NEON AQUATIC SAMPLING STRATEGY

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	11/01/2016	ECO-04118	Initial Release
B	03/16/2022	ECO-06785	• Update to reflect change in terminology from relocatable to gradient sites; logo revised.

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1 DESCRIPTION

1.1 Purpose

NEON design documents are required to define the scientific strategy leading to high-level protocols for NEON components and linking NEON Grand Challenges to specific measurements. Many NEON in situ measurements and sample analyses can be made in specific ways to enable continental-scale science rather than in ways that limit their use to more local or ecosystem-specific questions. NEON strives to address the Grand Challenges of interactions and feedbacks between causes of change, including Climate Change, Land Use, and Invasive Species, and responses to change, which include Biogeochemistry, Biodiversity, Ecohydrology, and Infectious Diseases. Design documents flow from questions and goals defined in the NEON Observatory Design AD[01], to subsystem specific high-level documents, and ultimately to more detailed procedures described in Level 0 (L0; raw data) protocol and procedure documents, algorithm specifications, and calibration/validation (CalVal) and maintenance plans.

1.2 Scope

This document defines the rationale and requirements for the Aquatic Sampling Strategy in the NEON science design.

1.3 Acknowledgements

This document is based on the template for TOS science design modules. Some of the text in common sections is the same or similar and has been revised to apply to the NEON aquatic system. We thank Andrea Thorpe, Kate Thibault and Stephen Craft for helpful comments.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain information that is applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

AD [01]	NEON.DOC.000001	NEON Observatory Design
AD [02]		DOORS Requirements Database
AD [03]	NEON.DOC.002652	NEON Scientific Data Products Catalog

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]	NEON.DOC.000693	AOS Protocol and Procedure: Reaeration Measuring Diffusion of O_2 Across the Water-Air Interface
RD [04]	NEON.DOC.001085	AOS Protocol and Procedure: Stream Discharge
RD [05]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD [06]	NEON.DOC.001191	AOS Protocol and Procedure: Sediment Chemistry Sampling in Lakes and Non-Wadeable Streams
RD [07]	NEON.DOC.001193	AOS Protocol and Procedure: Sediment Chemistry Sampling in Wadeable Streams
RD [08]	NEON.DOC.001194	AOS Protocol and Procedure: Zooplankton Sampling in Lakes
RD [09]	NEON.DOC.001195	AOS Protocol and Procedure: Riparian Habitat Assessment in Lakes and Non-Wadeable Streams
RD [10]	NEON.DOC.001196	AOS Protocol and Procedure: Riparian Habitat Assessment in Wadeable Streams
RD [11]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD [12]	NEON.DOC.001199	AOS Protocol and Procedure: Surface Water Dissolved Gas Sampling
RD [13]	NEON.DOC.001295	AOS Protocol and Procedure: Fish Sampling in Wadeable Streams
RD [14]	NEON.DOC.001296	AOS Protocol and Procedure: Fish Sampling in Lakes
RD [15]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD [16]	NEON.DOC.001886	AOS Protocol and Procedure: Stable Isotope Sampling in Surface and Ground Waters
RD [17]	NEON.DOC.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD [18]	NEON.DOC.002792	AOS Protocol and Procedure: Secchi Disk and Depth Profile Sampling in Lakes and Non-wadeable Streams
RD [19]	NEON.DOC.002905	AOS Protocol and Procedure: Water Chemistry Sampling in Surface Waters and Groundwater
RD [20]	NEON.DOC.003039	AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen and Macroalgae Sampling
RD [21]	NEON.DOC.003044	AOS Protocol and Procedure: Aquatic Microbial Sampling

RD [22]	NEON.DOC.003045	AOS Protocol and Procedure: Periphyton, Seston and Phytoplankton Sampling
RD [23]	NEON.DOC.003046	AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
RD [24]	NEON.DOC.001588	D01 AIS Site Characterization Report
RD [25]	NEON.DOC.001589	D02 AIS Site Characterization Report
RD [26]	NEON.DOC.001591	D03 AIS Site Characterization Report
RD [27]	NEON.DOC.001648	AIS Site Characterization Report D04
RD [28]	NEON.DOC.002067	D05 AIS Site Characterization Report
RD [29]	NEON.DOC.001858	D06 AIS Site Characterization Report
RD [30]	NEON.DOC.001372	D07 AIS Site Characterization Report
RD [31]	NEON.DOC.001370	D08 AIS Site Characterization Report
RD [32]	NEON.DOC.001670	AIS Site Characterization Report D09
RD [33]	NEON.DOC.002056	D10 AIS Site Characterization Report
RD [34]	NEON.DOC.002416	D11 AIS Site Characterization Report
RD [35]	NEON.DOC.001669	AIS Site Characterization Report D12
RD [36]	NEON.DOC.002068	D13 AIS Site Characterization Report
RD [37]	NEON.DOC.001592	D14 AIS Site Characterization Report
RD [38]	NEON.DOC.001857	AIS D15 Site Characterization Report
RD [39]	NEON.DOC.001856	D16 AIS Site Characterization Report
RD [40]	NEON.DOC.003536	AIS Site Characterization Report D17
RD [41]	NEON.DOC.001671	AIS Site Characterization Report D18
RD [42]	NEON.DOC.001373	D19 AIS Site Characterization Report
RD [43]	NEON.DOC.003600	Aquatic Site Sampling Design – NEON Domain 01
RD [44]	NEON.DOC.003601	Aquatic Site Sampling Design – NEON Domain 02
RD [45]	NEON.DOC.003602	Aquatic Site Sampling Design – NEON Domain 03
RD [46]	NEON.DOC.003603	Aquatic Site Sampling Design – NEON Domain 04
RD [47]	NEON.DOC.003604	Aquatic Site Sampling Design – NEON Domain 05
RD [48]	NEON.DOC.003605	Aquatic Site Sampling Design – NEON Domain 06
RD [49]	NEON.DOC.003606	Aquatic Site Sampling Design – NEON Domain 07
RD [50]	NEON.DOC.003607	Aquatic Site Sampling Design – NEON Domain 08
RD [51]	NEON.DOC.003608	Aquatic Site Sampling Design – NEON Domain 09
RD [52]	NEON.DOC.003609	Aquatic Site Sampling Design – NEON Domain 10
RD [53]	NEON.DOC.003610	Aquatic Site Sampling Design – NEON Domain 11
RD [54]	NEON.DOC.003611	Aquatic Site Sampling Design – NEON Domain 12
RD [55]	NEON.DOC.003612	Aquatic Site Sampling Design – NEON Domain 13
RD [56]	NEON.DOC.003613	Aquatic Site Sampling Design – NEON Domain 14
RD [57]	NEON.DOC.003614	Aquatic Site Sampling Design – NEON Domain 15
RD [58]	NEON.DOC.003615	Aquatic Site Sampling Design – NEON Domain 16
RD [59]	NEON.DOC.003616	Aquatic Site Sampling Design – NEON Domain 17
RD [60]	NEON.DOC.003617	Aquatic Site Sampling Design – NEON Domain 18
RD [61]	NEON.DOC.003618	Aquatic Site Sampling Design – NEON Domain 19
RD [62]	NEON.DOC.003626	Aquatic Instrumentation System – Site Level Coordinates

2.3 Acronyms

Acronym	Definition
AIS	Aquatic Instrument System
AOS	Aquatic Observation System
AQU	Aquatic Science System
DD	Degree Days
DOORS	Dynamic Object Oriented Requirements System
EMAP	Environmental Monitoring & Assessment Program
MGC	Multivariate Geographic Clustering
NAWQA	National Water Quality Assessment
NEON	National Ecological Observatory Network
NRC	National Research Council
TIS	Terrestrial Instrument System
TOS	Terrestrial Observation System
USGS	US Geological Survey

3 INTRODUCTION

3.1 Overview of the Observatory

The National Ecological Observatory Network (NEON) is a continental-scale ecological observation platform with the mission to enable understanding and forecasting of the impacts of climate change, land use change and invasive species on continental-scale ecology by providing infrastructure and consistent methodologies to support research and education in these areas. NEON is designed to enable users, including scientists, planners, policy makers, educators, and the general public, to address the Grand Challenges in Environmental Sciences (National Research Council 2001, AD[01], **Figure 1**). NEON infrastructure and data products are strategically aimed at those aspects of the Grand Challenges for which a coordinated national program of standardized observations and experiments is particularly effective. The open access approach to the observatory's data and information products will enable users to explore NEON data in order to map, understand, and predict the effects of humans on the earth and understand and effectively address critical ecological questions and issues. Detailed information on the NEON design can be found in AD[01] and AD[02].

