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NEON AOS DESIGN OPTIMIZATION: FISH SAMPLING IN STREAMS AND LAKES

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1 Introduction

1.1 Background

Fish abundance estimates at the National Ecological Observatory Network’s (NEON) lakes and streams are challenging to standardize and measure both within and across sites. Standardizing for sampling strategies that minimize safety hazards while maximizing data completeness is necessary in order to maintain NEON’s mission to provide robust cross-continental fish data.

Fish abundance in streams and lakes is frequently derived using depletion sampling. Depletion sampling involves netting off a known area and then performing iterative electrofishing (EF) passes within the known area. Generally, the first pass will catch the most fish, while the second, third, and any successive passes will catch fewer and fewer fish until no more are captured. However, depletion sampling is also time consuming, and performing multiple EF passes until no fish are captured is uncommon. As a result, the standard rule is to complete three EF passes, which is deemed sufficient to estimate fish abundance values within a given sampling area (Bonar et al., 2009). Established statistical models are then used to estimate fish abundance with uncertainty. As a result, estimates from one reach may not be representative of the entire study area, so additional reaches are sampled using three-pass depletion EF. Thus, the NEON fish sampling strategy has established designated “fixed” reaches that are always sampled using three-pass depletion within a given stream or lake site, along with similarly sized “random” reaches that only have a single EF pass. The current fish protocol samples three fixed reaches. However, it is unclear whether three fixed reaches are always needed to confidently estimate a site’s change in fish abundance over time, or if the number of fixed reaches can be reduced to one in the event that all three cannot be sampled during a fishing bout.

The fish sampling design for NEON’s streams consists of ten ~100-meter-long reaches, while lakes have ten reaches that extend from the littoral zone to the pelagic zone. Three of the ten reaches are designated as fixed reaches, to be sampled using three-pass depletion twice annually. The remaining seven reaches are identified as random reaches, three of which are sampled during the fish bout. Random reach order was assigned randomly at the inception of NEON. Within a year, both the spring and fall bouts sample the same random reaches. The following year, the next set of three random reaches are fished following the provided random reach order. Originally, sampling three fixed reaches with three EF passes and three random reaches with one EF pass across NEON’s lakes and streams was scheduled across a five-day window with five to ten field scientists.

This sampling design was fully operational across five NEON lakes and 23 NEON streams starting in 2018, while three rivers (Flint River (FLNT), Black Warrior River (BLWA), Lower Tombigbee River (TOMB)), one stream (Como Creek (COMO)), and two lakes (Suggs Lake (SUGG) and Barco Lake (BARC)) are not sampled due to safety and/or permitting restrictions. Additionally, McDiffit Creek (MCDI), has a shorter permitted reach (~500m), so there are only five fish reaches total, with two fixed reaches and three random reaches. Teakettle Creek (TECR) has been shortened to ~760m due to access restrictions from dense vegetation and barriers (i.e., waterfalls), resulting in 8 reaches total with two fixed reaches and six random reaches.

Despite the best efforts from field scientists, the originally scheduled five-day fish sampling window was not sufficient time to complete the sampling at all three fixed and three random reaches, due to adverse weather, competing priorities, or other logistical constraints. In addition to these early incomplete bouts, the COVID-19 pandemic resulted in widespread staff shortages throughout 2020, 2021 and early 2022,

making it impossible to complete the full fish bout within the standardized five-day sample window. In 2021, continued resource constraints and competing priorities led to a decrease in the planned fish sampling window from five to three total days, with a reduced requirement to sample one fixed reach and three random reaches if all three fixed reaches could not be completed.

1.2 Study Aims

We explored how sampling only one fixed reach compared to sampling three fixed reaches impacts the ability to detect a 20% year over year trend in fish abundance in NEON streams, and how the same fixed reach reduction affected fish catch-per-unit-effort (CPUE) in lakes. For streams, we implemented a modeling exercise that simulated fish abundances with a 20% year over year trend, followed by a power analysis on these simulated trend data. We hypothesized that a reduction in sampling from three-fixed reaches to one-fixed reach would increase the time to achieving the power to detect trends across all NEON stream sites (Figure 1). We also implemented a similar power analysis at the individual reach level within stream sites to determine which reach was expected to exceed the 0.9 power threshold first and used that to inform the fixed reach prioritization. This specific modeling exercise is not applicable in lakes because their littoral to pelagic sampling extent cannot quantify the reach area that is needed to predict reach-level abundances. Thus, we quantified how CPUE changed when going from three fixed reaches to one fixed reach and then prioritized what fixed reaches to sample first by identifying what reaches had the highest overall CPUE.

