NEON AQUATIC SAMPLING STRATEGY

PREPARED BY	ORGANIZATION	DATE
Kaelin M. Cawley	AQU	04/10/2025
Michael SanClements	SCI	08/23/2022
Claire Lunch	SCI	07/25/2022
Keli Goodman	AQU	07/15/2022
Kelly Aho	AQU	04/05/2022
Kelly Hondula	AQU	04/05/2022
Nora Catolico	AQU	12/12/2020
Nick Harrison	AQU	12/12/2020
Dylan Monahan	AQU	12/12/2020
Guy Litt	AQU	12/12/2020
Bobby Hensley	AQU	12/12/2020
Bryce Nance	AQU	12/12/2020
Zach Nickerson	AQU	12/12/2020
Gregory House	AQU	12/12/2020
Stephanie Parker	AQU	02/17/2016
Ryan Utz	AQU	02/17/2016
Caren Scott	AQU	02/17/2016
Michael Fitzgerald	AQU	02/17/2016
Jesse Vance	AQU	02/17/2016
Brandon Jensen	AQU	02/17/2016
Charles Bohall	AQU	02/17/2016
Tracey Baldwin	AQU	02/17/2016

APPROVALS	ORGANIZATION	APPROVAL DATE
Kate Thibault	SCI	04/15/2025

RELEASED BY	ORGANIZATION	RELEASE DATE
Tanisha Waters	CM	05/01/2025

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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.
C	07/13/2022	ECO-06806	Updates based off comments from external users and updates to the design and implementation.
D	05/01/2025	ECO-07138	• Template update to rev J • Updated NEON logo • Minor formatting fixes

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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, Stephen Craft, Charlotte Roehm, Jenna Stewart, AQU TWG members, and the Reaeration and Metabolism TWG members for helpful comments. We thank Leslie Goldman and Colin Williams for their graphic design skills making Figure 3 & Figure 4. We thank Victoria Waits and John Musinsky for helping compile information for Table 1.

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 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 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 how US ecosystems are changing and support activities to address critical ecological questions and issues. Detailed information on the NEON design can be found in AD[01] and AD[02].

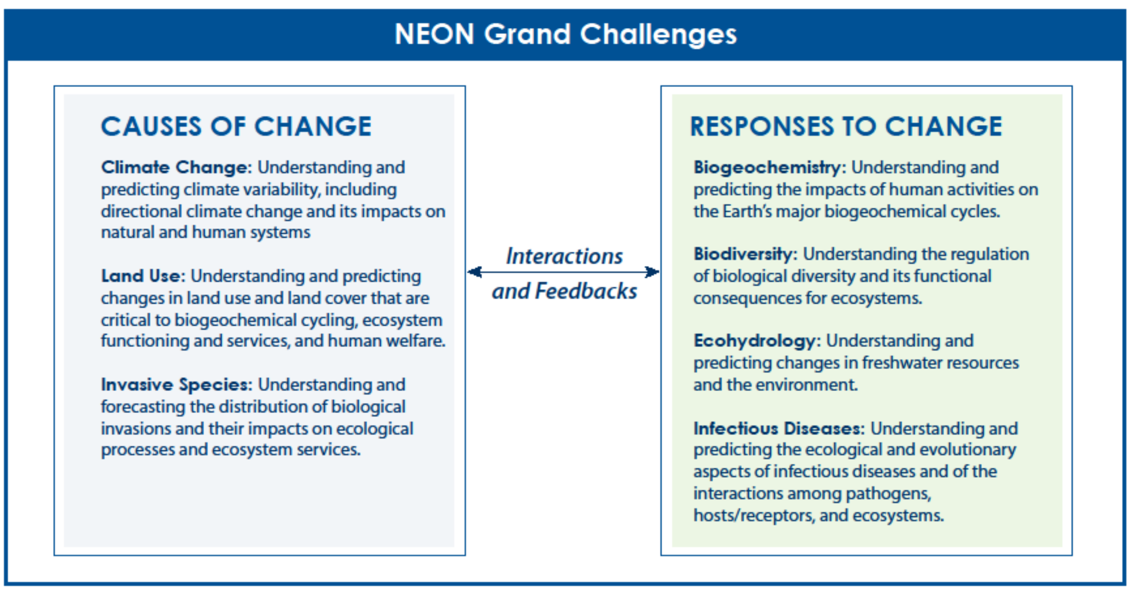


Figure 1. The seven Grand Challenges defined by the National Research Council (2001).

3.2 Components of the Observatory

Ecological variation is difficult to capture with synoptic sampling, monitoring studies, or remote sensing alone. Thus, a movement has developed toward research that integrates 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 (Carpenter 2008; Peters 2008). The combination of sampling strategies across temporal and spatial scales is an integral part of the National Ecological Observatory Network's (NEON) approach to addressing the

Grand Challenge questions (NRC 2001; Keller et al. 2008). The national-scale strategy adopted by NEON allows generalizing studies of individual aquatic ecosystems, past and present, to broader aquatic ecosystems in addition to representing local processes (McDowell 2015). The NEON design co-locates 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 delineated using multivariate geographic clustering (MGC) of ecoclimatic variance across the U.S. with uniform spatial distribution (Hargrove and Hoffman 1999, 2004). Within each Domain, data are collected from sites using a consistently applied set of sensors and sampling protocols. There are five science 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). Collocation of measurements associated with each of these components allows for linkage and comparison of data across the science components. For additional information on the AOP, TIS, and TOS components and NEON, see Keller et al. 2008, Kampe et al. 2010, Schimel et al. 2011, and Thorpe et al. 2016. NEON data are freely available from the data portal (data.neonscience.org) and packaged into over 180 data products described in the data product catalog (data.neonscience.org/data-product-catalog). All data download packages contain metadata in ecological metadata language (EML, Madin et al. 2007), a variable definition file, and a readme file.

Aquatic ecosystems exhibit physical, chemical, and biological variability over a range of spatial and temporal scales (Minshall 1988; Steele and Henderson 1994; Dudgeon et al. 2006). In an attempt to generate data to integrate multiple spatiotemporal scales, NEON developed a national-scale design to systematically sample national variability of ecological characteristics and to allow extrapolation of local observations to regions and the United States, including Alaska and Puerto Rico. The traceable links between this high-level NEON mission statement and the Observatory data provide a framework for the NEON design. The NEON aquatic sampling strategy informs measurements of aquatic biogeochemistry, biodiversity, and ecohydrology, collectively representing ecosystem responses to change driven by invasive species, land use, and climate change.

Overall, the NEON aquatic sampling strategy describes how NEON will collect standardized, high-quality data that can be used to understand national-scale aquatic ecology by following robust protocols for observations and designs for instrumented systems informed by ecological principles. Because NEON sites span climatic conditions from Arctic to tropical, a sampling strategy that results in comparable measurements across sites was required. The purpose of the Aquatic Sampling Strategy is to serve as a framework for configurations of AIS sensors and implementation of AOS protocols that fulfill the NEON goal by integrating high-level science requirements and logistical constraints.

The overarching strategy for NEON aquatic sites is to quantitatively capture physical, chemical, and biological variability at each local site over time. 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 all important drivers of ecological processes in aquatic ecosystems. Therefore, the aquatic sampling strategy relies on a combination of seasonal, riparian, and hydrologic conditions to determine the spatial and temporal sampling procedures meant to capture variability for a given NEON aquatic site. NEON instruments all aquatic sites with uniform monitoring equipment and field ecologists follow standardized, national-scale sampling protocols in a consistent manner to enable direct comparison among sites over the lifetime (30 years) of NEON operations. NEON classifies aquatic (AQU) sites into three main categories: (i) streams, (ii) rivers, and (iii) lakes (See section 3.5.2 for more details).

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 national 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 (**Table 1**). 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.