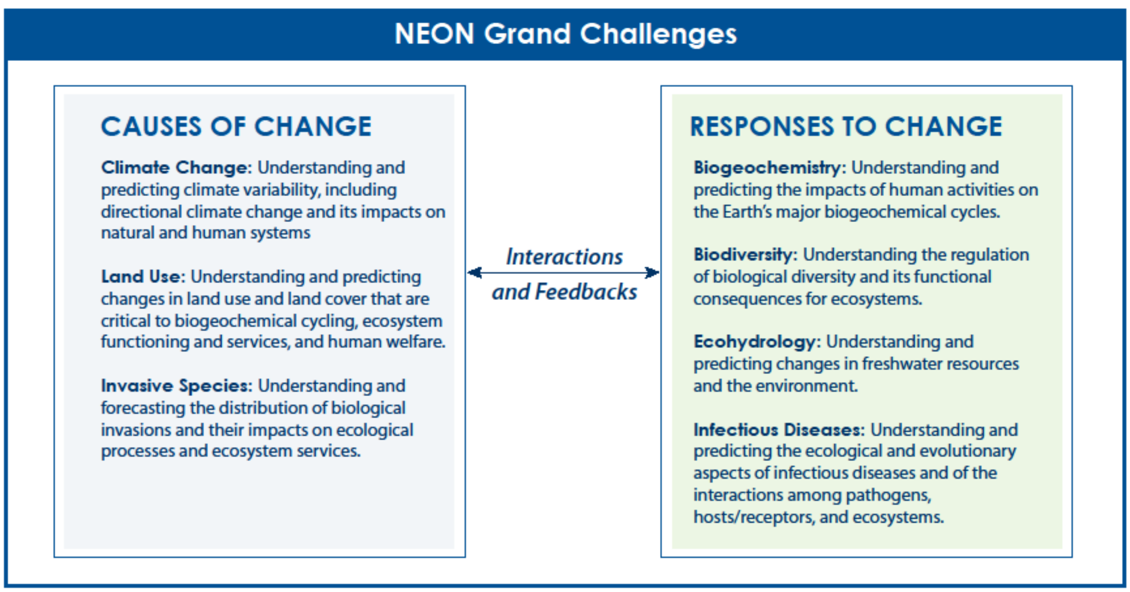

Figure 1. The seven Grand Challenges defined by the National Research Council (2001).

3.2 Components of the Observatory

Ecological variation is difficult to capture with synoptic sampling, monitoring studies or remote sensing alone. Recognition of this has resulted in a movement towards research approaches that utilize concurrent field based, in situ, aircraft mounted, and satellite based sampling strategies to measure physical, chemical, and biological parameters over large areas at efficient and ecologically relevant spatio-temporal scales (Peters 2008). The combination of sampling strategies across temporal and spatial scales is an integral part of NEON's approach to addressing the Grand Challenge questions (Keller et al. 2008). The continental-scale strategy adopted by NEON is necessary to determine whether studies of individual aquatic ecosystems, past and present, represent local processes or can be generalized to broader aquatic ecosystems (McDowell 2015). The design colocates measurements of atmosphere, soil, water, select organisms, and airborne observations. Observing change by integrating measures of the drivers and ecological responses will contribute to an improved understanding of ecological cause and effect (Vitousek 1997, Keller et al. 2008, Luo et al. 2011).

NEON is comprised of 20 Domains, which were delineated using multivariate geographic clustering (MGC) using ecoclimatic variance across the U.S. and spatially distributing it uniformly (Hargrove and Hoffman 1999, 2004). Data will be collected from sites within the NEON network using a consistent set of sensors and sampling protocols. Within each Domain, core sites were chosen that represent wildland conditions and will remain part of the observatory for the entire duration. Other gradient sites, which may be moved occasionally throughout the operation of NEON, were chosen to investigate environmental gradients and/or anthropogenic impacts, such as forestry activities.

There are five components of the observatory: the airborne observation platform (AOP), terrestrial instrument system (TIS), terrestrial observation system (TOS), aquatic instrument system (AIS), and aquatic observation system (AOS). Colocation of measurements associated with each of these components will allow for linkage and comparison of data products. For example, remote sensing data provided by the AOP will link diversity and productivity data collected on individual plants and riparian areas by the AOS. For additional information on these systems and NEON, see Keller et al. 2008 and Schimel et al. 2011.

3.3 Science Requirements

This science design is based on Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

3.4 Data Products

Execution of the protocols that are based on the Aquatic Sampling Strategy procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in NEON's Scientific Data Products Catalog (AD[03]).

3.5 The Aquatic Science System (AQU)

The NEON Aquatic Science System, consisting of the Aquatic Observation System, AOS, and Aquatic Instrument System, AIS, collectively referred to as "AQU". NEON AQU will quantify the impacts of climate change, land use, and biological invasions on freshwater populations and processes by sampling organismal community composition, measuring surface and groundwater chemistry, deploying micrometeorology and in situ water quality instrumentation in and around water bodies, and tracking habitat structure (AD[01], AD[02]). Instruments will be deployed in locations likely to capture as much variability as possible and will make measurements with sufficient temporal resolution to capture trends over the lifetime of NEON. Similarly, the biological sampling approach was selected to include organisms from representative aquatic habitats with varying life spans and trophic positions, and to allow for standardized comparisons across the continent. Many of the sensor-based and observational measurements will enable inference at regional and continental scales using statistical or process-based modeling approaches. Guided by NEON principles and requirements, the Aquatic Sampling Strategy provides a data collection framework that is statistically rigorous, operationally efficient, flexible, and readily facilitates integration with other data to advance the understanding of the drivers of and responses to ecological change.

3.5.1 Background

The colocation of instrumentation, field measurements, and sample collection will allow for the quantification of ecological parameters and the detection of trends in and around water bodies. AIS sensor instrumentation will be used to measure and record environmental conditions, such as air temperature and radiation levels, in areas surrounding aquatic water bodies, in surface water, and in groundwater, where possible. The AOP remote sensing payloads will annually record detailed spectral information around many of the NEON AQU sites with priority given to sites with proximity to terrestrial sites and watersheds with limited size. Neither an annual census, e.g., AOP remote sensing, nor temporally continuous measurements, e.g., AIS sensors capturing temporally continuous measurements, are appropriate for understanding all patterns of aquatic biogeochemistry and organisms. A complete census of these measurements at each site is impractical – microbes are ubiquitous and fish are mobile. Measurement of these types of ecological responses at sensor-like temporal frequencies is impossible and frequent observations at local scales would likely provide redundant information for a limited spatial extent. Hence, riparian vegetation, aquatic organisms, and water quality will be measured at discrete temporal and spatial units by human observers carrying out field-based observations at NEON aquatic sites, i.e., the AOS sampling design. Field observational data will aid in describing the ecological status and future trends NEON is designed to detect with a suite of measurements that cross diverse spatial and temporal scales.

3.5.2 Classes of Aquatic sites

NEON will collect data at 34 aquatic sites, including 24 wadeable streams, 7 lakes, and 3 non-wadeable rivers that are located within 19 of the 20 Domains. The aquatic sites were chosen to be representative of aquatic features and habitats in the United States within each NEON Domain. This means that the aquatic site(s) within each Domain may include wadeable streams (referred to as "streams" hereafter), non-wadeable streams (referred to as "rivers" hereafter), lakes, or a combination of aquatic feature types in order to best represent the diversity of aquatic ecosystems within a Domain. Lakes are chosen that have the representative characteristics of the region for size, hydrologic flow (i.e. seepage vs flow-through), and shoreline characteristics, with a minimum area of 5 acres and depth of 3 meters. Additionally, the buoy must be able to be deployed and continuously accessed in the deepest basin. Ideal stream reaches have a 1 km permitted length that is free of major flow obstructions or major tributaries. A 1 km reach is preferred to allow for the wide range of biological, chemical, and physical parameters being measured and to best capture within stream diversity across multiple habitat types (i.e., pools, riffles, and runs). Given the spatial requirements of aquatic protocols, as well as the dynamic and heterogeneous nature of streams, a reach smaller than 1 km is likely to result in samples that are not truly representative of the aquatic system being studied. River sites are chosen that allow year round access to a 1 km reach for field measurement and sampling similar to streams. Similar to lakes, river sites will be compatible with buoy deployment in a location that captures the main flow, but is outside of navigation channels, and have representative shoreline characteristics.

Core aquatic sites are located in areas that are not significantly influenced by built structures, e.g., bridges, or human activities that may impact water quality, such as urbanization, agriculture, or wastewater effluent. Gradient aquatic sites are located in order to capture environmental gradients, e.g., Domain 08 where a hydrologic continuum from headwater stream to large river was chosen. Most NEON aquatic sites are colocated with NEON terrestrial sites where aquatic features were available. Other aquatic sites are geographically separate from terrestrial sites because a representative aquatic site for the Domain was unavailable near the terrestrial site. In order to leverage existing infrastructure and long-term datasets, a few NEON sites are near other long-term ecological research areas, e.g., Toolik Lake and Konza Prairie. In the case of colocation of NEON and other research infrastructure there is direct communication between NEON and principal investigators in order to ensure no impacts to either research project. Logistical and permitting concerns were also important considerations for site selection as the use of land for infrastructure and field personnel must be secured for the tenure of NEON Operations.