Figure 1A exhibits how outputs of power to detect 20% year over year trends in fish abundance in streams vary through time under two scenarios: sampling only one fixed reach (purple line) or continued sampling of all three fixed reaches (green line) 25 years into the future. Between the two scenarios, the difference in time when each exceeds the 0.9 threshold indicates how much of an effect reducing to one fixed reach will have. A smaller Δt indicates that sampling one fixed reach has little reduction in our ability to detect a 20% year over year trends in fish abundance, while a larger Δt indicates that sampling one fixed reach substantially reduces our ability to detect the same trend. Figure 1B exhibits how the same power analysis can be adopted to determine which of the three fixed reaches is the optimal reach to sample before all others, if all three fixed reaches cannot be completed in a fishing bout (i.e., the reach that exceeds the 0.9 threshold first is the site's prioritized reach to sample at minimum or before all other fixed reaches).

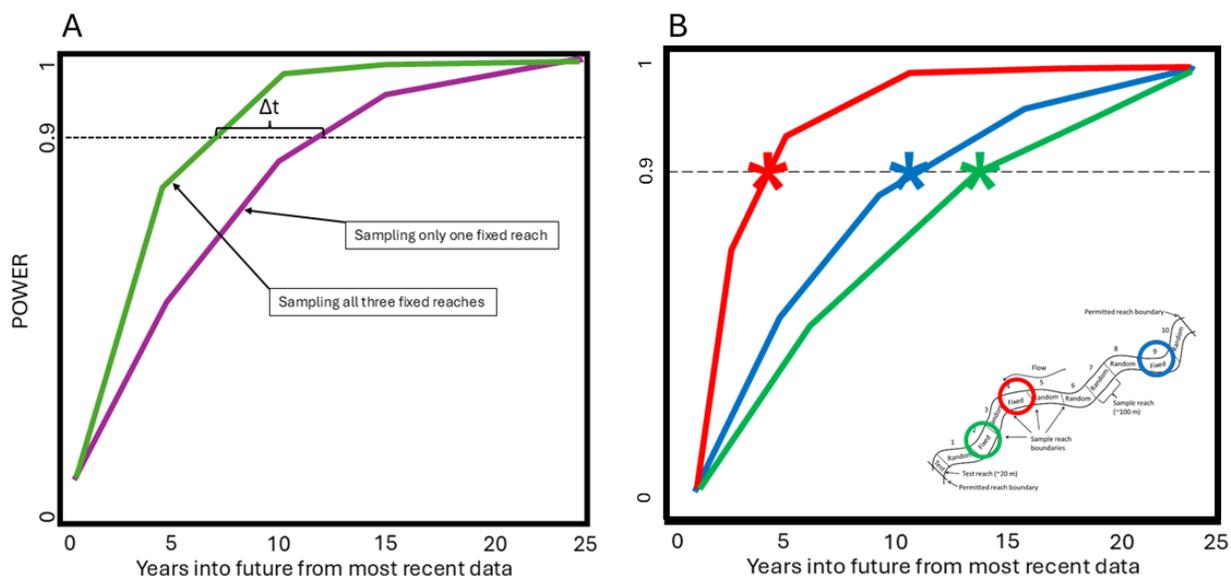


Figure 1: Example of power to detect a 20% year over year trends in fish abundance through time based on different scenarios. A represents the difference in power between sampling one and three fixed reaches. B represents the difference in power based on which reach is sampled every year at a given site to inform the optimal fixed reach to sample.

The focal questions addressed in this specific fish optimization exercise are:

1. How does reduced sampling from three fixed reaches to one fixed reach affect the power to detect a 20% year over year trend in fish abundance across all sampled stream sites?
2. How does reduced sampling from three fixed reaches to one fixed reach affect the CPUE across all sampled lake sites?
3. Among the three fixed reaches sampled at each site, which one yields data that would enable detection of a 20% trend detection year to year with power of 0.9 soonest for streams and which yields the highest overall CPUE for lakes?

