive round goby (Neogobius melanostomus). PLoS One **13**: e0191720. doi:10.5066/F7GH9H6F.Funding.

Nevers, M.B., Przybyla-Kelly, K., Shively, D., Morris, C.C., Dickey, J., and Byappanahalli, M.N. 2020. Influence of sediment and stream transport on detecting a source of environmental DNA. PLoS One **15**(12 December): 1–21. doi:10.1371/journal.pone.0244086.

Olds, B.P., Jerde, C.L., Renshaw, M.A., Li, Y., Evans, N.T., Turner, C.R., Deiner, K., Mahon, A.R., Brueseke, M.A., Shirey, P.D., Pfrender, M.E., Lodge, D.M., and Lamberti, G.A. 2016. Estimating species richness using environmental DNA. Ecol. Evol. **6**(12): 4214–4226. doi:10.1002/ece3.2186.

Pilliod, D.S., Goldberg, C.S., Arkle, R.S., and Waits, L.P. 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. **1130**(January): 1123–1130.

Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., Roset, N., Schabuss, M., Zornig, H., and Dejean, T. 2018. Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. Sci. Rep. **8**(1): 1–13. doi:10.1038/s41598-018-28424-8.

Rose, G.A., and Kulka, D.W. 1999. Hyperaggregation of fish and fisheries: how catch-per-unit-effort increased as the northern cod (Gadus morhua) declined. Can. J. Fish. Aquat. Sci. **56**: 118–127.

Rourke, M.L., Fowler, A.M., Hughes, J.M., Broadhurst, M.K., Dibattista, J.D., Fielder, S., Wilkes, J., and Elise, W. 2021. Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. Environ. DNA **Early View**: 1–25. doi:10.1002/edn3.185.

Sard, N.M., Herbst, S.J., Nathan, L., Uhrig, G., Kanefsky, J., Robinson, J.D., and Scribner, K.T. 2019. Comparison of fish detections, community diversity, and relative abundance using environmental DNA metabarcoding and traditional gears. Environ. DNA **1**(4): 368–384. doi:10.1002/edn3.38.

Schwarz, C.J., and Seber, G.A.F. 1999. A review of estimating animal abundance III. Stat. Sci. **14**: 1–126.

Sepulveda, A.J., Al-Chokhachy, Robert Laramie, M.B., Crapster, K., Knotek, L., Miller, B., Zale, A. V., and Pilliod, D.S. 2020. It's complicated…Environmental DNA as a predictor of trout and char abundance in streams. CJFAS **Early View**.

Shogren, A.J., Tank, J.L., Andruszkiewicz, E., Olds, B., Andrew, R., Jerde, C.L., and Bolster, D. 2017. Controls on eDNA movement in streams: Transport, Retention,

and Resuspension. Sci. Rep. **7**(December 2016): 1–11. Springer US. doi:10.1038/s41598-017-05223-1.

Spear, M.J., Embke, H.S., Krysan, P.J., and Vander, M.J. 2020. Application of eDNA as a tool for assessing fish population abundance. Environ. DNA (May): 1–9. doi:10.1002/edn3.94.

Spear, S.F., Groves, J.D., Williams, L.A., and Waits, L.P. 2015. Using environmental DNA methods to improve detectability in a hellbender (Cryptobranchus alleganiensis) monitoring program. Biol. Conserv. **183**: 38–45. Elsevier Ltd. doi:10.1016/j.biocon.2014.11.016.

Takeuchi, A., Iijima, T., Kakuzen, W., Watanab, S., and Yamada, Y. 2019. Release of eDNA by different life history stages and during spawning activities of laboratory-reared Japanese eels for interpretation of oceanic survey data. Sci. Rep. **9**(April): 1–9. doi:10.1038/s41598-019-42641-9.

Thalinger, B., Kirschner, D., Pütz, Y., Moritz, C., Schwarzenberger, R., Wanzenböck, J., and Traugott, M. 2021a. Lateral and longitudinal fish environmental DNA distribution in dynamic riverine habitats. Environ. DNA **3**(1): 305–318. doi:10.1002/edn3.171.

Thalinger, B., Rieder, A., Teuffenbach, A., Pütz, Y., Schwerte, T., Wanzenböck, J., and Traugott, M. 2021b. The Effect of Activity, Energy Use, and Species Identity on Environmental DNA Shedding of Freshwater Fish. Front. Ecol. Evol. **9**(February): 1–13. doi:10.3389/fevo.2021.623718.

Tiberti, R., Nelli, L., Brighenti, S., Iacobuzio, R., Rolla, M., Nelli, L., Brighenti, S., Iacobuzio, R., and Spatial, M.R. 2017. Spatial distribution of introduced brook trout Salvelinus fontinalis (Salmonidae) within alpine lakes: evidences from a fish eradication campaign. Eur. Zool. J. **84**(1): 73–88. Taylor & Francis. doi:10.1080/11250003.2016.1274436.

Turner, C.R., Uy, K.L., and Everhart, R.C. 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biol. Conserv. **183**: 93–102. Elsevier Ltd. doi:10.1016/j.biocon.2014.11.017.

Wilcox, T.M., Mckelvey, K.S., Young, M.K., Sepulveda, A.J., Shepard, B.B., Jane, S.F., Whiteley, A.R., Lowe, W.H., and Schwartz, M.K. 2016. Understanding environmental DNA detection probabilities : A case study using a stream-dwelling char Salvelinus fontinalis. Biol. Conserv. **194**: 209–216. The Authors. doi:10.1016/j.biocon.2015.12.023.

Wilcox, T.M., Young, M.K., McKelvey, K.S., Isaak, D.J., Horan, D.L., and Schwartz, M.K. 2018. Fine-scale environmental DNA sampling reveals climate-mediated interactions between native and invasive trout species. Ecosphere **9**(11). doi:10.1002/ecs2.2500.

Wood, Z.T., Erdman, B.F., York, G., Trial, J.G., and Kinnison, M.T. 2020. Experimental

assessment of optimal lotic eDNA sampling and assay multiplexing for a critically endangered fish. Environ. DNA **2**(4): 407–417. doi:10.1002/edn3.64.

Wood, Z.T., Lacoursière-roussel, A., Leblanc, F., Trudel, M., and Wood, Z.T. 2021. Spatial Heterogeneity of eDNA Transport Improves Stream Assessment of Threatened Salmon Presence , Abundance , and Location. **9**(April). doi:10.3389/fevo.2021.650717.

Wu, Q., Kawano, K., Uehara, Y., Okuda, N., Hongo, M., Tsuji, S., Yamanaka, H., and Minamoto, T. 2018. Environmental DNA reveals nonmigratory individuals of Palaemon paucidens overwintering in Lake Biwa shallow waters. Freshw. Sci. **37**(April): 307–314. doi:10.1086/697542.

Yates, M., Glaser, D., Post, J., Cristescu, M., Fraser, D., and Derry, A. 2020. The relationship between eDNA particle concentration and organism abundance in nature is strengthened by allometric scaling. Mol. Ecol. **Early View**. doi:10.1111/mec.15543.

Yates, M.C., Fraser, D.J., and Derry, A.M. 2019. Meta-analysis supports further refinement of eDNA for monitoring aquatic species-specific abundance in nature. Environ. DNA **1**(January): 5–13. doi:10.1002/edn3.7.

Zhang, S., Lu, Q., Wang, Y., Wang, X., Zhao, J., and Yao, M. 2020. Assessment of fish communities using environmental DNA: Effect of spatial sampling design in lentic systems of different sizes. Mol. Ecol. Resour. **20**(1): 242–255. doi:10.1111/1755-0998.13105.