Initial characterization of potential sites is made to ensure logistical practicality and scientific value; RD[24] – RD[42] contain initial characterization information about selected sites and, in some cases, sites that were investigated but not selected in each Domain. The selected NEON aquatic sites are then classified as a stream, river, or lake. Streams are stretches of flowing water that are safely wadeable during most, if not all, of the year. Rivers are stretches of flowing water that are not often, if ever, safely wadeable. Lakes are lentic bodies of water that may stratify for some period of time during the year. Because most water bodies at NEON aquatic sites have historic names that may not match exactly with the NEON classification, e.g., Arikaree River which is a NEON stream (i.e., wadeable) site, Table 1 provides a list of the site names, Domain locations and classes of NEON aquatic sites for reference. Because of hydrologic differences between the classes of NEON aquatic sites, it is necessary to create protocols that consistently capture the physical, chemical, and biological diversity in all locations while being adapted to each type of aquatic site, e.g., sampling multiple depths from a boat in a stratified lake versus collecting surface water samples while wading in a stream. Lakes, rivers and streams have historically been studied separately and the NEON Aquatic Sampling Strategy will create the ability to incorporate both lake, river and stream variability into understanding continental-scale ecological change (Jones 2010).

Table 1. Domain numbers, site names, locations and classes for all NEON aquatic sites.

Domain Number	Site ID	Site Name	Site Class	Domain Name	Type	State
01	HOPB	Hop Brook	Stream	Northeast	Core	MA
02	POSE	Posey Creek	Stream	Mid-Atlantic	Core	VA
02	LEWI	Lewis Run	Stream	Mid-Atlantic	Gradient	VA
03	BARC	Barco Lake	Lake	Southeast	Core	FL
03	SUGG	Suggs Lake	Lake	Southeast	Core	FL
03	FLNT	Flint River	River	Southeast	Gradient	GA
04	CUPE	Rio Cupeyes	Stream	Atlantic Neotropical	Core	PR
04	GUIL	Rio Guilarte	Stream	Atlantic Neotropical	Gradient	PR
05	CRAM	Crampton Lake	Lake	Great Lakes	Core	WI
05	LIRO	Little Rock Lake	Lake	Great Lakes	Gradient	WI
06	KING	Kings Creek	Stream	Prairie Peninsula	Core	KS
06	MCDI	McDiffett Creek	Stream	Prairie Peninsula	Gradient	KS
07	WALK	Walker Branch	Stream	Appalachians	Core	TN
07	LECO	LeConte Creek	Stream	Appalachians	Gradient	TN
08	MAYF	Mayfield Creek	Stream	Ozarks Complex	Core	AL
08	BLWA	Black Warrior River	River	Ozarks Complex	Gradient	AL
08	TOMB	Lower Tombigbee River	River	Ozarks Complex	Gradient	AL
09	PRPO	Prairie Pothole	Lake	Northern Plains	Core	ND
09	PRLA	Prairie lake	Lake	Northern Plains	Gradient	ND
10	ARIK	Arikaree River	Stream	Central Plains	Core	CO
11	PRIN	Pringle Creek	Stream	Southern Plains	Core	TX
11	BLUE	Blue River	Stream	Southern Plains	Gradient	OK
12	BLDE	Blacktail Deer Creek	Stream	Northern Rockies	Core	MT
13	COMO	Como Creek	Stream	Southern Rockies	Core	CO
13	WLOU	West St. Louis Creek	Stream	Southern Rockies	Gradient	CO
14	SYCA	Sycamore Creek	Stream	Desert Southwest	Core	AZ
15	REDB	Red Butte Creek	Stream	Great Basin	Core	UT
16	MART	Martha Creek	Stream	Pacific Northwest	Core	WA
16	MCRA	McRae Creek	Stream	Pacific Northwest	Gradient	OR
17	BIGC	Upper Big Creek	Stream	Pacific Southwest	Core	CA
17	TECR	Teakettle Creek	Stream	Pacific Southwest	Gradient	CA
18	OKSR	Oksrukuyik Creek	Stream	Tundra	Core	AK
18	TOOK	Toolik Lake	Lake	Tundra	Gradient	AK
19	CARI	Caribou Creek	Stream	Taiga	Core	AK

3.5.3 AQU's Contributions Beyond NEON

In addition to fulfilling the NEON goal, the AQU System is secondarily designed to be comparable with local, state, and national aquatic monitoring programs, such as the National Water Quality Assessment (NAWQA), which is a program of the U.S. Geologic Survey (USGS), and the Environmental Monitoring & Assessment Program (EMAP), which is a program of the U.S. Environmental Protection Agency (EPA) (Leahy et al. 1990, Gilliom et al. 1995). The existing programs are primarily dedicated to surface and groundwater quality and their suitability as water resources (e.g., drinking water sources) without the integrated terrestrial and airborne ecological approach of NEON. These programs were often created in response to concerns about specific environmental impacts, such as herbicides (Barbash et al. 2001), or to understand specific hydrologic basins, such as the great lakes (Read et al. 2010). Thus, existing scientific infrastructure is inadequate to enable the investigation of the aquatic component of connected ecological processes (Carpenter 2008, Peters 2008). However, the sampling protocols and procedures employed by these monitoring programs are well tested and robust after decades of use and refinement, making them excellent models for the Aquatic Sampling Strategy at NEON. Together, NEON and existing environmental monitoring programs will complement each other to enable a more holistic understanding of aquatic ecosystems. The AQU Sampling Strategy is also, and uniquely, designed to accommodate auxiliary investigation by independent observers and Principle Investigator (PI)-driven research leveraging the publicly available NEON observations and allowing for the possibility of adding infrastructure for additional measurements and/or experiments.

3.5.4 Purpose and Scope

The purpose of the Aquatic Sampling Strategy is to serve as a framework for implementation of AOS protocols and configurations of AIS sensors that fulfill the NEON goal by integrating high-level science requirements and logistical constraints. The Aquatic Sampling Strategy may also be used to establish the requisite resources, physical infrastructure, and cyber infrastructure necessary to meet science requirements.

3.5.5 Documentation Defining AQU

At the observatory level, *The NEON Observatory Design*, AD[01], describes the derivation of NEON's high level science requirements from the Grand Challenges and the high level science implementation, education plan and data products plan for NEON, including those which apply to the AQU system. Within DOORS, AD[02], the Observatory design is translated to explicit requirements that apply to all levels of NEON subsystems (**Figure 2**).

At the AQU system level *The Aquatic Sampling Strategy,* presented in this document, applies the high level design elements outlined in AD[01] and AD[02] to the AQU system and serves as a framework for site level design documents (**Figure 2**). AOS sampling protocols are step-by-step illustrated instructions for field technicians to follow in order to ensure consistent process quality and excellent data quality. For the AIS component of the AQU system, configuration documents are created as a record of sensor settings, data streams, and frequency of data collection that will be applied across the AQU system. To the extent possible, the AIS configuration is consistent with TIS configuration documents such that a given sensor is configured similarly across the entire NEON network.

At the site level for the AOS, each Domain has an *Aquatic Site Sampling Design Document* that covers spatial and temporal factors that dictate AOS sampling at each site within the Domain (RD[44] - RD[61] for Domains 01 - 19, respectively). Ecological variables such as temperature, vegetation, and hydrology drive the conclusions of the site specific sampling design documents. These site specific documents can be updated during NEON Operations in order to adapt to changing hydrologic conditions at Aquatic sites, while maintaining the continuity of the sampling designs to enable trend detection.

At the site level for the AIS, the *NEON Aquatic Instrumentation System – Site Level Coordinates* document contains information for all NEON AQU sites that describe the specific locations of sensors at each site (RD[62]). Physical site structure and hydrology drive the spatial location chosen for sensor installations, as described below (Section 5).