Table 1. NEON terrestrial site(s) nearest to each NEON aquatic site, the upstream watershed area, and the AOP coverage level of each watershed. The watershed is defined as the area of land draining into each water body from the most downstream sensor set (streams and rivers) or the littoral 2 sensor set (lakes). Watershed shapefiles can be downloaded from the NEON spatial data & maps page. See Algorithm Theoretical Basis Document (ATBD): Watershed delineation for NEON aquatic sites (NEON.DOC.005246) for details on how the watershed areas in this table were determined. The future AOP flight schedules can be used to determine the most likely timing of the next flight over an aquatic site and/or watershed. *Indicates a terrestrial site that is within the upstream watershed of the aquatic site. At some sites the nearby tower is located on the other side of a ridge (e.g., COMO) or the tower is located downstream of the aquatic sensors (e.g., MART) but is not within the upstream watershed, whereas the TALL tower is over 1,000 km away from TOMB but is within the watershed. ǂ TOOK inflow drains about 46.6 km^2 of the entire catchment, see Kling et al. (2000) for more details of the inflow hydrology at the site.

Domain	Site	Site Type	Nearby Terrestrial Site(s)	Distance to Tower(s) (km)	Area of Watershed (km^2)	Watershed AOP Coverage	AOP plans to completely capture watershed, if current coverage is partial
D01	HOPB	Stream	HARV	14.8	12.0	Complete	
D02	LEWI	Stream	BLAN	8.7	11.9	Complete	
D02	POSE	Stream	SCBI*	0.8	2.0	Complete	
D03	BARC	Lake	OSBS	2.4	31.3	Complete	
D03	FLNT	River	JERC	3.5	14,999	Partial	No
D03	SUGG	Lake	OSBS	2.6	39.6	Complete	
D04	CUPE	Stream	LAJA	13.7	4.3	Complete	
D04	GUIL	Stream	GUAN	23.8	9.6	Complete	
D05	CRAM	Lake	UNDE	5.2	0.6	Complete	
D05	LIRO	Lake	STEI	55.4	0.9	Complete	
D06	KING	Stream	KONA, KONZ*	1.0, 3.5	13.0	Complete	
D06	MCDI	Stream	KONZ	20.0	22.6	Complete	
D07	LECO	Stream	GRSM*	0.4	9.1	Complete	
D07	WALK	Stream	ORNL*	0.8	1.1	Complete	
D08	BLWA	River	DELA, TALL*	0.6, 59.4	16,159	Partial	No
D08	MAYF	Stream	TALL*	1.9	14.4	Complete	
D08	TOMB	River	LENO, DELA*, TALL*	0.4, 8.4, 1410	47,085	Partial	No
D09	PRLA	Lake	DCFS	1.2	3.4	Complete	

Domain	Site	Site Type	Nearby Terrestrial Site(s)	Distance to Tower(s) (km)	Area of Watershed (km^2)	Watershed AOP Coverage	AOP plans to completely capture watershed, if current coverage is partial
D09	PRPO	Lake	WOOD	0.8	2.1	Complete	
D10	ARIK	Stream	STER	92.5	2,632	Partial	No
D11	BLUE	Stream	CLBJ	145.4	322	Partial	Possible
D11	PRIN	Stream	CLBJ	19.8	48.9	Complete	
D12	BLDE	Stream	YELL	4.0	37.8	Complete	
D13	COMO	Stream	NIWO	3.9	3.6	Complete	
D13	WLOU	Stream	MOAB	351.9	4.9	Complete	
D14	SYCA	Stream	SRER	213.5	280	Partial	No
D15	REDB	Stream	ONAQ	87.2	16.7	Complete	
D16	MART	Stream	WREF	3.4	6.3	Complete	
D16	MCRA	Stream	ABBY, WREF	167.5, 174.3	3.9	Complete	
D17	BIGC	Stream	SOAP	2.8	10.9	Complete	
D17	TECR	Stream	SJER	65.3	3.0	Complete	
D18	OKSR	Stream	TOOL	9.3	57.8	Complete	
D18	TOOK	Lake	TOOL	10.3	67.6[‡]	Complete	
D19	CARI	Stream	BONA	0.1	31.0	Complete	

3.5.2 Classes and Selection of Aquatic sites

NEON classifies aquatic (AQU) sites into three main categories: (i) streams, (ii) rivers, and (iii) lakes. Streams are considered stretches of lotic water that are safely wadeable during most, if not all, of the year. Rivers are classified as stretches of flowing water that are not often, if ever, safely wadeable. Lakes are classified as lentic bodies of water that may or may not stratify depending on meteorological and hydrologic conditions during the year. NEON encompasses 34 aquatic sites, including 24 wadeable streams, 3 non-wadeable rivers, and 7 lakes, that are located within 19 of the 20 Domains (D20, Pacific Tropical, does not have an aquatic site due to logistical and permitting issues). Wetlands are not a specific type of water body in the aquatic subsystem. However, > 200 terrestrial observation system (TOS) plots are classified as wetlands using the National Land Cover Database (NLCD) in Domains D01, D03, D04, D05, D08, D09, D18, and D19.

Aquatic sites were chosen from a pool of locations identified by the research community to have representative features, habitats, and ecological properties of their respective Domain. Each NEON aquatic site possesses key attributes to facilitate research that can leverage Observatory generated data. Lakes and rivers were chosen to represent the hydrologic and watershed characteristics of the region for size, hydrologic flow (e.g., seepage vs. flow-through lakes), and shoreline characteristics. Streams and rivers were chosen to capture diversity across multiple habitat types (i.e., pools, riffles, and runs) common to a Domain or region. Many prospective sites had a history of ecological research nearby and the neonscience.org website has more detailed information about the selected sites. Where feasible, AQU sites were selected to be nearby the NEON terrestrial sites. NEON then considered logistical factors, such as keeping the distance between Domain office locations and sites to less than a 4-hour drive, and availability of infrastructure, such as line power and communication services. Lastly, final site selection was contingent on permits that guaranteed land-use and land-access from the site hosts for the duration of planned Observatory operations.

Because most water bodies at NEON aquatic sites have historic names that may not reflect the three-tier NEON classification, e.g., Arikaree River which is a NEON stream site (i.e., wadeable), **Table 2** provides a list of NEON site names, Domains, and classes of NEON aquatic sites. Hydrologic differences between the classes of NEON aquatic sites necessitate protocols that 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 (Jones, 2010) and the NEON aquatic sampling strategy aims to integrate lake, river, and stream variability to enable understanding continental-scale ecological change.

Table 2. Domain numbers, NEON site names, and locations, and classes for all NEON aquatic sites.

