AOS PROTOCOL AND PROCEDURE: AQUATIC DECONTAMINATION

PREPARED BY	ORGANIZATION	DATE
Stephanie Parker	AQU	10/22/2013
Jesse Vance	AQU	10/23/2013

APPROVALS	ORGANIZATION	APPROVAL DATE
Charlotte Roehm	AQU	1/26/2015
Dave Tazik	SCI	1/25/2016
Kirsten Ruiz	OPS	1/22/2015
Mike Stewart	SE	1/22/2015

RELEASED BY	ORGANIZATION	RELEASE DATE
Jennifer DeNicholas	CM	01/26/2015

See configuration management system for approval history.

The National Ecological Observatory Network is a project solely funded by the National Science Foundation and managed under cooperative agreement by NEON, Inc. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	04/28/2014	ECO-01640	Initial release
B	01/26/2015	ECO-02636	Migration to new protocol template

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 OVERVIEW

1.1 Background

Decontamination of aquatic sensors and equipment is imperative at NEON aquatic sites because sensors are planned to be rotated among NEON sites once per year, and field equipment may be used in up to three different field sites per domain. Biofouling on permanently-mounted sensors and aquatic invasive species on both sensors and equipment are concerns that can be remedied by stringent cleaning and decontamination procedures.

The long-term deployment of aquatic sensors provides potential substrate and habitat for organisms. Biofouling may result in the accumulation of multiple species of aquatic organisms. This creates a potential for the spread of invasive species when sensors are brought into contact with other sensors during batch calibrations and/or moved between sites. Precautions must be taken to not alter aquatic chemistry and/or biological assemblages during monitoring efforts. In freshwater, biofouling is typically "soft" and easily addressed because there are few calcifying organisms. Likely contaminants include bacteria, protozoans and algae. Any organisms adhering to the surface of sensors must be removed and bacterial spores and viruses should be deactivated through cleaning and disinfecting procedures.

Aquatic invasive species (also known as aquatic nuisance species) are those that cause economic or environmental harm, or degrade human health (ISAC 2006). Invasive species can also threaten natural resources by having significant effects on aquatic habitats such as shading, oxygen depletion, or predation, and often upset the ecological balance of ecosystems by outcompeting and often extirpating native organisms. The U.S. Fish and Wildlife Service reports that there are approximately 10,000 non-indigenous aquatic species in U.S. waters, resulting in the severe degradation of aquatic habitats throughout North America (USFWS 2012). Such factors contribute to 37% of naturally-occurring freshwater fish species and 67% of freshwater mussels becoming at risk of extinction (Stein and Flack 1997).

Aquatic species of high concern (USFS 2010) throughout the U.S. include New Zealand mudsnails (*Potamopyrgus antipodarum*), chytrid fungi, zebra (*Dreissena polymorpha*) and quagga (*Dreissena rostriformis bugensis*) mussels, *Didymosphenia geminata*, whirling disease (*Myxobolus cerebralis*), and Eurasian watermilfoil (*Myriophyllum spicatum*). In freshwaters, other common invasive taxa include crayfish and fishes, often transported beyond their natural range by people. Some taxa, especially algae, plants, snails and mussels, are easily spread because they can reproduce from fragments (e.g., Eurasian watermilfoil). It is not uncommon for aquatic organisms to dry completely, rehydrate, and become viable again (e.g., New Zealand mudsnails).

Decontamination of aquatic sensors requires special considerations due to the sensitivity of construction materials. It is advantageous to calibrate all common sensors in the NEON network under the same conditions at the same time to limit error and bias. This batch calibration method requires the complete

removal of biological and chemical contaminants to prevent the transfer of potentially harmful biological constituents between aquatic sites. The information detailed in this document presents a decontamination protocol derived in part from the USGS National Water-Quality Assessment Program (NAWQA) and from consultation with sensor manufacturers. The protocol takes into account the effectiveness of disinfectants and the safety for field personnel, the environment, and the integrity of the sensors.