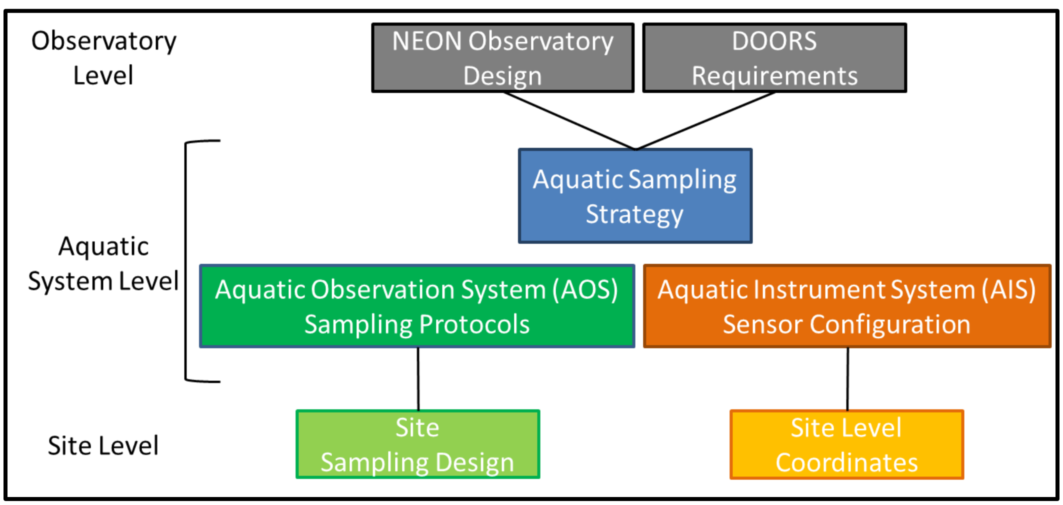

Figure 2. Overview of documents that define the scope of NEON science from the observatory-, AQU system-, AIS and AOS subsystem-, and site-levels.

4 OVERVIEW OF THE AQUATIC SAMPLING STRATEGY

NEON developed a continental-scale design to systematically sample national variability of ecological characteristics and to allow extrapolation of local observations to regional and continental scales. The traceable links between this high-level NEON mission statement and the Observatory data provide a framework for the NEON design. The Aquatic Sampling Strategy is part of this hierarchical structure. "Upstream" requirements and "downstream" data products provide context and constraints under which the strategy was developed. The NEON Aquatic Sampling Strategy is designed to measure aquatic biogeochemistry, biodiversity, and ecohydrology, collectively representing ecosystem responses to change driven by invasive species, land use, and climate change. Aquatic ecosystems exhibit physical, chemical, and biological variability over a wide range of spatial and temporal scales (Steele and Henderson 1994). Overall, the NEON Aquatic Sampling Strategy is to collect standardized, high quality data that can be used to understand continental-scale aquatic ecology by following robust protocols for observations and designs for instrumented systems that are informed by ecological principles.

The overarching strategy for NEON aquatic sites is to quantitatively capture physical, chemical, and biological variability at each local site. Hydrologic variables, such as stream flow or lake water density, meteorological conditions, such as air temperature and wind speed, and seasonal biological transitions, such as leaf-out and leaf-fall, are important drivers of ecological processes in aquatic ecosystems. Therefore, the Aquatic Sampling Strategy relies on a combination of seasonal, riparian, and hydrologic conditions to dictate the spatial and temporal sampling procedures that will capture the variability for a given NEON Aquatic site. All aquatic sites will be instrumented with uniform monitoring equipment and staff will follow standardized, national-scale sampling protocols in a consistent manner to enable direct comparison among sites over the lifetime of NEON operations. The details of the Aquatic Sampling Strategy at all NEON aquatic sites, both core and gradient, are detailed in the following sub-sections.

5 AQUATIC INSTRUMENT SYSTEM (AIS)

The Aquatic Instrument System (AIS) is designed to provide information about physical and chemical properties of aquatic water bodies, the surrounding atmosphere, and adjacent riparian areas. These instruments are capable of collecting a limited amount of information at limited spatial scales with high temporal resolution (many measurements per hour). These instruments are needed as aquatic variables can change on the order of minutes due to weather patterns and hydrologic drivers (Pellerin et al. 2012b, Sobczak and Raymond 2015). Instruments will measure meteorological conditions near aquatic water bodies and in situ sensors will simultaneously measure water quality parameters in order to allow direct integration and extrapolation between suites of measurements, thereby enabling researchers to elucidate drivers of aquatic change, which may exist outside of the water itself.

5.1 AIS Measurements

The AQU sensor suite is comprised of three primary sensor arrays: riparian meteorological stations, buoy mounted meteorological stations in lakes and rivers, and in situ aquatic sensors deployed to measure water quality parameters. Sensors are also deployed in groundwater wells and are discussed in section 7 of this document. Cameras will be used to capture AIS measurements of staff gauge levels at all NEON AQU sites. Riparian meteorological stations are deployed at all aquatic sites, and the meteorological sensors are configured the same as sensors deployed on NEON terrestrial towers and reported in the same data products, which include: wind speed and direction, air temperature, barometric pressure, relative humidity, shortwave radiation, and photosynthetically active radiation. In addition, precipitation gauges are deployed in riparian areas of aquatic sites that are not colocated with terrestrial sites. At lake and river sites, additional meteorological measurements are made above the water surface from buoy-mounted meteorological stations. However, because these measurements are made above the water surface rather than above land they are reported as different data products.

The following suite of sensors will be deployed in the water column to capture physical and chemical properties of water at NEON AQU sites (**Table 2**): a multi sonde equipped with sensors for conductivity, chlorophyll a, dissolved oxygen, fluorescent dissolved organic matter (fDOM), pH, and turbidity. In wadeable streams, a second multi sonde without an fDOM sensor will be deployed. Surface water nitrate concentration will be measured with an optically based sensor at all AQU sites. Physical measurements made below the water surface include upwelling and downwelling photosynthetically active radiation and water temperature. Pressure transducers will be used to record water pressure from which water surface elevation can be derived. In lakes and rivers, water temperature measurements will be made at multiple depths.

Table 2. AIS sensor measurements and locations.

Sensor Measurement	**Stream Stations**	**Lake Stations**	**River Stations**	**Groundwater Wells**
Meteorological - shared with tower				
Wind speed and direction	Riparian	Riparian	Riparian	
Air temperature	Riparian	Riparian	Riparian	
Barometric pressure	Riparian	Riparian	Riparian	
Relative humidity	Riparian	Riparian	Riparian	
Photosynthetically active radiation	Riparian	Riparian	Riparian	
Shortwave radiation	Riparian	Riparian	Riparian	
Meteorological - above water, AQU only				
Wind speed and direction above water		Buoy	Buoy	
Air temperature above water		Buoy	Buoy	
Barometric pressure above water		Buoy	Buoy	
Relative humidity above water		Buoy	Buoy	
Photosynthetically active radiation above water	Sensor sets #1 & #2	Buoy, inlet & outlet	Buoy, near shore	
Shortwave radiation above water		Buoy	Buoy	
In situ aquatic measurements				
Water temperature	Sensor sets #1 & #2	Buoy	Buoy	All
Conductivity	Sensor sets #1 & #2	Buoy	Buoy	All
Water level (pressure transducer based)	Sensor sets #1 & #2	Inlet & outlet	Near shore	All
Photosynthetically active radiation below water	Sensor sets #1 & #2	Buoy, inlet & outlet	Buoy, near shore	
Chlorophyll a	Sensor sets #1 & #2	Buoy	Buoy	
Dissolved oxygen	Sensor sets #1 & #2	Buoy	Buoy	
pH	Sensor sets #1 & #2	Buoy	Buoy	
turbidity	Sensor sets #1 & #2	Buoy	Buoy	
SUNA nitrate analyzer	Sensor set #2	Buoy	Buoy	
Fluorescent dissolved organic matter	Sensor set #2	Buoy	Buoy	

5.2 AIS Spatial Sampling Strategy

The goal of the AIS is to collect high temporal resolution data enabling the detection of ecosystem-level change over time. Physical, chemical, and biological processes in aquatic ecosystems are driven by interactions with the surrounding atmosphere, riparian area, and groundwater. The AIS is designed to support the integration of atmospheric, terrestrial and aquatic measurements at NEON aquatic sites whether or not they are located near a NEON terrestrial site.

Riparian meteorological stations will be deployed within a representative area of the riparian canopy surrounding streams, rivers, and lakes. Representative conditions are identified using a rapid riparian habitat assessment (Barbour et al. 1999). Briefly, for a given stream reach the determination of representative riparian canopy involves a survey of bank physical structure and riparian vegetation characteristics that are rated on a numeric scale to determine the most common riparian vegetation pattern. Once representative sites have been selected based on the riparian habitat assessment, logistical factors, such as land access and power supply availability, will be used to select the final station location. These meteorological sensors will be deployed in a manner consistent with best practices (EPA 1987, WMO 1983). Riparian meteorological stations will measure similar atmospheric variables as the terrestrial instrument system deployed on towers allowing comparison to those measurements and the creation of consistent data products between aquatic and terrestrial instruments. Even when terrestrial towers are relatively close, it is important to have a consistent measure of the meteorological conditions directly adjacent to aquatic water bodies using riparian meteorological stations. Riparian meteorological conditions have been linked to ecosystem processes, such as evapotranspiration (Hernandez-Santana et al. 2011, Kabenge et al. 2013), and are therefore required for understanding aquatic ecosystem processes that may be altered due to drivers of change on a decadal timescale.