Domain	Site ID	NEON Site Name	NEON Site Class	Domain Name	State	Alt Precip Site ID
01	HOPB	Lower Hop Brook NEON	Stream	Northeast	MA	HARV
02	POSE	Posey Creek NEON	Stream	Mid-Atlantic	VA	SCBI
02	LEWI	Lewis Run NEON	Stream	Mid-Atlantic	VA	BLAN
03	BARC	Lake Barco NEON	Lake	Southeast	FL	OSBS
03	SUGG	Lake Suggs NEON	Lake	Southeast	FL	OSBS
03	FLNT	Flint River NEON	River	Southeast	GA	JERC
04	CUPE[2,3]	Rio Cupeyes NEON	Stream	Atlantic Neotropical	PR	-
04	GUIL[2,3]	Rio Yahuecas NEON	Stream	Atlantic Neotropical	PR	-
05	CRAM	Crampton Lake NEON	Lake	Great Lakes	WI	UNDE
05	LIRO	Little Rock Lake NEON	Lake	Great Lakes	WI	UNDE
06	KING	Kings Creek NEON	Stream	Prairie Peninsula	KS	KONA & KONZ
06	MCDI[2]	McDiffett Creek NEON	Stream	Prairie Peninsula	KS	-
07	WALK	Walker Branch NEON	Stream	Appalachians	TN	ORNL
07	LECO	LeConte Creek NEON	Stream	Appalachians	TN	GRSM
08	MAYF	Mayfield Creek NEON	Stream	Ozarks Complex	AL	TALL
08	BLWA[4]	Black Warrior River NEON	River	Ozarks Complex	AL	DEAD
08	TOMB[4]	Lower Tombigbee River NEON	River	Ozarks Complex	AL	LENO
09	PRPO	Prairie Pothole NEON	Lake	Northern Plains	ND	WOOD
09	PRLA	Prairie Lake NEON	Lake	Northern Plains	ND	DCFS
10	ARIK[1,3]	Arikaree River NEON	Stream	Central Plains	CO	-
11	PRIN[1,3]	Pringle Creek NEON	Stream	Southern Plains	TX	-
11	BLUE[1,3]	Blue River NEON	Stream	Southern Plains	OK	-
12	BLDE	Blacktail Deer Creek NEON	Stream	Northern Rockies	WY	YELL
13	COMO	Como Creek NEON	Stream	Southern Rockies	CO	NIWO
13	WLOU[2]	West St Louis Creek NEON	Stream	Southern Rockies	CO	-

Domain	Site ID	NEON Site Name	NEON Site Class	Domain Name	State	Alt Precip Site ID
14	SYCA[2,3]	Sycamore Creek NEON	Stream	Desert Southwest	AZ	-
15	REDB[1,3]	Red Butte Creek NEON	Stream	Great Basin	UT	-
16	MART	Martha Creek NEON	Stream	Pacific Northwest	WA	WREF
16	MCRA[5]	McRae Creek NEON	Stream	Pacific Northwest	OR	-
17	BIGC[2]	Upper Big Creek NEON	Stream	Pacific Southwest	CA	-
17	TECR	Teakettle Creek - Watershed 2 NEON	Stream	Pacific Southwest	CA	TEAK
18	OKSR	Oksrukuyik Creek NEON	Stream	Tundra	AK	TOOL
18	TOOK[2]	Toolik Lake NEON	Lake	Tundra	AK	-
19	CARI	Caribou Creek NEON	Stream	Taiga	AK	BONA

[1] Sites with bulk primary precipitation collectors, weighing gauge surrounded by a double fence inter-comparison reference (DFIR).

[2] Sites with bulk secondary precipitation collectors, tipping bucket.

[3] Sites with wet deposition collectors, heated and cooled samplers for chemical and isotopic analysis.

[4] Sites with meteorological measurements collected at a collocated terrestrial site. For BLWA, query Dead Lake (DEAD) for relevant meteorological data. For TOMB, query Lenoir Landing (LENO) for relevant meteorological data.

[5] NEON does not produce precipitation data at MCRA or a nearby alternate NEON site. Users are encouraged to use precipitation data from H.J. Andrews Experimental Forest, which is administered by Oregon State University, the USDA Forest Service's Pacific Northwest Research Station, and the Willamette National Forest

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), the Environmental Monitoring & Assessment Program (EMAP) and National Aquatic Resource Surveys (NARS), which are a programs of the U.S. Environmental Protection Agency (EPA), and the National Science Foundation's long-term ecological research program (LTER) (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).

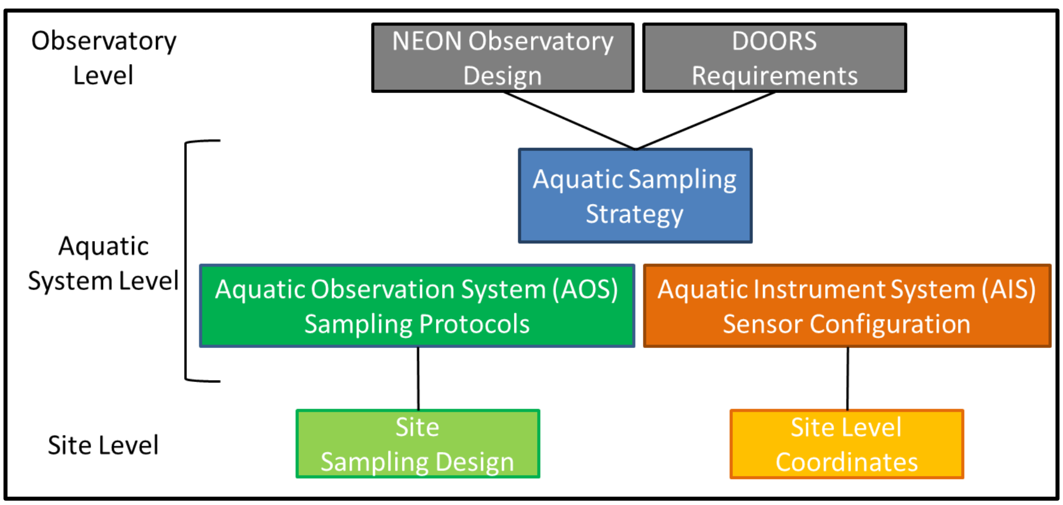
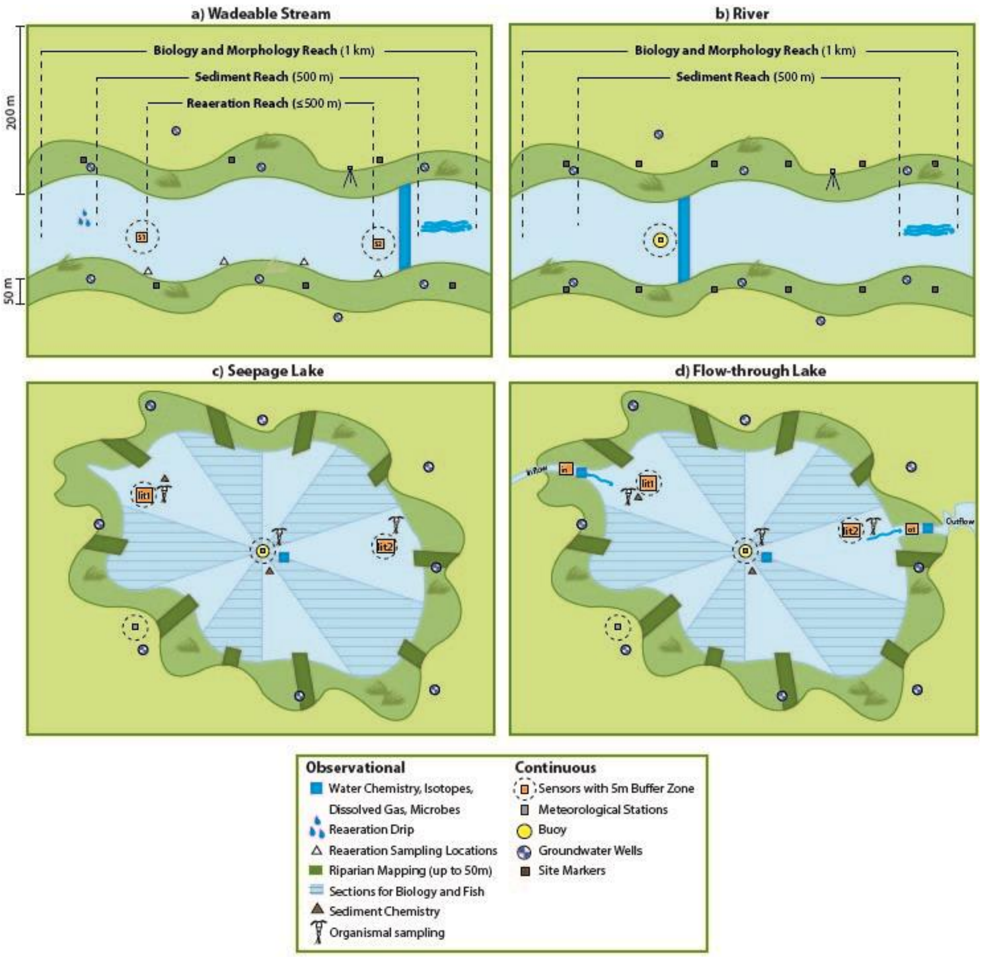


Figure 2. Overview of documents that define the scope of NEON science from the observatory-, AQU system-, AIS and AOS subsystem-, and site-levels.