1.3 Rationale

Statistically rigorous analyses are needed to assess the capacity of NEON data to address Observatory goals and to guide sampling design optimization efforts. Initial spatial and temporal sampling designs for the NEON Terrestrial Observation System (TOS) and Aquatic Observation System (AOS) were developed in collaboration with Technical Working Groups (TWGs) comprised of community experts, and these sampling designs were captured in Science Design documents (RD[01], etc.). The initial designs relied on analysis of published datasets (where relevant), analysis of NEON prototype data collection efforts, and subject matter expertise.

Now that the NEON Observatory has matured and moved into full operations, it is critical for TOS and AOS initial design assumptions to be tested with multiple years of data collected from NEON sites. Analy-

sis and evaluation of the data provides a feedback loop that enables assessment of the Design relative to Observatory goals. Moreover, results of these analyses allow the NEON TOS and AOS to effectively prioritize sampling in the face of uncertain budgets and labor availability.

1.4 Reference Documents

RD[01]	NEON.DOC.000001	NEON Observatory Design
RD[02]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[03]	NEON.DOC.001296	AOS Protocol and Procedure: FSL – Fish Sampling in Lakes
RD[04]	NEON.DOC.001295	AOS Protocol and Procedure: FSS – Fish Sampling in Wadeable Streams

1.5 Abbreviations

Acronym	Definition
NEON	National Ecological Observatory Network
EF	Electrofishing
AOS	Aquatic Observation System
TOS	Terrestrial Observation System
TWG	Technical Working Group

2 Methods

2.1 Data Acquisition

Fish count data from the NEON ‘Fish electrofishing, gill netting, and fyke netting counts’ data product (DP1.20107.001) were collected following the standard NEON protocols (RD[03] & RD[04]). Stream wetted width data, which were used to model capture probabilities in the simulated fish abundance data, were obtained from the NEON Reaeration data product (DP1.20190.001). All data for these analyses are published and publicly available via the NEON Data Portal. Data from 2016 through 2025 were retrieved from the NEON Data Portal on 2025-12-23 using the R `neonUtilities::loadByProduct()` function.

2.2 Data preparation

We removed ‘samplingImpractical’ records from the `fish_fieldData` table. We joined the `fish_perFish`, `fish_bulkCount` and `fish_perPass` records by the `eventID`, `namedLocation`, and `passNumber` fields, and then flagged instances where the second or third pass from a reach was missing or incomplete or yielded increased fish captures relative to the first or second pass, respectively. Once these flagged samples (<5% of the total records) were removed, we developed a data matrix with the total number of fish collected across all three passes, event ID, `namedLocation`, the year of collection, the reach length of the stream during fish sampling, and the wetted width of the stream collected closest to the fishing bout. These tallied pass counts and covariates were all used in the model calibration, validation, and power analysis scenarios below.

2.2.1 Model workflow for streams

Our goal was to evaluate the time it would take to gain the statistical power to detect a 20% year over year trend in fish abundance when sampling three fixed reaches per bout compared to one fixed reach. While any number of reaches (one, two, or three) could be simulated, we focused on the comparison of one versus three fixed reaches in this review to retroactively test the changes to the protocol that were implemented in 2021. First, we derived reach-level abundance estimates for each valid three pass fishing effort with empirical NEON fish capture data collected among all fixed locations across all stream sites. We then used the same three pass capture data to calculate the capture probabilities for each EF pass that occurred within each fish sampling effort by fitting a Multinomial-Poisson Mixture Model to the raw fish capture data for each pass. Then, the derived abundance estimates and capture probabilities were used to simulate potential fish abundances for each site 25 years into the future at five-year increments. Finally, the statistical power to detect a linear 20% year over year trend was calculated on the simulated abundance data. These simulations were run under two scenarios, one with only sampling one fixed reach with three pass depletion and a second scenario where NEON maintains sampling of three fixed reaches with three pass depletion.

2.2.2 Model calibration for abundance and capture probabilities

The power analysis required empirically derived capture probabilities and estimated abundance across sites and years to serve as informed priors to generate our simulations of projected fish capture data. Estimated fish abundance by each three pass depletion event at each site was applied to existing data with the Carle–Strub method (Carle and Strub 1978) within the FSA package (Ogle 2017) in R (R Core Team 2023).