Currently, there is no single standardized protocol available for cleaning equipment that targets all biofouling measures and aquatic invasive species of concern. In general, bleach-cleaning methods used by the USGS National Water-Quality Assessment Program (NAWQA) are effective; however, this method is relatively ineffective against New Zealand mudsnails. Hot water cleaning is also used by several states for boats and trailers and appears to be a relatively effective treatment for most aquatic invasive organisms. All cleaning methods should be followed by a period of complete drying.

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills 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.

Execution of this protocol 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 the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

This protocol is based on the protocols of the U.S. Forest Service Region 4 Fire Operation Guidelines to Prevent the Spread of Aquatic Invasive Species, the USEPA National Streams Assessment (NRSA; USEPA 2007), the USGS National Water-Quality Assessment (NAWQA) program (Moulton et al. 2002), and the National Park Service Guidelines for Prevention of Introduction and Spread of Aquatic Threats (NPS 2006).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.014051	Field Audit Plan
AD[07]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[06]	NEON.DOC. 00####	NEON Aquatic Field Maintenance Plan (TBW)
RD[07]	NEON.DOC.000692	AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling in Wadeable Streams
RD[08]	NEON.DOC.001202	AOS Protocol and Procedure: Aquatic Plant and Macroalgae Sampling in Lakes and Non-wadeable Streams
RD[09]	NEON.DOC.000691	AOS Protocol and Procedure: Periphyton and Seston Sampling in Wadeable Streams
RD[10]	NEON.DOC.001203	AOS Protocol and Procedure: Algae Sampling in Lakes and Non-Wadeable Streams
RD[11]	NEON.DOC.000690	AOS Protocol and Procedure: Macroinvertebrate Sampling in Wadeable Streams
RD[12]	NEON.DOC.001204	AOS Protocol and Procedure: Macroinvertebrate Sampling in Lakes and Non-wadeable Streams
RD[13]	NEON.DOC.001201	AOS Protocol and Procedure: Microbes in Wadeable Streams
RD[14]	NEON.DOC.001200	AOS Protocol and Procedure: Microbes in Lakes and Non-wadeable Streams
RD[15]	NEON.DOC.001295	AOS Protocol and Procedure: Fish Sampling in Wadeable Streams
RD[16]	NEON.DOC. 001296	AOS Protocol and Procedure: Fish Sampling in Lakes
RD[17]	NEON.DOC.00####	AOS Protocol and Procedure: Packaging and Shipping Sensors
RD[18]	NEON.DOC.000694	AOS Protocol and Procedure: Surface Water Chemistry Sampling in

		Wadeable Streams
RD[19]	NEON.DOC.000693	AOS Protocol and Procedure: Reaeration – Measuring Diffusion of O_2 Across the Water-Air Interface
RD[20]	NEON.DOC.001190	AOS Protocol and Procedure: Lake and Non-wadeable Stream Water Chemistry
RD[21]	NEON.DOC.001193	AOS Protocol and Procedure: Sediment Chemistry of Wadeable Streams
RD[22]	NEON.DOC.001085	AOS Protocol and Procedure: Stream Discharge
RD[23]	NEON.DOC.001219	AOS Protocol and Procedure: Groundwater Chemistry Sampling from Observation Wells
RD[24]	NEON.DOC.001162	AOS Protocol and Procedure: NEON Lake Riparian Mapping
RD[25]	NEON.DOC.001163	AOS Protocol and Procedure: NEON Stream Riparian Mapping
RD[26]	NEON.DOC.001164	AOS Protocol and Procedure: NEON Bathymetric Mapping Protocol
RD[27]	NEON.DOC.001153	AOS Protocol and Procedure: NEON Aquatics Wadeable Stream Morphology Mapping