4 OVERVIEW OF THE AQUATIC SAMPLING STRATEGY

NEON developed a national-scale design to systematically sample national variability of ecological characteristics and to allow extrapolation of local observations to regional and national 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 national-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 using homogeneous sensor suites 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



		Streams		Rivers		Lakes	
	Automated Instrument Measurements	Upstream U	Downstream D	Buoy B	Near Bank NS	Buoy B	Littoral L
	PAR at water surface	✓	✓	✓	⊘	✓	⊘
	PAR below water surface	⊘	⊘	✓	✓	✓	✓
	Elevation of surface water (pressure transducer based)	✓	✓	⊘	✓	⊘	✓
	Temperature in surface water	✓	✓	⊘	✓	⊘	✓
	Temperature at specific depth in surface water (depths vary by site)	⊘	⊘	✓	⊘	✓	⊘
	Water quality: specific conductivity, chlorophyll a, dissolved oxygen content, pH, turbidity, and fluorescent dissolved organic matter (fDOM)	✓ (no fDOM)	✓	✓	⊘	✓	⊘
	Nitrate in surface water	⊘	✓	✓	⊘	✓	⊘
	Groundwater wells: specific conductivity, water temperature, elevation of groundwater	✓ Up to 8 per field site					
M	Meteorological measurements: wind speed and direction, air temperature, barometric pressure, relative humidity, shortwave radiation, and photosynthetically active radiation (PAR)	✓ One on bank		✓ One on bank, One on buoy		✓ One on bank, One on buoy	

Figure 3. General diagram illustrating the sampling and sensor locations for all classes of NEON aquatic sites.

5 AQUATIC INSTRUMENT SYSTEM (AIS)

The AIS platform provides sensor-derived information about physical, biological, and chemical properties of aquatic water bodies, the surrounding atmosphere, and adjacent riparian areas (**Figure 3**). Instruments in the AIS system collect information at point spatial scales with high temporal resolution, i.e., many measurements per hour (**Table 3**). These instruments can monitor variables that can vary on the order of minutes, such as changing weather patterns or streamflow (Pellerin et al., 2012, Sobczak and Raymond, 2015).

5.1 AIS Measurements

The sensor suite deployed at aquatic sites is composed of four primary sensor arrays: riparian meteorological stations, buoy mounted meteorological stations in lakes and rivers, underwater aquatic sensors measuring water quality parameters, and a network of shallow groundwater monitoring wells (**Figure 3**). Sensors are deployed consistent with well-established methods (EPA, 1987; WMO, 1983), which improves ease of comparing data from other networks with NEON and more details about the sensor infrastructure that NEON uses in streams can be found in Hensley et al., (2021). The Sensors deployed in groundwater wells are discussed in the Groundwater Instrumentation and Sampling section below.

Riparian meteorological stations deployed at aquatic sites are outfitted with a subset of the sensors installed on NEON terrestrial towers using identical configurations and are published in the same data product (**Figure 3**). Some aquatic sites have precipitation sensors, a double fence intercomparison reference (DFIR) or tipping bucket, installed if they are located over 10 km away from a NEON terrestrial site or if the terrestrial and aquatic sites are in areas with different climatic conditions. At river and lake sites, similar meteorological measurements are also made above the water surface from buoys (**Figure 3**). At stream sites, photosynthetically active radiation is measured above the water surface at or near the submerged sensor sets

A submerged sensor suite captures chemical and physical properties of surface water at all NEON aquatic sites. Transducers record water pressure, from which NEON algorithms derive water surface elevation. Water quality is measured at each site using a multiparameter sonde. Additionally, all AQU sites measure surface water nitrate concentrations with an optically based sensor. At lake and river sites, photosynthetically active radiation is measured 0.5 m below the water surface and a chain of temperature thermistors records temperature throughout the water column.

Although the AIS sensors are rugged field instruments, NEON recognizes the need for sensor preventive and corrective (i.e., repair) maintenance. AIS sensor maintenance takes place at all sites on a semi-monthly basis initially and will be optimized for each site and season as more data becomes available to ensure proper sensor function while efficiently using field personnel time. Seasonal deployment and/or removal of some sensors takes place at select sites where significant ice occurs, i.e., in streams that freeze solid (Oksrukuyik Creek) or nearly so (Caribou Creek) or lakes that freeze over (Toolik Lake, Prairie Pothole, Prairie Lake, Crampton Lake, and Little Rock Lake). Because NEON removes AK, ND, and WI lake pontoon buoys before ice-on, NEON collects additional temperature and conductance profile data in a continuously deployed subsurface-moored temperature chain system. The subsurface moored temperature and conductance assembly has the advantage of collecting data below ice in the winter, but as a result requires occasional manual data retrieval before and after ice periods.

Table 3. AIS measurement frequencies for in situ aquatic sensors and meteorological sensors.

In situ Measurement	**Collection Frequency**	**Publication Frequency***
PAR	1 Hz	1- and 30-minute
Elevation of surface water	1 per minute	1-, 5-, and 30-minute
Continuous discharge	1 per minute**	1 per minute**
Water temperature	1 Hz	1-, 5-, and 30-minute
Water quality (conductivity, turbidity, pH, DO, fDOM)	1 per minute	1 per minute
Nitrate	Burst of 20 per 15 minutes	15-minute
Buoy, where different		
PAR	2 per minute	1- and 30-minute
Water temperature	1 per minute	1- and 30-minute
Water quality (conductivity, turbidity, pH, DO, fDOM, chla)	1 per 5 minutes	1 per 5 minutes
Groundwater Wells		
Groundwater specific conductivity, temperature, and elevation	1 per 5 minutes	1 per 5 minutes
Meteorological Measurement	**Collection Frequency**	**Publication Frequency***
Wind speed and direction	1 Hz	2- and 30-minute
Air temperature, PAR, relative humidity, shortwave and longwave radiation	1 Hz	1- and 30-minute
Barometric pressure	0.1 Hz	1- and 30-minute
Buoy, where different		
Wind speed and direction	Burst of 40 per minute	2- and 30-minute
Shortwave and longwave radiation	2 per minute	1- and 30-minute
Air temperature, relative humidity, barometric pressure	1 per minute	1- and 30-minute

* Where publication frequency and collection frequency are different users are directed to the Algorithm theoretical basis document (ATBD) for each data product for details of the algorithm used for converting the data frequency.

** Continuous discharge at TOMB is reported using data from a nearby USGS gauge station (which reports data at hourly or 30-minute frequencies) due to downstream hydrologic controls that dynamically alter the relationship between stage and discharge.

5.2 AIS Spatial Sampling Strategy

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 change on a decadal timescale. Meteorological station locations were chosen to capture conditions in the riparian area surrounding NEON aquatic sites using a rapid riparian habitat assessment. NEON then considered logistical factors such as land access and power supply availability to select the final meteorological station location.

To address meteorological differences between riparian areas and open water at lakes and rivers, such as shading from riparian vegetation versus unshaded open water (Vannote et al., 1980, Lauck et al., 2005), NEON collects additional meteorological measurements on the buoys (**Figure 3**). Additionally, in lakes and large rivers, where lake size, wind speed and barometric pressure influence gas exchange at the water surface (Raymond et al., 2012; Klaus and Vachon, 2020) measurements made in the riparian area may not be representative of open water conditions because of the presence of trees. Thus, these secondary meteorological measurements made in open water are critical for interpreting measurements being made below the water surface in lakes and rivers.