After abundance for each three pass fishing event was estimated with the Carle–Strub method, we developed a data matrix of raw fish counts from the EF three pass depletion data with additional covariates including year, reach identification, and length of the reach. Each of the covariates was used as response variables in a Multinomial-Poisson Mixture Model within the `ubms` package in R (Kellner et al., 2021). The `ubms` package is used to fit Bayesian hierarchical models of animal abundance and occurrence without mark recapture data using the `rstan` package (Carpenter et al., 2017). Here we used the `stan_multinomPois()` function, which is useful to estimate capture probabilities for data collected via survey methods such as removal, as is the case with NEON’s fish data methods, and then extracted the capture probabilities using the `getP()` function within `ubms`. Thus, our Multinomial-Poisson Mixture Model used to extract the capture probabilities for the historical fishing events was modeled for fish abundance N at site i in year t with a Poisson distribution:

$$N_{i,t} \sim \text{Poisson}(\lambda_{i,t}) \quad (1)$$

$$\log(\lambda_{i,t}) \sim \mu + \alpha_t + \beta_t + \log(\text{reachlength}/100) \quad (2)$$

$$\alpha_i \sim \text{Normal}(0, \sigma_\alpha^2) \quad (3)$$

$$\beta_t \sim Normal(0, \sigma_\beta^2) \quad (4)$$

where μ was the overall mean abundance, α_i was a random site effect with variance of σ_α^2 , and β_t was a random year effect with variance of σ_β^2 . A reach length offset term was also included as the logarithm of reach length (m) standardized by 100 m to account for varying lengths among reaches within the sites.

The output capture probability from the above model calibration was then modeled to generate parameter estimates that were used in the one-fixed reach and three-fixed reach scenarios. Here, capture probability (p) at site i in year t was modeled on a logit scale with a mixed-effects approach and included one model covariate, which has been modeled before as a means to predict capture probability (Hanks et al., 2018):

$$\text{logit}(p_{i,t}) \sim \gamma + \delta + \beta_t + (\text{streamwidth}_{i,t}) + \varepsilon_{i,t} \quad (5)$$

$$\varepsilon_{i,t} \sim Normal(0, \sigma_\varepsilon^2) \quad (6)$$

where γ is the overall mean capture probability and δ is the coefficient of stream width (m) at site i and year t , and $\varepsilon_{i,t}$ is the residual error with variance equal to σ_ε^2 . Including stream width in capture probability estimates is necessary because it could be negatively affected with increasing width as fish have more space to escape from the electrical field (Hanks et al., 2018).

2.2.3 Simulated scenarios and power analyses

The capture data from both one-fixed reach and three-fixed reaches were simulated based on the above derived capture probabilities model parameters and abundances. The 20% year over year trends in abundance was added in order to assess the statistical power to detect this trend under the one-fixed reach or three-fixed reach three-pass sampling scenarios. Following Dauwalter et al. (2009), an exponential growth model was used in which the population was assumed to increase annually with a constant rate over time for each site (r_i), and thus Equation 1 was modified to:

$$\log(N_{i,t}) \sim \mu + \alpha_t + \beta_t + \log(1 + r_i) \times t \quad (7)$$

where t continues to represent the year into the sampling effort. Annual rate of decline was assumed to vary among sites (r_i) because it is unlikely that all sites across NEON would undergo the same 20% year over year increasing abundance trend. With varying rates of a 20% year over year trends, an interaction between sites and years was implicitly incorporated into the above equation.

Capture probability (p_i, t) at site i in year t was simulated based on equations 5 and 6, where values of stream width were randomly drawn from a normal distribution with mean = 0 and SD = 1. Finally, three-pass capture data (y_i, t, j) at site i , year t , and pass j were simulated following binomial distributions based on successive depletion of individuals through passes:

$$y_{i,t,1} \sim \text{Binomial}(N_{i,j}, p_{i,t}) \quad (8)$$

$$y_{i,t,2} \sim \text{Binomial}(N_{i,j} - y_{i,t,1}, p_{i,t}) \quad (9)$$

$$y_{i,t,3} \sim \text{Binomial}(N_{i,j} - y_{i,t,2} - y_{i,t,1}, p_{i,t}) \quad (10)$$