2.3 Acronyms

Acronym	Definition
ANS	Aquatic Nuisance Species
IAS	Invasive Aquatic Species
ISAC	Invasive Species Advisory Committee
NAWQA	National Water-Quality Assessment (USGS)
NISC	National Invasive Species Council
NPS	National Park Service
PRT	Platinum Resistance Thermometer
uPAR	Underwater Photosynthetically Active Radiation
USEPA	U.S. Environmental Protection Agency
USFS	U.S. Forest Service
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
v/v	Volume-to-volume percentage

2.4 Definitions

Aquatic nuisance species (ANS): Non-native species that threaten native communities, ecological stability, and commercial and recreational activities in infested waters

Biofouling: The gradual accumulation of water borne organisms, such as bacteria, protozoans, algae, and crustaceans on the surfaces of engineering structures (e.g., sensors and infrastructure) in water resulting in impairment or degradation. Can also be used to describe fouling activities from birds (e.g., aquatic biofouling)

Equipment: Items used for sampling at NEON sites that are not installed permanently at the site, rather they are carried to and from the site each sampling day (e.g., waders, nets, buckets, boats).

Invasive species: Often used as a synonym for aquatic nuisance species (ANS), any species or viable biological material (including fragments, seeds, spores) that is transported into an ecosystem outside of its historic range, that reproduces and spreads rapidly into new locations causing economic or environmental harm, or harm to human health

Sensor: An instrument used for measuring a chemical or physical parameter of a given medium, such as water or air. Examples include temperature, conductivity, pH, dissolved oxygen, etc.

Veligers: Planktonic larvae of snails and mussels.

3 METHOD

Aquatic invasive species are a threat to freshwater ecosystems across the continent. Invasive species are easily transported by boats and other recreational watercraft, water from live wells, sensors and equipment (see Definitions, Section 2.4), and waders across ecosystems. NEON's field-based operating procedures prohibit the use of felt-soled waders and wading boots for its field staff, and requires cleaning and drying of all boats and equipment between site visits. Equipment will be drained and cleaned by hand at each field site at the end of each sampling day, and decontaminated at the Domain Support Facility if they will be used at a different site on the next field visit. For any equipment returning to the same site the next day, chemical decontamination is not necessary. Sensors installed for long-term deployment require decontamination only when removed from the field for repairs and/or calibration at NEON HQ and redeployments. Hand-held sensors require decontamination between sampling at different sites within the Domain. Sensors shall be decontaminated at the Domain Support Facility.

The cleaning procedures presented here are designed for sites where the status of invasive species is unknown (i.e., invasive species have not yet been documented). At sites where invasive species have been documented, more rigorous chemical cleaning procedures may be necessary (Appendix C). Some aquatic field sites may require, as part of the land-use agreement, a dedicated set of sensors and field equipment that are used only at that particular site (e.g., D13 Como Creek, Appendix C).

A combination of manual removal of debris, chemical cleaning, and complete drying are typically effective for decontaminating sensors and equipment. Species of concern are outlined below and specific cleaning methods are presented in Table 4.

Whirling disease (*Myxobolus cerebrali*) – whirling disease is caused by a myxospore parasite and primarily affects salmonid fishes. Spores are generally transported through fish parts and organic matter. Flushing of equipment and removal of debris and organic material significantly decreases the number of spores on equipment.

Chytrid fungus (*Batrachochytrium dendrobatidis*) – Chytridiomycosis, or chytrid disease, has caused a massive decline in amphibian diversity since 1998. Chytrids are microscopic and can be transported in water, but can be destroyed using bleach and hot water.

New Zealand mudsnails (*Potamopyrgus antipodarum*) – New Zealand mudsnails were introduced to North America in 1987. They are small (4-6 mm) outside of their native range and thrive in disturbed, silted streams (Figure 1). Because of their small size, they are easily transported in waders and nets. Bleach treatment is not effective, in mudsnail-infested waters stronger ammonium compounds may need to be used.

Figure 1. New Zealand mudsnails are easily spread due to their small size (photo credit: Wikipedia.org).