Submerged sensor arrays are spatially distributed to measure in both pelagic and littoral areas of lakes and rivers, and in the thalweg of streams (**Figure 3**). This spatial design facilitates the calculation of key ecological drivers and processes at the reach scale of streams, lakes, and rivers, including aquatic ecosystem metabolism, discharge, nutrient fluxes, sediment transport, hyporheic exchange, and heat energy balance, among others. The NEON buoys are installed in the deepest location in the main basin of lakes. At river sites, in situ buoy mounted sensors are installed outside of navigation lanes within the main channel (**Figure 3**). Stratification and light availability, which often vary with depth, can drive nutrient distributions and dissolved gas concentrations, both of which are important for biological communities (Engelhardt and Kirillin, 2014). Lakes exhibiting prolonged thermal stratification (Toolik Lake, AK, Little Rock Lake, WI, and Crampton Lake, WI) use buoys with a winch profiler system for continuous water quality measurement near the water surface and across multiple depths. At the other buoy sites, former profiling water quality measurements became fixed to approximately 0.5 m depths beginning in 2020 owing to a lack of prolonged thermal stratification detected from previous NEON monitoring. The nitrate sensor and underwater PAR sensors on the buoys are fixed at 0.5 m below the water surface to capture conditions in the photic zone. The temperature chain hanging from the pontoon buoy's platform consists of thermistor depths covering the water column's vertical profile during seasonal low water conditions, and the total length is designed to prevent the temperature chain dragging on the water body bottom. In addition to the buoy-based measurements at lakes, surface water elevation, temperature, and down welling underwater PAR are measured where conditions represent the littoral zone. At rivers, a near-shore sensor station records pressure that is converted to surface water elevation.

At stream sites, longitudinal variation is more important than changes in aquatic characteristics with depth, so two sensor sets are installed at upstream and downstream locations rather than having multiple sensors with different positions in the water column. Channel morphology and hydrologic characteristics are considered in determining the distance between the upstream and downstream sensor stations and the in-stream placement. Sensors are located in well-mixed pools that maximize the duration of continuous water quality and water level measurements (Hensley et al., 2021), with a downstream riffle characterized by stable downstream channel controls and laminar flow. The upstream and downstream AIS sensor stations are selected to capture a 30 - 45 minute travel time during median flow to support two-station metabolism modeling using NEON data products (Riley & Dodds, 2013).

5.3 AIS Temporal Sampling Strategy

The AIS sampling frequency is designed to capture flux estimates of organic matter, nutrients, and suspended solids under conditions that are likely quantitatively important yet challenging to sample with traditional grab sampling approaches. Continuous aquatic sensor readings are invaluable for streams and rivers where constituent fluxes may vary over short time scales, especially during high-flow and storm events, which 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 addition to storm events, NEON users can use high temporal resolution sensor data to produce higher-level derived estimates, such as stream metabolism, annual fluxes, and lake stratification (Utz et al., 2013). Metabolism measurements, for instance, have been employed to quantify long-term integrated signals of energy cycling in aquatic ecosystems (Carpenter et al., 2005; Roberts, 2007; Staehr et al., 2012). Such models require frequent measurements of dissolved oxygen and temperature throughout diel cycles (Riley and Dodds, 2013; Grace et al., 2015). The AIS sensors collect high temporal resolution data, on the order of a measurement per second for meteorological data and a measurement per minute for some in situ physical and chemical data (**Table 3**). The sensors for these high frequency measurements are rigorously calibrated and data is averaged, QAQC flagged and assigned uncertainty for publication based on data product specific Algorithm Theoretical Basis Documents (ATBDs), also see Csavina et al. (2017) and Sturtevant et al. (2022).

6 AQUATIC OBSERVATION SYSTEM (AOS)

The Aquatic Observation System (AOS) within NEON includes field measurements and sample collections executed by field personnel at NEON Domains. The standardization of the AOS protocols across all sites is key to the success of NEON, as well as part of its novelty. Although the timing of protocol execution may vary at NEON aquatic sites due to site-specific seasonal hydrology or permitting constraints, e.g., sampling restrictions may be enforced at some sites during fish spawning seasons. Shared field equipment used at multiple sites is decontaminated following US Forest Service, EPA, and USGS guidelines to prevent the spreading of invasive or endemic species between sites where equipment is shared (Parsons, 2008; USFS, 2016).

The AOS is designed to complement the AIS by providing additional information about biological, physical, and chemical parameters of aquatic water bodies and surrounding riparian areas (**Table 4**). Observational measurements collected and recorded by AOS provide additional insight into aquatic ecosystems that vary on longer timescales (e.g., riparian vegetation which varies seasonally), require time-intensive laboratory analyses (e.g., alkalinity titrations or ash free dry mass), or do not currently have in situ monitors available (e.g., genetic and elemental composition of biological organisms). Additionally, some AOS data can aid AIS data quality assurance and development of site-specific relationships in cases where NEON collects samples for analysis of similar constituents (e.g., dissolved organic carbon grab samples and fDOM sensor data). The AOS sample collection occurs over ecologically relevant and practical spatio-temporal scales, which represents the goal of the sampling strategy described below.

6.1 AOS Measurements

Critical physical properties of NEON aquatic ecosystems are quantified as part of the AOS system. These physical properties and collection methods vary by site type. For example, secchi depth and handheld meter-based temperature profiles are collected in lakes and rivers, but not in streams given their shallow depths. Discharge is measured at all stream and river sites across a variety of water stages to create site-specific stage-discharge rating curves. Measurement methods differ between site type. Discharge methods employed at NEON aquatic sites primarily follow standard USGS protocols (Rantz, 1982; Turnipseed and Sauer, 2010; Mueller et al., 2013). In streams, wading surveys, acoustic doppler current profiler (ADCP), and salt-dilution methods can all be used depending on conditions, while in rivers only ADCP surveys are conducted off a piloted boat. At stream sites, salt-based discharge measurements are conducted at the time of the gas exchange (i.e., reaeration) protocol using simultaneous gas and conservative salt tracer injections to evaluate physical drivers of oxygen fluxes. Gas exchange can be estimated using the change in downstream gas concentration normalized to conservative salt tracer concentration using the NEON data package download and an R package created and maintained on the NEON GitHub repository (github.com/NEONScience/NEON-reaeration). Bathymetry and morphology are physical properties that NEON monitors at all aquatic sites to create maps and metrics that delineate habitat distribution and physical features. In streams, morphology is surveyed over the permitted reaches using high-resolution total station surveying equipment. In rivers and lakes, bathymetry is mapped using a GPS and sonar system.

Chemical parameters collected by AOS are meant to comprehensively characterize water quality. As such, organic constituents, inorganic constituents, the mass of dissolved and suspended solids, particulate (^{15}N, ^{13}C) and water isotopes (^{2}H, ^{18}O), and dissolved gas concentrations (CO_2, CH_4, N_2O) all fall within AOS measurement protocols (see github.com/NEONScience/NEON-dissolved-gas for an R package developed to convert reference and equilibrated gas concentrations to aquatic dissolved gas concentrations *A DOI will be generated using Zenodo if accepted for publication and Aho et al. (2021) for a processed dataset for data collected through 2020). At each Domain support facility, NEON personnel measure pH and perform alkalinity titrations to reduce the time between sample collection and analysis. Sediment is collected from AQU water bodies for physical grain size and chemical analyses for a range of inorganic and organic constituents. Chemical properties of surface water, algal, and plant matter are also determined, including dissolved cations, anions, nutrients, elemental analysis (C, H, N, O) and stable isotopes (^{15}N, ^{13}C, ^{2}H, ^{18}O, and ^{34}S in algae).