In rivers and lakes, the increased width of open water results in concomitant increases in light exposure due to decreased shading from riparian vegetation (Vannote et al. 1980, Lauck et al. 2005). Thus, the meteorological conditions in the riparian area and at the open water surface in rivers and lakes can be quite different. In these systems, in addition to the on-shore meteorological station, NEON will deploy buoys capable of measuring meteorological conditions to capture this difference in conditions.

In addition to measuring the riparian and terrestrial meteorological drivers of aquatic ecosystem change, the AIS is designed to measure physical, chemical, and biological processes occurring within aquatic ecosystems, which are often dynamic. At stream sites, the AIS subsystem sensor sets will be located based on hydrologic characteristics, i.e., discharge and velocity values, measured at each site enabling consistent travel times between the two sensor sets. In addition to surface water sensors, buoy mounted sensors capable of depth profiles will be installed at rivers and lakes in the deepest location in the main basin (Pellerin et al. 2012a). From these depth profiles, temperature and stratification conditions can be determined, which can drive nutrient distributions and dissolved gas concentrations, which are both important variables for biological communities (Engelhardt and Kirillin 2014). Depth profiles have historically been collected from the deepest points in lakes for EPA monitoring programs and mooring at the deepest part of the lake or representative area (outside of boat travel lanes) in the river channel allows for sampling the depth characteristics of lakes and rivers in addition to surface water characteristics.

5.3 AIS Temporal Sampling Strategy

In streams, rivers, and lakes the flux of constituents may vary over short time scales, especially during logistically challenging conditions, e.g., storms that may increase flows to unsafe levels for field personnel. In these cases, sensor readings that can continuously measure aquatic conditions are highly valuable. Storm events can be responsible for large portions of the total annual flux of nitrate and organic carbon in streams (Inamdar et al. 2011, Carey et al. 2014, Dhillon and Inamdar 2014). In order to capture the variability in these systems, high temporal resolution data collection using in situ sensors is required. Metabolism measurements, for instance, have been a valuable long-term integrated signal of energy cycling in aquatic ecosystems (Carpenter et al. 2005, Staehr et al. 2012). These metabolism measurements require frequent measurements of dissolved oxygen throughout diel cycles (Riley and Dodds 2013, Grace et al. 2015). The use of high resolution temporal sampling will enable NEON to produce data that are robust and comparable between Domains and aquatic ecosystem types. The AIS sampling frequency will be designed to allow NEON to capture accurate flux estimates of organic matter and nitrate under conditions that are likely quantitatively important yet challenging to sample with traditional "grab" sampling approaches. In addition to storm events, these high temporal resolution sensor data can be used by the community to derive higher level derived data, such as stream metabolism, constituent fluxes, and lake stratification (Utz et al. 2013). Because the AIS sensors are capable of collecting high temporal resolution data (on the order of a measurement per second) our data analysis strategy will also include developing procedures for smoothing, filling in gaps, accounting for calibration drift, biofouling, and reducing noise in order to increase accuracy and precision of the values reported by NEON for use by the scientific community (AD[03] and data product specific Algorithm Theoretical Basis Documents (ATBDs)). Thus, the temporal sampling at meteorological stations, buoys, and aquatic sensors will be optimized for the specific data product being created. The AIS sensors are designed to be rugged field instruments, but will require regular, preventive and repair maintenance to function properly over time. AIS sensor maintenance will take place at all sites on a bi-weekly basis initially and will then be optimized for each site and season as more data becomes available in order to ensure proper sensor function while efficiently using field personnel time. Seasonal deployment/removal of many sensors will take place at sites where icy winter conditions occur.

6 AQUATIC OBSERVATION SYSTEM (AOS)

The standardization of the Aquatic Observation System (AOS) protocols across all sites is key to the success of NEON, as well as part of its novelty, and must be maintained at all sites through time. Although the timing of protocol execution may be varied at NEON aquatic sites due to different seasonal hydrology or logistical constraints of the permit (e.g., sampling restrictions may be enforced at some sites during fish spawning seasons), protocols have been developed to ensure data comparability over temporal and spatial scales.

The AOS is designed to complement the AIS by providing additional information about biological, physical, and chemical features of aquatic water bodies and surrounding riparian areas. These observational measurements provide additional insight into aspects of aquatic ecosystems that vary on longer timescales (e.g., riparian vegetation which varies seasonally), require time-intensive laboratory analyses (e.g., alkalinity titrations), or do not currently have in situ monitors available (e.g., elemental composition of biological organisms). AOS samples are collected by field personnel over ecologically relevant and practical spatio-temporal scales, which are described in the following sections.

6.1 AOS Measurements

The AOS subsystem is comprised of collecting measurements and samples for physical, chemical, and biological analysis from lakes, rivers, streams, and, as described in section 7 below, groundwater wells. Physical properties of water bodies captured by the AOS include secchi depth and handheld meter-based temperature and depth profiles in lakes and rivers. In streams, discharge measurements will be made with a handheld velocity meter and also using a salt based method, when applicable. Salt based discharge measurements will be made at the time of physical reaeration determinations using simultaneous gas and conservative salt tracer injections to evaluate physical drivers of Oxygen fluxes. Reaeration will be estimated using the change in downstream gas concentration normalized to conservative salt tracer concentration and rates will be published as a data product. Bathymetry in lakes and rivers will be mapped using sonar. Stream morphology measurements will be made using surveying equipment, and this information will be used to create maps of physical structure and habitat.

Chemical parameters that will be measured from samples collected through the AOS include organic constituents, inorganic constituents, mass of dissolved and suspended solids, particulate (^{15}N, ^{13}C) and dissolved isotopes (^{2}H, ^{18}O), and dissolved gas concentrations (CO_2, CH_4, N_2O). Alkalinity titrations will be performed at the Domain labs. Sediment will be collected from AQU water bodies for physical grain size analysis and chemical analysis for a range of inorganic and organic constituents. Chemical properties of surface water, algal, and plant matter will also be determined, including elemental analysis (C, H, N, O) and stable isotopes (^{15}N, ^{13}C, ^{2}H, ^{18}O, and ^{34}S in algae). A full list of the parameters that will be returned from external laboratories can be found in relevant Algorithm theoretical basis documents (ATBD) for each data product available in the NEON resource/document library on the data portal website.

Biological organisms that were selected as sentinel taxa for the AQU system include benthic and water column microbes, algae (periphyton, seston, and phytoplankton), aquatic plants, zooplankton, aquatic macroinvertebrates, and fish. Each of these groups of organisms will be sampled for community composition using field identifications (fish), microscopy-based taxonomic identification (algae, zooplankton, plants, macroinvertebrates), or DNA sequencing (marker gene sequences and metagenomes of microbes), where applicable. A subset of fish, zooplankton and macroinvertebrate samples will also be sequenced for additional taxonomic information. In addition to the aquatic organisms mentioned above, a rapid riparian assessment will be performed annually (during the AOP flyover window at peak greenness) for dominant and subdominant riparian plant species, vegetation composition and cover, and bank characteristics.

Higher level derived data such as models can be derived for NEON AQU sites by combining physical, chemical, and biological attributes of AOS data products with AIS data streams, which will be available on the NEON data portal. For example, at stream sites data users can model 2-station metabolism, which is a highly desired data set used to understand ecosystem function. Stream metabolism measurements quantify the amount of primary production and respiration occurring within a reach (both benthic and water-column) by measuring changes in oxygen (O_2) concentration within a stream segment (part of the AIS subsystem described above). Changes in dissolved oxygen concentration can occur from both biological, such as primary production (O_2 gain in the water column) and respiration (O_2 loss), and physical, such as gas exchange with the atmosphere (i.e., reaeration, the gain of O_2, or deaeration, the loss of O_2) as oxygen diffuses across the water-air interface. In order to understand the biological controls on oxygen within our systems, we must first account for the physical controls (e.g., reaeration or gas exchange). By having field technicians measure reaeration rates between the two AIS stream sensor sets, the physical component of estimating in-stream metabolism can be captured.

Table 3. AOS measurement and sample collection types and frequencies.