Zebra (*Dreissena polymorpha*) and **Quagga (*Dreissena rostriformis bugensis*) mussels** – Ecologically similar and closely related, zebra and quagga mussels were introduced to the Great Lakes in ballast water in 1988. Quagga mussels were first discovered in Lake Mead, Nevada and represent the first confirmed introduction of a dreissenid species in the western United States (Wong and Gerstenberger 2011). Individuals are relatively small, <1.5 inches, and can survive 3-5 days of desiccation (Figure 2). Bleach cleaning and hot water are effective in killing mussels on equipment.

Figure 2. Quagga mussels have spread into Western U.S. waters, and are resistant to desiccation (photo: cisr.ucr.edu).

"Didymo" (*Didymosphenia geminata*) – Although native to many cold-water streams in North America, the range of Didymo has been expanding and it was listed as a nuisance species 2005. Large mats of the diatom filter water and alter stream habitat (Figure 3). Didymo is microscopic and can be spread in a small amount of water, so bleach cleaning of equipment and waders is important in restricting the spread of the species.

Figure 3. *Didymosphenia geminata* is a stalked-diatom that forms large mats in streams (photo: stopans.org).

Eurasian watermilfoil (*Myriophyllum spicatum*) – Eurasian watermilfoil was introduced to North America in the 1940s, and quickly spreads in lakes (Figure 4). Spread by plant fragments on boat motors or equipment. Fragments easily regenerate in freshwaters. Thorough cleaning and inspection of equipment and boats, along with high-pressure wash, are the most effective treatment.

Figure 4. Eurasian watermilfoil spreads easily among lakes and ponds due to fragmentation (photo: dnr.wi.gov).

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[06]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[07]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Deployable sensor decontamination occurs prior to being returned to NEON HQ for repairs, calibration or redeployment. This is scheduled to occur once per year. Additional occurrences will be on an as needed basis. Hand-held sensors will be decontaminated after sampling each day, and before moving to a new body of water.

Sampling of groundwater wells requires rinsing of the down well pump and flow through cell prior to each well sampled at the same site, and full decontamination before using at another site.

Decontamination of equipment occurs after sampling each day, and before moving to a new body of water.

Decontamination must occur after sampling in a body of water and before moving to the next body of water, with enough time between for equipment to completely dry. For equipment at Domains with only one site, follow the same cleaning procedures for consistency.

4.2 Criteria for Determining Onset and Cessation of Sampling

All sites within the NEON Domain are initially listed as "unknown" with respect to the presence of invasive species of concern. Any NEON personnel working on site (including contractors hired by NEON) shall follow the decontamination procedures detailed in this protocol. Sites that have been confirmed to have invasive species of concern shall always have the sensors and equipment utilized in the field decontaminated following more stringent procedures determined specifically for that site.

4.3 Sampling Specific Concerns

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met:

Sensors:

1. Manual removal of visible invasive species and debris from sensors and infrastructure shall be performed while at the site (e.g. technicians must wipe off biofilms, debris, etc.).
2. Sensors shall be packed and transported to Domain Support Facility for chemical decontamination procedures.

Equipment:

1. Initial draining and manual removal of visible invasive species and debris from all equipment must be performed while at the site (e.g., technicians must drain the boat and buckets, and remove plants from the boat and boat trailer while still at the boat ramp).
2. If multiple sites are to be sampled in one day, equipment **must** be thoroughly cleaned and sun-dried in the field before moving to the next site. If equipment cannot be adequately cleaned and dried in the field (e.g., it is raining and the equipment will not dry), return to the Domain Support Facility to clean equipment and sample the next site another day. Alternatively, a second set of equipment may be used when two sites are to be sampled within the same day.
3. If invasive species are known to be present at the site, all equipment must be returned to the Domain Support Facility for thorough cleaning and drying before reuse at any other site.

5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

Eye protection and gloves should be worn while cleaning using chemicals (e.g., bleach). Take special care not to burn skin when using hot water. Follow proper chemical disposal guidelines per the domain-specific Chemical Hygiene and Biosafety Manual (AD[03]).