Fish data were simulated by site with the standardized NEON optimization 20% year over year trend in abundance, the numbers of fixed reaches (1 or 3), and numbers of years into the future from the most recent empirically collected data (5, 10, 15, 20, and 25 years). The annual 20% rate of abundance trend was set to vary among study sites with a mean value $\pm 2.5\%$ to introduce uncertainty into the simulations. For example, the rates a site with the fixed annual 20% year over year trend was drawn randomly between 17.5% and 22.5% when the mean rate of abundance change was 20% annually ($r_i \approx \text{Uniform}[0, 0.05]$ in equation 7). All unique combinations of levels of percent annual abundance increases, numbers of fixed reaches, and the number of years were simulated, and data were generated 2000 times for each unique combination.

2.2.4 Power analysis assessment in streams

The primary objective was to assess the difference in time (Δt from Figure 1A) at which the simulations exceeded 0.9 statistical power to detect 20% year over year trends between sampling one vs. three fixed reaches using three-pass electrofishing at all fished NEON stream sites. Power was defined as the proportion of 2000 simulations in which the coefficient of numeric year was significantly positive at $p = 0.05$.

2.2.5 Lake site catch-per-unit-effort analysis

The above modeling exercise was not applicable in lakes because their littoral to pelagic sampling extent cannot quantify the reach lengths and widths that are needed to predict reach-level abundances and capture probabilities. Lake reaches with three-pass depletion are sectioned off with block nets and fished with an electrofisher, however, they are deployed along the shoreline and not along a littoral to pelagic gradient, and thus abundance trends cannot be applied here.

As a result, we quantified how much CPUE changed when going from three fixed reaches to one fixed reach and then prioritized which fixed reach to sample first by identifying which reach had the highest overall CPUE. CPUE was calculated at each lake site by summing the total number of fishes captured among electrofishing, mini-fyke netting and gill netting (i.e., passive netting) divide by the sum of the electrofishing times and passive netting times. CPUE was then calculated under one scenario with only one fixed reach being sampled and another scenario where three fixed reaches were sampled. We then evaluated whether there was a significant difference in the CPUE between the two scenarios using a Welch's t-test.

3 Results and recommendations

3.1 Question 1: Reduced fixed reaches and abundance trend detection in NEON streams

- How does decreasing from three fixed reaches to one fixed reach affect the ability to detect a 20% year over year trend in fish abundance in streams?

We evaluated the impact of reducing the fixed stream reaches from three to one on the ability to detect a 20% year over year trend in fish abundance in streams, as reductions may be necessary due to logistical challenges throughout the life of the Observatory. Our results indicate that reducing the number of fixed reaches from three to one increased the time at which the streams were able to exceed the 0.9 power threshold of detecting a 20% year to year trend by a median of 2.5 years, from 9.5 years to 12 years (Figure 2). Therefore, the original sampling design of three fixed and three random reaches remains the target for all sites. However, if an adaptation to a site sampling plan occurs such that one fixed reach can be sampled instead of three, fish abundance trends can still be detected within the lifespan of the observatory.