Biological organisms sampled for the AQU system include benthic and water column microbes, algae (periphyton, seston, phytoplankton, and macroalgae), aquatic plants, zooplankton, aquatic macroinvertebrates, and fish. Each assemblage is sampled for community composition using either field identifications (plants and fish), microscopy-based taxonomic identification (algae, zooplankton, plants, macroinvertebrates), or DNA sequencing (marker gene sequences and metagenomes of microbes and metabarcoding of macroinvertebrates and zooplankton). A subset of fish specimens is DNA sequenced and included in the Barcode of Life (BOLD) database. Additionally, a rapid riparian assessment for dominant and subdominant riparian plant species, vegetation composition and cover, and bank characteristics is performed annually during the NEON airborne observation platform (AOP) flyover peak greenness window, which is determined by the dominant vegetation classes at nearby terrestrial sites (Musinsky et al., 2022). Current and planned AOP coverage of the NEON AQU sites is summarized in **Table 1**.

Table 4. AOS measurement and sample collection types and frequencies.

Sampling Module	Streams	Lakes	Rivers	Groundwater
Physical				
Discharge	up to 24	Up to 24*	up to 12	
Gas Exchange (i.e., reaeration)	6			
Rapid Habitat Assessment	1			
Morphology	1 per 5 years			
Bathymetry		1 per 5 years	Up to 1	
Active Layer Depth				Up to 16**
Biological				
Aquatic Plants	3	3	3	
Macroinvertebrates	3	3	3	
Zooplankton		3		
Periphyton and Phytoplankton	3	3	3	
Benthic Microbes	3			
Surface Water Microbes	12	6*	6	
Fish	2***	2***		
Riparian Assessment	1	1	1	
Chemical				
Water Chemistry	26	12*	26	2
Dissolved Gas in Water	26	12*	26	
Isotopes	26	12*	26	2
Sediment Chemistry	2	2	2	
Aquatic Plants	1	1	1	
Periphyton and Phytoplankton	3	3	3	

*Discharge and water chemistry, dissolved gas, isotopes, and microbe sampling are performed at the inflow and outflow of TOOK, which is the only lake with a defined inflow and outflow. All other lakes sites are seepage lakes, where discharge is not collected and grab samples are only collected at the buoy.
** Active layer dept is only measured at sites with permafrost (CARI, OKSR, TOOK).
*** Fish are not sampled at D13 Como Creek (COMO), D03 Lake Barco (BARC) and D03 Lake Suggs (SUGG) anytime or during bout 1 at D16 Martha Creek (MART) and McRae Creek (MCRA).

6.2 AOS Spatial Sampling Strategy

Like the AIS design that encompasses both riparian, water surface, shallow groundwater, and in situ aquatic sensor measurements (see section 3 above), AOS sampling takes place in and adjacent to water bodies at the NEON aquatic sites. For a list of terrestrial sites that are near or within the watershed of aquatic sites, thus enabling the linkage of terrestrial and aquatic ecological data. To quantify locational variance and heterogeneity of landscape-scale ecological processes at each NEON aquatic site, the AOS spatial design uses the same location for measuring physical and chemical variables while allowing flexibility in identifying representative habitat (i.e., riffle, run, or pool) for biological sampling at locations that can vary over time. AOS sampling is distributed throughout the stream reach in a manner that attempts to prevent interference but maximize linkages and extrapolation with other AIS and AOS operations. For example, water chemistry sampling occurs immediately downstream of the sensor set to allow for comparisons between grab samples and sensor measurements while avoiding suspending sediment from foot traffic that may affect sensor measurements. Boats and/or docks are used to facilitate sampling at rivers and lakes near buoys.

The AOS sampling strategy includes measurements of riparian vegetation, which is integrally linked to aquatic ecosystem processes, similar to AIS riparian meteorological measurements (Williamson et al., 2008). Field ecologists use a densiometer at stream and river sites to estimate riparian canopy cover. Along streams and rivers, riparian assessment takes place at approximately equidistant transects along the stream channel and covers up to 15 m from the water's edge (Fitzpatrick et al., 1998). Transects were established at the initiation of each site and will be maintained throughout NEON operations, if possible. Riparian assessments for lakes take place in permanently established wedges designed to divide the lake perimeter approximately evenly. Riparian assessment campaigns are meant to enable the linking of aquatic sites to the remotely sensed data collected by the NEON AOP, which detects vegetation patterns at most NEON sites using hyperspectral and lidar sensors alongside high-resolution imagery on an annual to triennial basis.

To calculate physical and chemical fluxes, the concentration of dissolved and particulate constituents is required in addition to the flow measurements (see section 5: Linking AIS and AOS Data to Produce Continuous Discharge Estimates for more details on flow estimates). In shallow, well-mixed streams and non-stratified lakes and rivers, one sample is usually representative of the entire water column, while in deeper, stratified lakes multiple samples are collected at varying depths to capture the chemical variability across stratified layers. In lakes and rivers, one sample is collected from a depth of 0.5 m to align with the AIS Buoy sensor location. When lakes or rivers are stratified, another sample is collected from the mid-point of the hypolimnion if the hypolimnion is at least 2 m thick, but less than or equal to 4 m thick. If the hypolimnion is greater than 4 m thick, a composite sample is created from water collected at one fourth and three fourths of the hypolimnion depth (**Figure 4**). The samples collected from different depths in stratified rivers and lakes for AOS complement the vertically profiling sensors deployed through AIS. For comparability and extrapolation of water chemistry parameters in streams, samples are collected at the primary sensor location (Sensor Set 2). Additionally, water chemistry samples are collected at the inflow and outflow locations at Toolik Lake. The Toolik Lake outflow sampling location is located at the lake-outflow interface, just inside the lake.

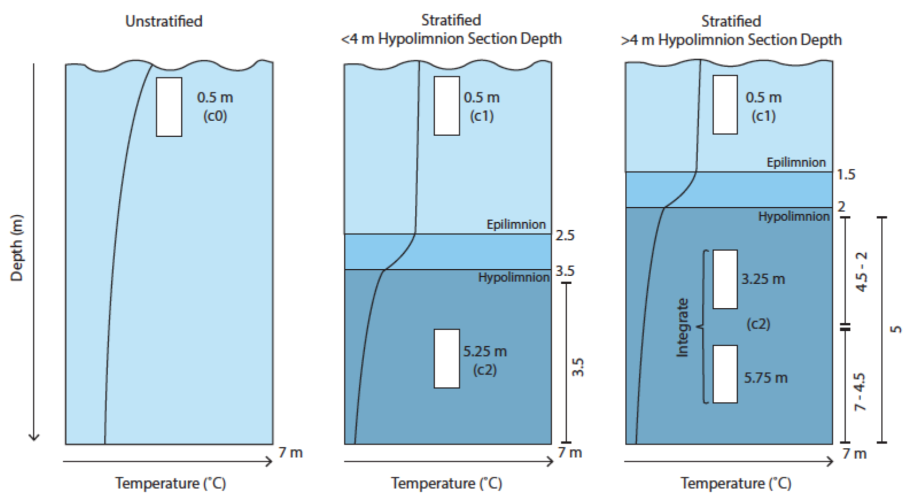


Figure 4. Example of unstratified and stratified lake water column sampling depths with placement of thermocline. Hypolimnion sampling is determined by the hypolimnion section depth (i.e., thickness). If hypolimnion thickness is < 4m and > 2 m, one sample at midpoint of hypolimnion is collected. If hypolimnion thickness is > 4 m an integrated sample is collected.