Sampling Module	Stream Sampling	Lake Sampling	River Sampling	Groundwater Well Sampling
Physical				
Discharge	24 times per year			
Reaeration	6 times per year			
Rapid Habitat Assessment	1 time per year			
Morphology	1 time every 5 years			
Bathymetry		Up to 1 time per year	Up to 1 time per year	
Biological				
Aquatic Plants	3 times per year	3 times per year	3 times per year	
Macroinvertebrates	3 times per year	3 times per year	3 times per year	
Zooplankton	3 times per year	3 times per year	3 times per year	

Periphyton and Phytoplankton		3 times per year	3 times per year	
Periphyton and Seston	3 times per year			
Benthic Microbes	3 times per year			
Fish	2 times per year	2 times per year		
Surface Water Microbes	12 times per year	6 times per year	6 times per year	
Riparian Assessment	1 time per year	1 time per year	1 time per year	
Chemical				
Water Chemistry	26 times per year	12 times per year	12 times per year	1-2 times per year
Dissolved Gas in Water	26 times per year	12 times per year	12 times per year	
Isotopes	26 times per year	12 times per year	12 times per year	1-2 times per year
Sediment Chemistry	3 times per year	3 times per year	3 times per year	

6.2 AOS Spatial Sampling Strategy

AOS sampling will take place in and around water bodies at the NEON Aquatic sites, similar to the AIS design that encompasses both riparian, water surface, and in situ aquatic sensor measurements (see sections 6.1.1 & 6.1.2). In order to understand locational variance and heterogeneity of landscape-scale ecological processes at each NEON Aquatic Site, the AOS spatial design is consistent for measuring physical and chemical variables while allowing flexibility for identifying representative habitat (i.e., riffle, run, or pool) for biological sampling. AOS sampling will be distributed throughout the stream reach in a way that prevents interference but maximizes linkages and extrapolation with other AIS and AOS operations, e.g., water chemistry sampling will occur immediately downstream of the sensor set to avoid kicking up sediment during stream wading that may impact sensor measurements while still allowing for linkages between grab samples and continuous measurements (**Figure 3**). Equipment will be decontaminated in order to prevent the spreading of invasive or endemic species between sites where equipment is shared (RD [05]). Boats and/or other infrastructure will be used to facilitate sampling at rivers and lakes near buoys and other infrastructure equipped with AIS sensors.

The AOS sampling strategy includes measurements of riparian vegetation (RD[09] & RD[10]), which is integrally linked to aquatic ecosystem processes, similar to AIS riparian meteorological measurements (Williamson et al. 2008). AOS will annually assess riparian vegetation in order to detect changes in riparian canopy, which is an important driver of aquatic biogeochemical and biological processes (Vannote et al. 1980). Along streams and rivers, riparian assessment will take place at equidistant transects (Fitzpatrick et al. 1998) that will be established at the initiation of each site and maintained throughout NEON Operations. Riparian assessments for lakes will take place in permanently established wedges designed to evenly divide the lake perimeter (**Figure 3**). These riparian assessment campaigns will also have the added advantage of linking AQU sites to the remotely-sensed data collected through the NEON airborne observation platform (AOP), which will also detect vegetation patterns at the majority of NEON sites through optical sensors on an annual basis.

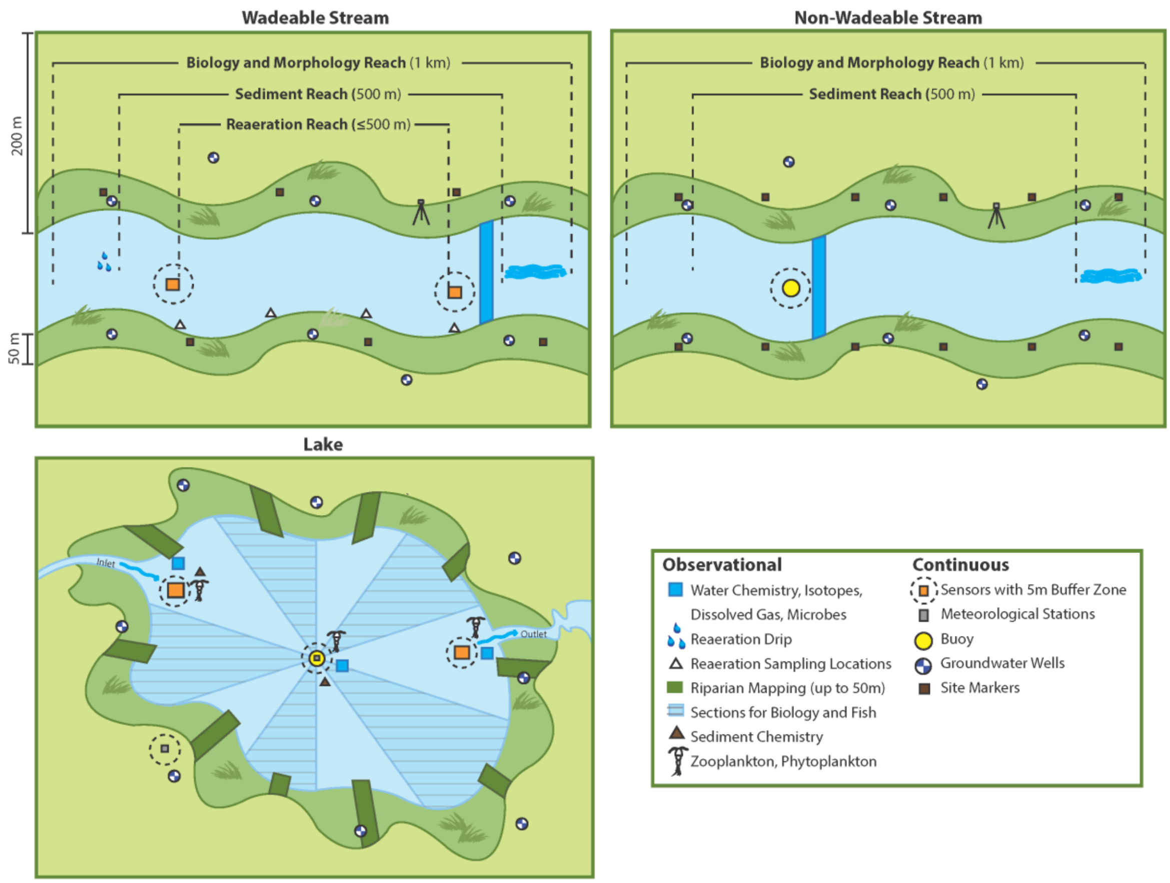

Figure 3. General diagram illustrating the sampling locations for all three classes of NEON Aquatic sites.

Similar to riparian assessment, the AOS sampling design for physical and chemical aquatic parameters is based on using established locations throughout the tenure of NEON Operations. Fluxes of nutrients, carbon, and other constituents in streams, rivers, and lakes are quantitatively significant on a global scale (Cole et al. 2007, Alexander et al. 2008). The AOS sampling design will enable calculation of the fluxes and flows of chemically and biologically relevant constituents in aquatic ecosystems and is built on the tenet that the physical structure of streams, rivers, and lakes is in a dynamic equilibrium, i.e., as the current carries sediment downstream, sediment from upstream is deposited, replacing the lost sediment (Curry 1972, Vannote et al. 1980). Thus, over relatively short timespans (on the order of months), when no major flooding or drying events occur, stream and river channel morphology tends to be stable. One of the major components of calculating fluxes of constituents in water is the amount of water traveling in the water body (i.e., discharge or the velocity of water multiplied by the cross-sectional area of the stream at a given location). By collecting multiple measurements of discharge, channel area, and stream stage (i.e., water height) over a range of discharge levels, a stage-discharge rating curve can be developed for a specific cross section. This cross section location will remain fixed so that the stage-discharge relationship can be checked and revised regularly, when necessary (e.g., following major disturbance events), for the duration of NEON Operations (RD[11] & RD[17]). The discharge methods employed at NEON aquatic sites primarily follow the USGS discharge protocols (Rantz 1982, Turnipseed and Sauer 2010). These rating curves are then linked to continuous measurements of water level (AIS data product) to calculate continuous discharge. In lakes, inflow and outflow locations will be identified, when possible, and samples and measurements will be made at these boundaries in addition to sampling at a central point in the main basin of the lake.

In order to calculate fluxes, the concentration of dissolved and particulate constituents (i.e., water chemistry) is required in addition to the flow measurements described in the previous paragraph (RD [16], RD[12], RD[19]). In shallow, well mixed streams one sample can be considered to be representative of the entire water column, while in deeper, stratified (i.e., where there are significant changes in temperature with depth) lakes multiple samples will be collected to capture the chemical variability across stratified layers (RD[18]). The samples collected from different depths in stratified rivers and lakes for AOS will complement the vertically profiling sensors deployed through AIS. For comparability and extrapolation of water chemistry parameters, samples will be collected in conjunction with continuously monitoring sensors, i.e., at the primary sensor location (Sensor Set 2) in streams and at all infrastructure in rivers and lakes, including the inflow, outflow, and buoy location at the deepest part of the lake.