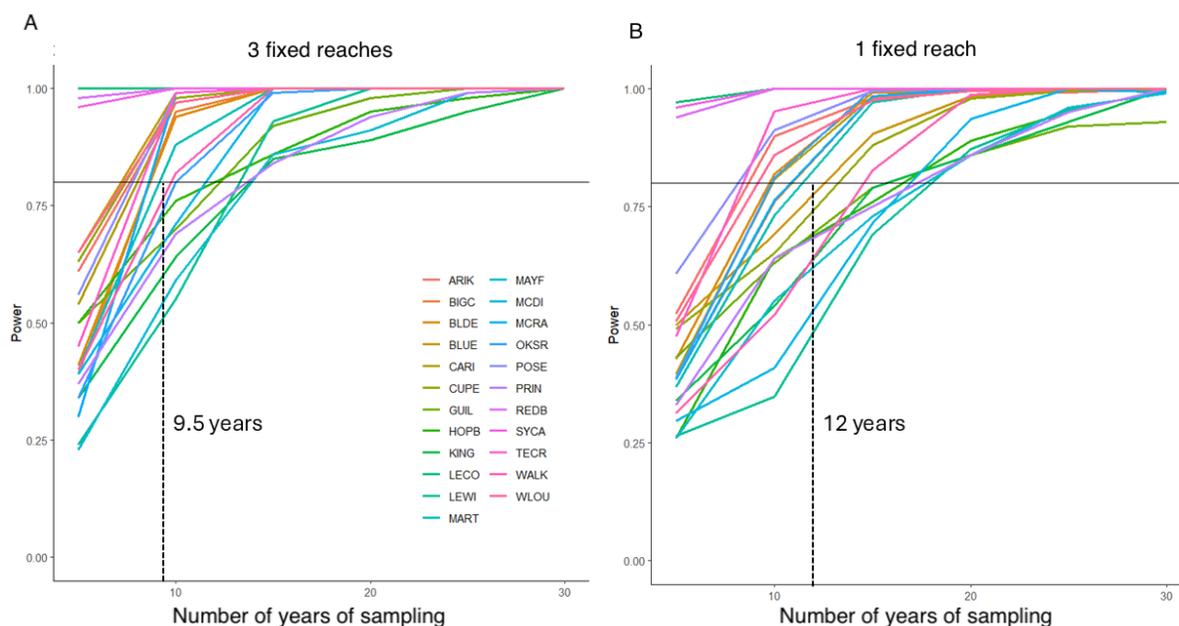


Figure 2: Power to detect 20% year over year changes in fish abundance with increasing years of sampling by NEON site. The target power threshold of 0.9 is indicated by a solid horizontal line and the dashed vertical line indicates the median number of years of sampling needed to reach this threshold. Panel A shows the results when 3 fixed reaches are sampled each year, while Panel B shows the results if only 1 fixed reach is sampled.

3.2 Question 2: Reduced fixed reaches and catch-per-unit-effort changes in NEON lakes

- How does reduced sampling from three fixed reaches to one fixed reach affect the CPUE across all sampled lake sites?

In lakes, there was no significant difference ($p\text{-value} > 0.05$) in the site-level CPUE between three fixed reaches and only one fixed reach, indicating that a reduction in the number of fixed reaches in lakes would have little effect on the CPUE for the site (Figure 3). Therefore, the original sampling design of three fixed and three random reaches remains the target for all lake sites. However, if a site were to need to adapt their sampling to just one fixed reach, the sampling will yield comparable fish CPUE data.

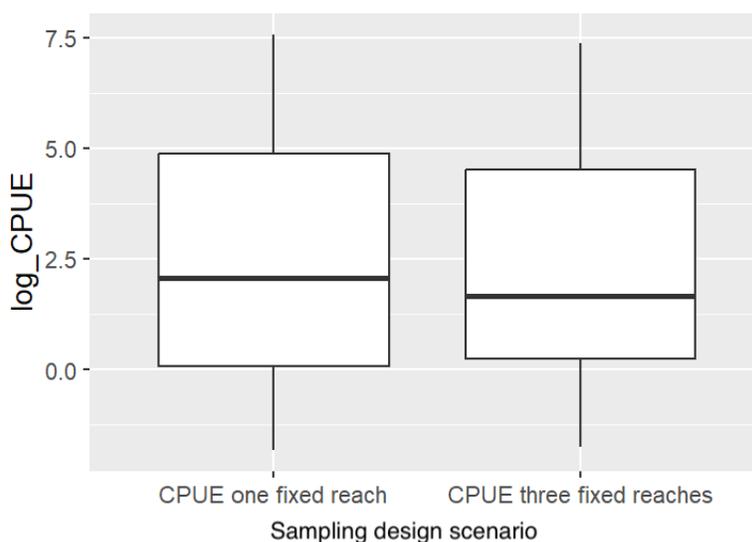


Figure 3: Comparison of lake Catch Per Unit Effort (CPUE) between sampling one fixed reach and three fixed reaches.

3.3 Question 3: Identifying the priority fixed reach at NEON’s streams and lakes

- Among the three fixed reaches sampled at each site, which one yields data that would enable detection of a 20% trend detection year to year with power of 0.9 soonest for streams and which yields the highest overall CPUE for lakes?