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

Table 1. Equipment list – Decontaminating sensors

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
	R	Hand-held thermometer	Measuring water temperature for the bleach cleaning method	1	N
	R	5 gal Bucket	Wash solution container	4	N
	R	1 L Volumetric flask	Preparing a 5% (v/v) hydrochloric acid (HCl) solution	1	N
	R	100 mL Graduated cylinder	Preparing a 5% (v/v) hydrochloric acid (HCl) solution	1	N
	R	1 L Wash bottle	Wash solution and rinsing container	4	N
	R	1 L Nalgene bottle	Storing a 5% (v/v) hydrochloric acid (HCl) solution	2	N
	R	10 L Nalgene carboy	Acid waste container	1	N
	R	Soft, antistatic brush	Removing debris from sensors	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	YSI EXO conductivity cell cleaning brush	YSI sonde decontamination	1	N
Consumable Items					
	R	Phosphate-free detergent (Alconox or Liquinox)	Decontaminating aquatic sensors (except uPAR)	1 gallon	N
	R	Hydrochloric acid, 37%	Decontaminating aquatic sensors (except uPAR)	4 L	Y
	R	Isopropyl alcohol, 70%	Decontaminating aquatic sensors (except uPAR)	4 L	Y
	R	Deionized water	Rinsing sensors	4 gallon	N
	R	White vinegar	Softening mineral deposits	1 gallon	N
	R	Cotton Swab	Removing large debris from sensor housings	1 box	N
	R	Kimwipes	Applying cleaning solutions to sensors	3 boxes	N
	R	Compressed air (keyboard) duster	Drying conductivity cells of the Aqua and Level Trolls	1	N

R/S=Required/Suggested

Table 2. Equipment list – Decontaminating equipment

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
	S	1 gallon home and garden compression sprayer	Applying bleach or ammonium-based solution	All	1	N
	S	24 oz. hand sprayer	Applying bleach or ammonium-based solution	All	1	N
	R	Plastic mixing/catch basin	Containing bleach or ammonium-based solution	All	2	N
	R	Nylon-bristled kitchen brush (separate from the brush used for macroinvertebrate sampling)	Removing debris from equipment	All	1	N
	R	Safety glasses	Safe handling of chemicals	All	1	N
Consumable Items						
	R	Chlorine bleach (6% NaClO)	Bleach solution	All	1 gallon	Y
MX100586	S	Quaternary ammonium disinfectant (ADBAC + DDAC)	Ammonium-based solution	New Zealand mudsnails	1 gallon	Y
	R	Nitrile gloves	Safe handling of chemicals	All	1 box	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	White label tape	Labeling solutions	All	1 roll	N
	R	Black marker (Sharpie)	Labeling solutions	All	1	N

R/S=Required/Suggested

6.2 Training Requirements

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

This protocol requires one field technician who has been trained in decontamination practices and to recognize plant fragments and debris on field equipment.

6.3 Specialized Skills

N/A

6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

Sensors: Decontamination of deployable sensors will require one field technician for 30 minutes at the field site and 30-90 minutes at the Domain Support Facility (variance based on reagent preparation). Handheld sensors will require 30 minutes at the Domain Support Facility.

Equipment: Decontamination for equipment requires one field technician for 30 minutes at the field site after each day of sampling and 30-90 minutes at the Domain Support Facility. Equipment must be fully dried (e.g., overnight) before next use.

7 STANDARD OPERATING PROCEDURES

SOP A Field Decontamination

All boots, waders, sensors, equipment (e.g., buckets, nets, and motors), boats, and trailers must be properly cleaned, disinfected, and dried between uses at sites to prevent transport of invasive species from one body of water to another. The basic premise of decontamination for aquatic invasive species is threefold: Drain → Clean → Dry. A combination of draining, manual removal, chemical disinfection, and drying will be used to remove and kill invasive species.

Felt-soled waders and wading boots are prohibited for NEON field staff, NEON designated visitors, and subcontracted personnel due to the difficulty of removing small invasive materials.