In addition to being conduits and reactors for transport and transformation of chemical constituents, water bodies also provide habitat for a variety of biological organisms. The habitat structure of streams, rivers, and lakes is a function of many factors including flow regime, light regime, riparian inputs (e.g., large woody debris), and sediment characteristics (e.g., silt versus large cobbles). Thus, quantifying and characterizing sediment is an important component of the NEON Aquatic Sampling Strategy (RD[06], RD[07]). Sediments are chemically important as they can sorb nutrients such as phosphate (Reddy et al. 1999), control the bioavailability of contaminants (Eggleton and Thomas 2004), and bind metals (Chapman et al. 1998). Sediments are also important to aquatic organisms, e.g., some submerged aquatic vegetation, macroinvertebrates, and fish have preferences for specific habitat types (Baptista et al. 2001, Koch 2001, Kawanishi et al. 2015, Leps et al. 2015). In order to capture the variability and composition of sediments in aquatic ecosystems, NEON will sample sediments from depositional zones identified at each of the specific Aquatic sites depending on their flow and geomorphological structure. Surface sediments will be collected in accordance with EPA EMAP and USGS NAWQA protocols (Wilde 2005). Heterogeneity of sediments is common, and the NEON AOS sampling will composite multiple sediment samples within the reach in order to increase the probability of detecting trace elements and to enhance the comparability of data among sites (Collins et al. 1997, Palazon et al. 2015).

Collecting information about biological community composition in aquatic ecosystems is an important component of the AOS sampling strategy as it is a critical link in understanding the ecology of aquatic ecosystems in order to detect drivers of change and their ecological impacts (RD[20], RD[23], RD[21], RD[22], RD[14], RD[08], RD[13]). Distinct habitat structures have historically been classified in lotic and lentic ecosystems, such as pools, riffles, and runs in streams (Rosgen 1994) and the littoral and pelagic zones in lakes (Schindler and Scheuerell 2002). Based on the types of habitat present at each NEON aquatic site, the exact locations where biological sampling will take place will be determined in order to capture site-specific variability for a given type of organism. Biological samples will be collected from the most common habitat types, determined from annual site-specific rapid habitat assessments. For plants, 10 transects will be established across the stream at specific representative habitat types (with 5 transects for each of the two dominant habitat types or all 10 if there is only one dominant habitat type). These transects will be distinct from the discharge and water chemistry transects in order to avoid sampling areas that are regularly disturbed. Specifically, benthic macroinvertebrate, zooplankton, and periphyton samples will be collected quantitatively, i.e., a known area or volume will be sampled so that organism density can be derived, from representative habitat types. Fish sampling will take place at a combination of three fixed and seven random sampling areas due to their rapid mobility. For all types of organisms, multiple samples will be taken from each representative habitat type in order to ensure that the sampling is robust and representative of variability in the system.

6.3 AOS Temporal Sampling Strategy

Due to variable patterns in precipitation, discharge, vegetation, temperature, and light across the suite of NEON aquatic sites, AOS temporal sampling will be based on specific local drivers of variability similar to those used to delineate the 20 ecohydrologic NEON Domains (AD[01]). Specifically, hydrologic, climatic, and biological metrics derived from historical data will be used to determine the timing of AOS sampling at each NEON Aquatic site and will be described in the *Aquatic Site Sampling Design Documents* (RD[44]-RD[61] for Domains 01-19, respectively).

Analysis of hydrologic metrics were performed using data obtained from the closest USGS gauge to each site of a similar sized catchment that had a minimum of 15 years of data. Non-metric Multidimensional Analysis was used to order sample units along hydrologic regime gradients characterized using six flow metrics: 1) the annual coefficient of variation, 2) annual high flow pulse count with pulses defined as three times the annual median flow, 3) a baseflow index defined as the seven-day minimum flow divided by the mean flow for each year, 4) flow predictability (Colwell 1974), 5) flow constancy divided by predictability, and 6) the mean duration of high flow pulses. The suite of hydrologic metrics was selected based on low inter-metric redundancy and prior success in relating such a system to life history strategies of stream organisms (Mims and Olden 2012). Baseflow (normal flow) was calculated according to the USGS standard definition as the 25th and 75th percentile of variation of percent exceedance (Searcy 1959).

Climatic conditions were derived from mean daily temperature records collected from the National Oceanographic and Atmospheric Administration National Climatic Data Center land-based station datasets. A minimum of ten consecutive years of air temperature data from the weather station closest to each Aquatic Site was chosen. Degree days (DD) are used as a measure of heating or cooling, and are often used to predict plant or insect development times. DD were determined using the air temperature dataset described above and calculated on a base of 0°C (Vannote and Sweeney 1980) following the formula:

$$DD = T_{mean} - T_{base} \quad (1)$$

Where, T_{mean} is the mean daily temperature and T_{base} is the temperature at which growth begins. Cumulative DD were then calculated. Because water temperature data were not available for all NEON Aquatic sites, air temperature was used as a proxy in this analysis. Biological bout sampling dates were determined at 5-15%, 45-55%, and 85-95% of cumulative degree days (Mackay and Kalff 1969, Chester and Robson 2011, Heino et al. 2003).

Biological metrics, including leaf out and leaf fall, were defined using moderate resolution imaging spectroradiometer (MODIS) and Normalized Difference Vegetation Index (NDVI) phenology data product (MOD13Q1). Phenology data for a 3 km by 3 km area centered around each Aquatic Site were acquired for the period 2001-2009. Sample dates were centered around mean onset of greenness (spring) and prior to mean low greenness (autumn; Ganguly et al. 2010).

Ultimately, AOS temporal sampling will be frequent enough to capture annual variability at a site while acknowledging that there are logistical constraints on how frequently samples can be collected in the field for different protocols. The detection of change relies on capturing natural variability in order to separate this natural variability from ecological variation due to climate change, land-use change, and invasive species (AD[01]). Sampling should also coincide and integrate with other NEON sample collection activities outside of the Aquatic system, e.g., atmospheric sampling, and optimize the use of field team time and resources, e.g., collecting water quality samples while out for sensor maintenance tasks. One overarching design component of the AOS temporal sampling strategy is based on the concept of individual protocols that are performed independent of other protocols and sets of protocols that are performed over a fixed time interval of multiple days, e.g., biological and sediment sampling, and referred to as a "bout." Sampling bouts ensure comparability and linkages across measurements that are related; specific seasonal bouts are described in more detail in the following section.

The frequency of independent protocols will vary depending on the sample or measurement being collected and the characteristics of each NEON Aquatic Site. At all NEON Aquatic sites, 12 fixed-frequency samples will be collected for water chemistry, dissolved gases, stable isotopes, and surface microbes on a monthly basis. AOS water chemistry samples will be collected on Tuesday, if possible, to match with government protocols, e.g., NAWQA and EMAP. Sample collection frequency at NEON Aquatic sites is similar to that selected by NAWQA (Gilliom et al. 1995) and has been shown to produce unbiased load estimates (Robertson and Roerish 1999). At stream and river sites, where hydrological and chemical dynamics are often driven by seasonal factors, an additional set of 14 chemistry samples will be collected to capture water quality (i.e., chemistry, dissolved gasses, and isotopes) during dynamic periods of the hydrograph, which has been shown to be a valuable strategy when using data to estimate flux values (Harmeson and Barcelona 1981). The selection of when to collect these samples will be based on the cumulative hydrograph at each site with sampling efforts temporally focused on times of rapid change to capture flushing responses, i.e., steep slopes in the cumulative hydrograph (Gilliom et al. 1995, King and Harmel 2003) and elevated discharge to capture major times of export. For some sites, such as snowmelt dominated areas, this may mean weekly sampling during spring freshet. For other sites, such as those where flow is more consistent over the year, a bi-weekly sampling approach through the year will be applied to capture the annual variability.

At stream sites two rating curves will be developed: 1) stage-discharge rating curve to be used for determining water fluxes, which rely on flow rates, and 2) reaeration rate rating curves, including reaeration-discharge rating curves) used in the calculations of continuous stream metabolism (RD[03], RD[04]). During the development of rating curves, i.e., the first year of Operations for the site, measurements will be taken with relatively high frequency, approximately bi-weekly for discharge and up to 10 times per year for reaeration, with the goal of capturing the entire seasonal range of flow magnitudes. Once initially established, the rating curves for flow and reaeration rates will be periodically checked a few times per year to ensure that they are within acceptable limits. The criterion for creating new rating curves will be a deviation of greater than 10% across three different measurements. At some NEON Aquatic sites dramatic hydrologic events will also trigger an assessment of rating curves based on flow and/or precipitation, which can both be important drivers of stream or river channel physical structure (Leopold et al. 1964). Reaeration rating curves will need to be verified and potentially re-calculated during seasons when factors other than flow may be an important driver of reaeration rates, e.g., during fall when litter fall may have a large influence (Roberts et al. 2007).