3.3.1 Prioritized fixed reach in streams

Among all stream sites, the capture data from at least one of the three fixed reaches enabled trend detection with power exceeding the 0.9 threshold and thus could be identified as the priority fixed reach (Figure 3). The within-site (i.e., reach-level) power analyses exhibited strong variation for 20% year over year trend detection at some sites (Figure 3B), while other NEON sites exhibited little variability where all reaches had already exceeded the 0.9 threshold (Figure 3D). The reach that exceeded the 0.9 threshold line first is the priority fixed sampling reach.

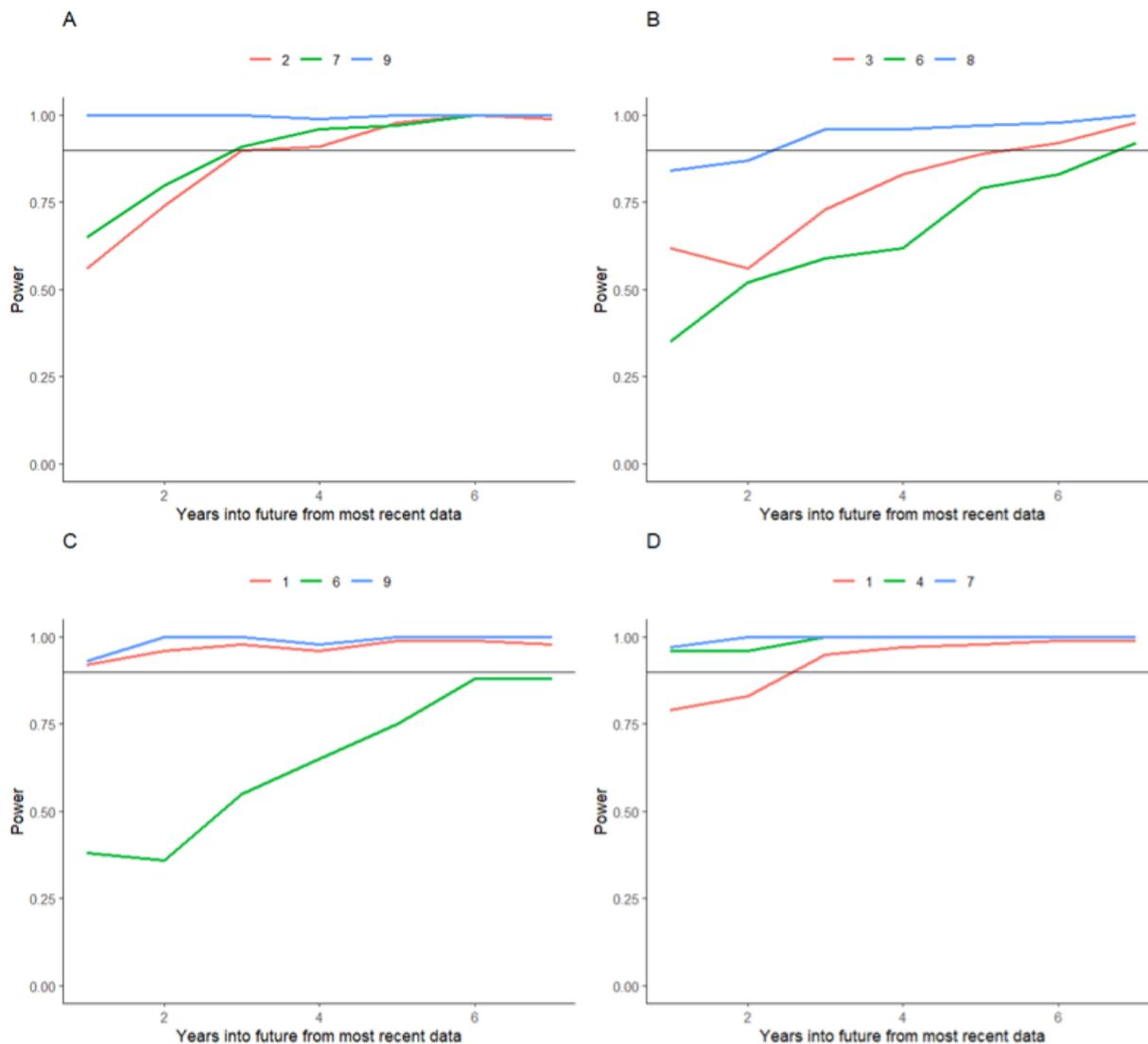


Figure 4: Examples of power analyses used to identify the priority fixed reach at each site. Plot A is Posey Creek (POSE), plot B is Rio Cupeyes (CUPE), plot C is Arikaree River (ARIK), and plot D is West St. Louis Creek (WLOU).