A.1 Field Site Decontamination

1. Remove any large debris from sensor housings at the field site with a dry, nylon-bristle brush or cotton swab.
2. Before leaving the site, DRAIN all buckets, coolers, waders, boots, and any other equipment that can hold water.
   a. Remove the drain plug and drain transom well, bilges, and live wells (if present).
   b. Follow factory guidelines for flushing water from the boat motor.
   c. Empty water out of buckets, coolers, etc.
3. CLEAN all debris off equipment, including waders, nets, and brushes by hand. Leave debris at the site (discard on the shoreline or in the water).
   a. Remove any visible plants or plant fragments, animals, mud, or other debris.
   b. Check net seams and edges of sieve for hidden organisms.
   c. You may rinse mud and debris off of surfaces using water from the site and the nylon kitchen brush.
4. CLEAN all debris off boat, motor, and trailer by hand. Leave debris at the site (discard on the shoreline or in the water).
   a. Remove any visible plants or plant fragments, animals, mud, or other debris.
   b. You may rinse mud and debris off of surfaces using water from the site and/or the nylon kitchen brush.
   c. Check trailer, axle, wheel wells as well as the boat, motor, propeller, anchor, ropes, and paddles.
5. If possible, drive through a self-service car wash facility on the way back to the Domain Support Facility and spray the boat and trailer with high pressure hot water (no detergent).

SOP B Decontamination at Lab

B.1 Sensors

All sensors must be cleaned and decontaminated before being returned to HQ for servicing and/or calibration, or when being moved to a new body of water. Sensors are serviced at least once per year, but may be removed from the site more often if offsite troubleshooting and repairs or unscheduled calibrations are needed. The underwater PAR (uPAR) sensor housing is made of material that is sensitive to the general decontamination procedure and therefore has a sensor-specific procedure (see SOP B.2).

1. When sensors are being recovered and removed from the site for servicing or calibration, treat them by following the guidelines in Table 3. Sensors are transported to the Domain Support Facility for chemical decontamination to minimize chemical transport to the field site.

Table 3. General cleaning methods for decontamination of aquatic sensors (except uPAR)

Sensor Decontamination Procedure				
Step	**Action**	**Method**	**Solution**	**Delivery**
1	Remove debris	Dry brush	N/A	Manual removal
2	Remove soils and organic material	Detergent solution wash	0.2% Liquinox (v/v) or Alconox (w/v)	Wash bucket; wetted Kimwipes and brush as needed
3	Rinse	Rinse	Tap water	Rinse bucket; wash bottle
4	Remove inorganic chemical contaminants, mineral deposits, biofilms	Acid solution wash	HCl 5 % (v/v)	Wash bottle; wetted Kimwipes and brush as needed
5	Rinse	Rinse	Deionized water	Rinse bucket; wash bottle
6	Remove organic chemical contaminants, disinfect surfaces	Alcohol solution wash	Isopropanol 70% (v/v)	Wash bottle
7	Rinse	Rinse	Deionized water	Rinse bucket; wash bottle

2. Prepare a 0.2% (v/v) detergent solution in a 5 gallon bucket.
   a. Measure out either 378.5 mL of Liquinox or 37.85g Alconox phosphate-free detergent and pour into the 5 gal bucket.
   b. Fill the bucket with tap water, mixing halfway.
   c. Label the bucket “SOAP”.

3. Prepare a 5% (v/v) hydrochloric acid (HCl) solution and store in a 1 L Nalgene bottle. (NOTE: At this concentration, 37% HCl quickly evaporates forming a gas that can be irritating to lungs and eyes. Handling this should be done in under a fume hood or at the very least a well-ventilated area. Acid causes burns on contact and safety glasses and gloves are required.)