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). Measurements of physical properties recorded by the AOS are also meant to characterize key habitat variables for as many aquatic organisms as possible. 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). Quantifying and characterizing sediment is an important component of the NEON aquatic sampling strategy. Sediments are chemically important as they can adsorb nutrients such as phosphate (Reddy et al., 1999), control the bioavailability of contaminants (Eggleton and Thomas, 2004), and bind metals (Chapman et al., 1998). To holistically capture sediment composition in aquatic ecosystems, NEON samples recently deposited sediment for analysis of chemical and physical properties at upstream and downstream locations in streams and rivers and near the littoral and buoy locations in lakes (Wilde, 2005).

Collecting information about biological community composition in aquatic ecosystems is a critical link in detecting drivers of change and understanding their ecological impacts. Distinct habitat structures that support partially disparate assemblages have 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). The exact location for biological sampling is based on representative habitat types and their relative cover at each NEON aquatic site to capture site-specific variability for a given organism. Biological samples are collected from the two most common habitat types, which is determined from annual site-specific rapid habitat assessments and 5-year stream morphology surveys. For wadeable stream aquatic plants, 10 transects are established spanning the stream reach at specific, representative habitat types with 5 transects in each of the two most dominant habitat types. Transects are located a minimum of 5 meters from the discharge and water chemistry transects to avoid sampling areas that are regularly disturbed. In lakes and rivers, aquatic plants are surveyed and collected at 10 randomly chosen points within the portion of the lake or river that is known to support plant life. Benthic macroinvertebrate, periphyton, and benthic microbe samples are collected from the two most dominant habitat types of a stream and in the pelagic and littoral areas of lakes and rivers. Surface microbes are collected near stream, lake, and river infrastructure. Zooplankton and phytoplankton samples are collected from the pelagic water column of lakes near sensor infrastructure, and seston is collected for chlorophyll analysis near stream infrastructure. Macroinvertebrate, zooplankton, periphyton, microbe, and phytoplankton samples are collected quantitatively: a known area or volume is sampled so that organism density can be derived from each representative habitat type. Fish sampling takes place at a combination of three fixed (i.e., sampled every year) and three random (i.e., locations rotate between years) sampling areas to ensure full representation of the assemblage despite their rapid mobility relative to other organisms.

6.3 AOS Temporal Sampling Strategy

NEON aquatic sites represent a vast range of patterns in precipitation, discharge, vegetation, temperature, and light, which requires an AOS temporal sampling strategy based on specific local drivers of variability. Specifically, hydrologic, climatic, and biological metrics derived from historical data are used to determine the site-specific timing of AOS sampling at each NEON aquatic site and is described in the Aquatic Site Sampling Design Documents (RD[43] to RD[61]). For methodologic details of this process, see Parker and Utz (2022).

The AOS temporal sampling represents an attempt to balance annual variability at a site with the logistical constraints on how frequently samples can be collected during a field season. The detection of change relies on separating natural variability from drift in variables due to climate change, land-use change, and invasive species. One key temporal design component of the AOS sampling strategy involves the concept of individual protocols that are performed independently and sets of protocols that are performed over a fixed time interval of multiple days, e.g., biological and sediment sampling during defined windows 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 varies depending on the sample or measurement being collected and the characteristics of each NEON aquatic site. Monthly samples are collected for water chemistry, dissolved gases, and stable isotopes. Surface water microbes are collected monthly with water chemistry in streams but only every other month in rivers and lakes. AOS monthly water chemistry samples are collected on Tuesday, if possible, to match with government protocols such as the National Water-Quality Assessment (NAWQA, Leahy et al., 1990; Gilliom et al., 1995), which has been shown to produce unbiased load estimates (Robertson & Roerish, 1999). At stream and river sites, where hydrologic and chemical dynamics are often driven by seasonal factors, an additional set of 14 chemistry samples are collected to capture water quality (i.e., chemistry, dissolved gasses, and isotopes) during dynamic periods of the hydrograph, which is a valuable strategy when using data to estimate flux values (Harmeson & Barcelona, 1981). The selection of when to collect these flow-weighted samples is based on the annual hydrograph at each site, with sampling efforts temporally focused on times of rapid change to capture flushing responses. Examples of periods when sampling is more intensive due to hydrologic conditions include the rising and falling limbs in the seasonal discharge hydrograph (Gilliom et al., 1995; King and Harmel, 2003) and prolonged periods of elevated discharge to capture major times of export. For some sites, such as snowmelt-dominated areas, this may result in weekly sampling bouts during spring freshet. At Lake and river sites, the AOS chemistry suite is collected in coordination with AOP flyover, when scheduled for a particular year (Musinsky, 2016; Musinsky et al., 2022).

At all aquatic sites, three seasonal sampling bouts for biological collections and two sampling bouts for sediment sampling take place annually. The seasonal bouts are site-specific, month-long, periods during which multiple AOS biological and sediment sampling protocols are implemented, allowing for logistical flexibility of protocol execution while ensuring that data are collected close enough in time to be compatible and relatable. Biological sampling includes aquatic plants, macroinvertebrates, periphyton and phytoplankton, benthic microbes, and zooplankton (lakes only), over the course of multiple days. During bouts 1 (spring) and 3 (autumn), biological sampling is followed by sediment chemistry and fish sampling to minimize benthic disturbance to the site. Fish sampling takes up to five days to complete. Bouts are timed to take place during spring, mid-summer, and autumn, which are defined by degree-days and vegetation greenness (Parker and Utz, 2022).

AOS annually assesses riparian vegetation to detect changes in riparian canopy cover and composition, which is an important driver of aquatic biogeochemical and biological processes (Vannote et al., 1980). Riparian habitat assessment is conducted during the period of peak greenness that aligns with the AOP flyover schedule (Musinsky, 2016; Musinsky et al., 2022). Lake and river bathymetry takes place during peak greenness or biological bout 2, with the goal of collecting data within two weeks of aquatic plant sampling. However, in streams, the channel morphology 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. Bathymetry and stream morphology mapping occurs at each site at least every five years, unless a significant event such as a hurricane or flood changes the morphology of the site, which triggers a new survey.

7 LINKING AIS AND AOS DATA TO PRODUCE CONTINUOUS DISCHARGE ESTIMATES

Fluxes of nutrients, carbon, and other constituents in streams, rivers, and lakes are a significant part of global budgets (Cole et al., 2007, Alexander et al., 2008). One of the major components of calculating fluxes of constituents in water involves quantifying the amount of water traveling through the water body (i.e., discharge, or the volumetric flow rate of water transported through a given cross-sectional area at a given location). The aquatic sampling design attempts to enable calculation of the fluxes and flows of chemically and biologically relevant constituents in aquatic ecosystems by providing continuous stream flow data derived from AIS surface water elevation sensors. Empirical discharge measurements, which are collected at all stream and river sites as part of AOS, are used to formulate a stage-discharge rating curve for each site. The stage-discharge rating is developed using a Bayesian model to fit an exponential curve to the staff gauge readings and flow measurements collected by field personnel (Le Coz et al., 2013). Rating curves are then applied to continuous stage data from AIS to calculate continuous discharge for a site. Cross-section locations remain fixed so that the stage-discharge relationship can be verified annually and updated on an as–needed basis for the duration of NEON Operations.