The following table provides the recommended fixed reaches among all NEON stream sites that are to be prioritized to be fished first (Priority), second (Secondary), and then third (Tertiary).

Domain	Site	Priority Fixed Reach	Secondary Fixed Reach	Tertiary Fixed Reach
D01	Hop Brook	10	4	6
D02	Lewis Run	1	9	4
D02	Posey Creek	9	2	7
D04	Rio Guilarte	5	2	9
D04	Rio Cupeyes	8	6	3
D06	Kings Creek	1	4	3
D06	McDiffett Creek	4	2	NA
D07	LeConte Creek	1	10	4
D07	Walker Branch	5	1	3
D08	Mayfield Creek	9	2	7
D10	Arikaree River	9	1	6
D11	Pringle Creek	10	3	7
D11	Blue River	4	7	2
D12	Blacktail Deer Creek	2	3	6
D13	West St. Louis Creek	7	4	1
D14	Sycamore Creek	4	6	10
D15	Red Butte Creek	4	6	9
D16	McRae Creek	4	8	6
D16	Martha Creek	4	7	1
D17	Teakettle Creek	5	6	NA
D17	Upper Big Creek	1	7	4
D18	Oksrukuyik Creek	9	3	6
D19	Caribou Creek	10	1	6

Table 1: Prioritized fixed reach order for NEON streams if fewer than three fixed reaches can be sampled in a fishing bout. TECR and MCDI are NA for their tertiary fixed reach as they only have two fixed reaches given their shorter total reach length.

3.3.2 Prioritized fixed reach in lakes

We were also able to identify the highest overall CPUE among the three fixed reaches within each lake site and use that to determine the priority fixed reach to sample at each lake site. The following table provides the recommended fixed reaches that are to be prioritized to be fished first (Priority), second (Secondary), and then third (Tertiary).

Domain	Site	Priority Fixed Reach	Secondary Fixed Reach	Tertiary Fixed Reach
D05	Crampton Lake	3	6	10
D05	Little Rock Lake	9	7	1
D09	Prairie Lake	6	1	9
D09	Prairie Pothole	8	1	5
D18	Toolik Lake	10	7	3

Table 2: Identified prioritized fixed reach order for lakes in the event that fewer than three fixed reaches can be sampled in a fishing bout.

4 Discussion

This power analysis of simulated fish data across the NEON stream sites was a successful tool to compare the power to detect changes in abundance over time across two levels of sampling. This modeling exercise has been implemented before to test how three pass vs. one pass depletion sampling changes the detection of abundance trends (Hanks et al., 2018). Here, we adopted a similar workflow to Hanks et al., 2018, but we held pass number consistent at three EF passes and tested how reducing the number of depletion sampling (i.e., fixed) reaches from three to one changed detection of 20% abundance trends within a site.

We have retroactively analyzed and shown that reducing the total number of fixed reaches sampled from three to one had some, but not substantial, effect on the total number of years (median Δt of 2.5) at which the NEON sites would reach the 0.9 power threshold of detecting a 20% year over year trend in abundance. Moving forward, the aim for each stream site is to sample three fixed reaches and three random reaches. If limitations arise during the sampling effort, these analyses provide direction on sampling reach prioritization to ensure the most efficient and effective sampling to detect abundance trends over time. At lakes, we are unable to evaluate abundance trends as done in streams, but we did show that there was not a significant change in CPUE when sampling either one or three fixed reaches. We were able to isolate the highest overall CPUE among all fixed reaches and provide guidance for what reaches should be prioritized if full sampling cannot occur at lake sites.

5 Acknowledgements

I am grateful to the NEON's Aquatic Biology Technical Working Group and the NEON Optimization working group for providing valuable insight to the analyses conducted in this report. I also appreciate the prior modeling effort from Hanks et al. (2018) who made their work accessible such that it could be adopted across other cross-continental monitoring efforts.

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