At all stream, river, and lake Aquatic sites, three seasonal sampling bouts will take place each year (**Figure 4**). The seasonal bouts are specified time periods, usually a month long, during which multiple AOS protocols can be executed in order to allow logistical flexibility for time consuming protocols while still ensuring that they are collected close enough in time to be comparable. Bouts 1 and 3 will entail collecting biology samples, which include aquatic plants and macroinvertebrates followed by periphyton, benthic microbes, and zooplankton (rivers and lakes only), over the course of at least two days followed by sediment collection, which will take another whole day, and ending with fish sampling, which will take multiple days to complete. On each sampling day a team of at least two field staff will be active. Bouts 1 and 3 will be timed to take place during "spring" and "autumn", which are defined by degree days and vegetation greenness (**Figure 4**)**.** Spring sampling, i.e., seasonal bout 1, will commence as temperature and light levels begin to increase promoting an increase in primary productivity and/or a change in riparian phenology, which will be especially useful at sites where winter does not involve freezing temperatures (Mackay and Kalff 1969, Chester and Robson 2011). For high elevation and/or high latitude sites ice-off is an important threshold that must occur prior to bout 1 sampling. Seasonal bout 2 will take place during "summer", which is determined based on maximum greenness of vegetation and riparian phenology and may be a time of relatively low flow for some ecosystems and will not include fishing. Bout 2 may include riparian habitat assessment if the peak greenness window overlaps with bout 2 for a site. Autumn sampling, i.e., bout 3, will commence when light levels begin to decrease and temperatures cool, resulting in water column physical shifts, e.g., decreased lake stratifications, and biological community shifts, e.g., shredding insects proliferate as leaf litter inputs increase (Heino et al. 2003). Lake and river bathymetry will take place during bout 2 along with the riparian habitat assessment. However, in streams the channel geomorphology mapping can take place when flows are low and vegetation is least dense to decrease the effort required for field personnel to travel in and around the Aquatic site; this may be in the fall or between biology bouts 1 and 2. As with the AOS spatial design, initial evaluation of data will be required for each NEON Aquatic site in order to determine the actual calendar days that correspond to the ecological conditions under which the AOS sampling bouts will take place in the future.

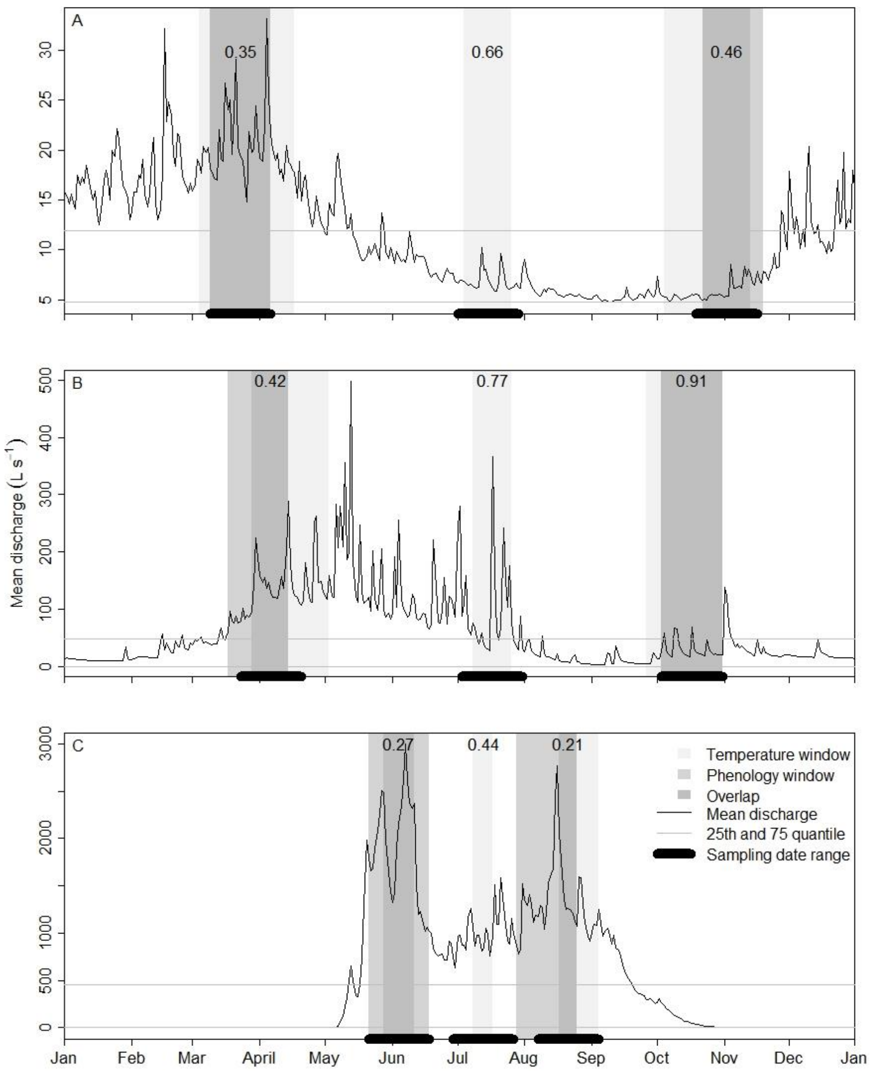

Figure 4. An example of the NEON organismal sample strategy in three different types of streams. A) D07 Walker Branch is a low baseflow stream. B) D06 Kings Creek is representative of consistent baseflow stream. C) D18 Oksrukuyik Creek represents snowmelt-dominated systems.

7 GROUNDWATER

Similar to the connections between aquatic ecosystems and riparian areas, there are important linkages and feedbacks between groundwater and streams, rivers, and lakes (Brunke and Gonser 1997, Winter 1999). Thus, the Aquatic Sampling Strategy also includes measurements of groundwater parameters with both AIS and AOS measurements to capture high resolution temporal changes as well as more detailed water quality characteristics on a seasonal basis. Several groundwater wells (up to eight) will be installed at each NEON Aquatic site in such a way that the magnitude and direction of groundwater flow can be calculated from sensor measurements. The groundwater well spatial design will be informed by site visits where topographical, meteorological, and logistical considerations can be assessed because these factors are important to the success of a groundwater monitoring campaign (Rosenberry and LaBaugh 2008). Wells will be located both in areas near the stream for capturing hyporheic flow (Boulton et al. 1998) and further from water bodies, depending on local conditions to enable sampling less directly connected groundwater flow paths (Sophocleous 2002). Groundwater wells will be installed to form triangles from which groundwater direction, gradients, and delay can be derived by data users from high temporal resolution groundwater elevation, temperature, and specific conductance (Rodhe and Seibert, 2011). In all locations around NEON Aquatic sites, the groundwater wells will be drilled to a depth ~ 3-6 feet below the seasonal low groundwater level to ensure that the full seasonal hydrograph can be captured.

In addition to the in situ sensor data capturing groundwater hydraulic gradients, groundwater samples will be collected on a semi-annual basis for the same suite of water chemistry parameters measured on surface water samples, which are described above in section 6. Because the suite of groundwater quality parameters will be similar to that of the surface water, direct comparisons can be made knowing the direction and magnitude of groundwater and hyporheic flow from the in situ sensors. These groundwater samples will be collected based on USGS NAWQA methods in accordance with existing best practices to ensure that the sample is representative of the particular zone, i.e., groundwater or hyporheic, from which water is sampled (Koterba et al. 1995). The timing of groundwater sample collection will be driven by the seasonal and cumulative hydrograph of the stream, river, or lake at the specific NEON Aquatic site as surface water hydrology is often linked to groundwater hydrology and water quality (Soulsby et al. 2009). In general, groundwater sample collection will be temporally timed to capture seasonal variability and match with the 25% and 75% (± 5%) of cumulative annual flow seasonal sampling bouts for AOS described above in section 6.4.2. For some NEON Aquatic sites, especially those in agriculturally dominated areas, seasonal groundwater withdrawals for human uses may drive groundwater height and flow direction, which will likely impact surface water level, temperature, and chemistry (Alley et al. 2002, van Roosmalen et al. 2009).

8 LOGISTICS AND ADAPTABILITY

The Aquatic Sampling Strategy document outlines how to achieve science goals while keeping protocol implementation consistent for all Aquatic sites despite the three different classes and dynamic hydrologic characteristics present at the suite of NEON Aquatic sites. *Aquatic Site Sampling Design Documents* have been designed with spatial and temporal information that meets the requirements of this overall sampling strategy in order to be consistent between Aquatic sites. However, these site specific locations and dates may be altered based on changes in ecological factors, e.g., baseflow or maximum greenness, over the lifetime of the observatory.

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