8 SHALLOW GROUNDWATER INSTRUMENTATION AND SAMPLING

Like the connections between aquatic ecosystems and riparian areas, there are important linkages and feedbacks between groundwater and the surface water of streams, rivers, and lakes (Brunke & Gonser, 1997; Winter, 1999). As such, the aquatic sampling strategy also includes measurements of groundwater parameters of both AIS and AOS data to capture high-resolution temporal changes and more detailed water quality characteristics on a seasonal basis. Up to eight shallow groundwater wells are installed at most NEON aquatic sites apart from four sites due to logistical and permitting issues (LeConte Creek, McRae Creek, Teakettle 2 Creek, and Rio Cupeyes,

Table 5). The spatial orientation of groundwater wells at each NEON aquatic site was informed by topographical and logistical considerations and is meant to capture hyporheic and shallow, unconfined aquifer hydrology and chemistry. Across the network, the spatial orientation includes wells located throughout the sampling reach. Near-stream wells are meant to capture hyporheic flow (Boulton et al., 1998) and are specifically located near S1 and S2 infrastructure to enable direct comparisons to surface water temperature and chemistry data. The inclusion of wells located further from water bodies allows for characterization of the magnitude and direction of groundwater flow, and depending on local conditions, enable sampling of less directly connected groundwater flow paths (Sophocleous, 2002). In all locations around NEON aquatic sites, the groundwater wells were drilled to refusal, ideally ensuring that the full seasonal hydrograph can be captured.

Each well is equipped with a data logger to capture changes in groundwater elevation, temperature, and fluid electrical conductivity every 5 minutes. Data loggers are located 0.50 m from the bottom of the screened interval for wells greater than 3 m total depth, while shallow wells less than 3 m total depth have the data logger located 0.2 m from the bottom of the well screen. Groundwater well elevation is measured and recorded with an accuracy of 0.005 m relative to the site benchmarks.

One exception to the method described above is at NEON permafrost sites (Caribou Creek, Oksrukuyik Creek, and Toolik Lake) where dynamic water table boundaries present significant challenges in measuring absolute groundwater elevation. This is due to surface elevation fluctuations from frost heave and seasonal fluctuations in the underlying active layer, which is the ground layer above the permafrost that seasonally freezes and thaws. In permafrost ecosystems, the active layer is a primary ecohydrologic driver, influencing nutrient cycles, biological processes, and downstream water quality. For these NEON sites, key measurements of the active layer including the depth to liquid water from the ground surface, if present, and depth to the active layer thaw are collected weekly. Like groundwater elevation in stationary ecosystems, these data are useful for addressing water table related questions of groundwater availability and seasonal variability. As in all NEON wells, data loggers capture temperature and fluid electrical conductivity.

In addition to the in situ sensor data capturing physical groundwater characteristics, groundwater samples are collected twice per year for water chemistry ions, nutrients, dissolved carbon, and water isotopes. Water chemistry samples are collected from a subset of four wells at each NEON site with four or more total wells, or from three wells at sites containing three wells total. For rivers and wadeable streams, the sampling wells are selected in attempt to cover all the following categories: upstream, downstream, right bank, and left bank. Preference is also given to wells that are closer to the surface water chemistry sampling locations to enable direct comparison. For lakes, the sampling wells are selected with two wells located near each of the two littoral sensor sets. Consistent sampling of the same set of four wells at each site is necessary for evaluation of seasonal responses in groundwater constituent concentrations. Because the suite of groundwater quality parameters is 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. The groundwater samples are 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). Groundwater samples are collected within 48 hours of the surface water samples. The seasonal timing of groundwater sample collection is 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). At river and stream sites, groundwater sample collection is temporally timed to capture seasonal variability at 25% and 75% (± 5%) of cumulative annual discharge. 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). The timing of groundwater chemistry sampling will be reassessed once a minimum of three years of water table data are available directly from the NEON wells to ensure that seasonal variability in chemical and hydrologic conditions is captured.

Table 5. Well Depths at the time of installation.

Domain	Site	Number of Wells	Well Depth (m) for each well							
			1	2	3	4	5	6	7	8
01	HOPB	4	2.3	2.2	1.9	2.2				
02	POSE	8	3.0	3.0	4.2	3.0	3.0	4.4	3.4	3.1
02	LEWI	8	5.5	5.4	5.4	5.6	6.8	6.9	5.4	5.4
03	SUGG	8	14.7	14.8	13.1	14.7	14.9	11.0	17.6	13.0
03	BARC	8	12.3	11.6	10.7	10.2	11.6	9.2	13.1	13.3
03	FLNT	7	11.5	13.0	11.5	11.5	11.6	13.1	13.1	
04	GUIL	6	12.9	9.8	7.2	8.3	6.4	8.7		
04	CUPE	0								
05	CRAM	8	9.2	4.0	6.2	3.8	12.3	12.3	4.6	6.2
05	LIRO	7	14.6	10.2	8.7	10.0	7.0	11.6	11.5	
06	KING	8	9.7	6.6	8.5	8.5	7.2	9.1	8.6	8.4
06	MCDI	8	8.2	10.0	10.1	8.1	7.0	8.1	6.6	7.1
07	WALK	3	4.3	2.3	4.5					
07	LECO	0								

Domain	Site	Number of Wells	Well Depth (m) for each well							
			1	2	3	4	5	6	7	8
08	MAYF	8	3.6	3.8	4.5	4.0	4.0	4.3	4.4	4.2
08	TOMB	3	16.7	16.1	16.2					
08	BLWA	3	13.0	13.2	13.5					
09	PRPO	8	7.2	8.1	9.4	6.9	7.6	7.7	8.2	6.3
09	PRLA	8	10.1	10.2	10.5	7.8	12.3	10.7	15.8	7.6
10	ARIK	6	9.1	9.4	9.4	8.9	9.3	8.9		
11	PRIN	6	8.2	6.2	8.7	6.6	5.0	8.6		
11	BLUE	7		5.5	10.3	5.6	5.5	7.0	4.7	5.5
12	BLDE	8	2.2	2.6	2.4	2.9	2.1	2.3	2.4	2.4
13	COMO	4	2.5	2.3	1.9	2.7				
13	WLOU	8	2.2	2.4	2.7	3.0	2.4	2.1	2.4	2.8
14	SYCA	4	6.7	6.6	6.0	6.1				
15	REDB	5	4.6	4.6	4.5	4.3	4.4			
16	MCRA	0								
17	BIGC	6	5.5	6.6	8.2	4.1	4.4	5.7		
17	TECR	0								
18	TOOK	8	2.1	1.6	1.5	1.9	1.6	1.8	1.7	2.0
18	OKSR	8	2.0	1.6	2.0	1.7	1.6	2.1	1.6	1.8
19	CARI	8	3.3	3.2	3.1	2.7	2.9	2.9	2.7	3.5

9 LOGISTICS AND ADAPTABILITY

In the past decade, while NEON was under construction and in initial operations, the actual implementation does not perfectly reflect previously published plans for NEON and the aquatic subsystem (e.g., there is no longer an experimental STREON component, many derived data products are no longer part of the catalog, and “relocatable” sites are now, more accurately, called “gradient” sites). These changes illustrate some of the many challenges faced when building a long-term observatory that covers such a diverse set of ecological variables and broad geographic range, as well as the evolution of the Observatory to meet the advancing needs of the community. Among these changes are also many improvements (e.g., sensor infrastructure design and placement (Hensley et al., 2021) and data processing pipeline quality measures (Sturtevant, 2022)).

The aquatic sampling strategy outlined above details how NEON is achieving science goals while maintaining consistency for all aquatic sites despite their different classes and dynamic hydrologic characteristics. The future success of the observatory will likely rely on additional changes to balance the need to respond to changes in technology, scientific questions of interest, and scientific understanding while maintaining a consistent long-term dataset. One way the aquatic subsystem will document and respond to changing needs is with Aquatic Site Sampling Design Documents (RD[43] to RD[61]), which have been created with spatial and temporal information for each NEON Domain. These site-specific designs were often based on nearby historic data rather than information from precisely the same location as a NEON site. Following initial operations, data from NEON sites will be used, likely in conjunction with historic data, to verify and potentially update the site-specific sampling designs to maintain their original purpose based on ecological factors, e.g., baseflow or peak greenness. Over the lifetime of the observatory, NEON will continue to ensure that the aquatic sampling strategy is used to gather comparable datasets from sites covering arctic to tropical environments to allow for large-scale and long-term detection of ecological change.

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