   a. Fill a 1L volumetric flask with approximately 600 mL of tap water.
   b. Measure out 135mL of 37% HCl using a 100 mL graduated cylinder (e.g.100 mL & 35mL) and add to the volumetric flask.
   c. Swirl the volumetric flask to mix.
   d. Fill the volumetric flask to the 1 L line with tap water.
   e. Pour the solution into an appropriately labeled 1 L Nalgene for storage.
4. Label a catch basin and a 10 L carboy as “ACID WASTE (NO BLEACH!)”.
5. Prepare wash and rinse stations.
   a. Station 1. Add 3 gallons of tap water to a 5 gallon bucket and label it “SOAP RINSE”. Place the “SOAP” and “SOAP RINSE” buckets next to each other, with a wash bottle, a brush and Kimwipes.
   b. Station 2. Add 3 gallons of deionized water to a 5 gallon bucket and label it “ACID RINSE”. Place the “ACID RINSE” bucket with a catch basin, the acid solution, a wash bottle, a brush and Kimwipes.
   c. Station 3. Add 3 gallons of deionized water to a 5 gallon bucket and label it “FINAL RINSE”. Place the “FINAL RINSE” bucket with the alcohol solution, a wash bottle, and the catch basin from Station 2.
6. Wash the sensor with detergent solution at Station 1.
   a. Submerge the sensor in the “SOAP” bucket.
   b. Use a brush and/or Kimwipe to clean the exterior of the sensor.
      1) Remove all visible soils and organic materials.
      2) For YSI sonde, use the small brush to clean the conductivity cell.
7. Rinse the sensor with tap water at Station 1.
   a. Fill a wash bottle with tap water and label it appropriately.
   b. Submerge the sensor in the “SOAP RINSE” bucket.
   c. Working over the bucket, spray the sensor with tap water to remove any remaining detergent.
8. Wash the sensor with acid solution at Station 2.
   a. Fill a wash bottle with acid solution (5% HCl) and label it appropriately.
   b. Working over a catch basin, spray the sensor with acid solution.
   c. If mineral deposits are visible, apply light scrubbing with a brush and/or Kimwipe wetted with acid.
9. Rinse the sensor with deionized water at Station 2.
   a. Fill a wash bottle with deionized water and label it appropriately.
   b. Working over the catch basin, spray the sensor with water to remove acid solution.

c. Submerge the sensor in the "ACID RINSE" bucket and lightly scrub with a Kimwipe.
d. Working over the catch basin, spray the sensor with water to remove any remaining acid.

10. Wash the sensor with alcohol solution at Station 3.
    a. Move the catch basin from Station 2 to Station 3.
    b. Fill a wash bottle with alcohol solution (70% isopropanol) and label it appropriately.
    c. Working over the catch basin, spray the sensor with alcohol solution.
11. Rinse the sensor with deionized water at Station 3.
    a. Use the wash bottle filled with deionized water.
    b. Working over the catch basin, spray the sensor with water to remove alcohol solution.
    c. Submerge the sensor in the "FINAL RINSE" bucket and lightly scrub with a Kimwipe.
    d. Working over the catch basin, spray the sensor with water to remove any remaining alcohol.
12. Pour the contents of the catch basin into the "ACID WASTE" carboy for disposal.
13. Store the sensors according to the manufacturer's specifications (ER [01, ER [02], ER [03]).
    a. For YSI EXO2 Multisonde recovery:
        1) Fill the YSI EXO2 calibration cup half-way with deionized water.
        2) Submerge the sensors in the water and fasten the cup to the end of the sonde for storage and transport.
    b. For Aqua and Level Trolls:
        1) Dry the clean sensor.
            a) Wipe exterior with Kimwipes.
            b) Gently apply compressed air to conductivity cell to dry.
        2) Place protective red dust cap on the cable end
    c. For PRT:
        1) Dry the clean sensor.
        2) Wipe exterior with Kimwipes.
14. Package and ship sensors according to procedures outlined in RD[17].

B.2 uPAR Decontamination Procedure

Do NOT use alcohol, organic solvents, abrasives, or strong detergents to clean the diffuser element on the uPAR sensor. The acrylic material is highly sensitive and alcohol and organic solvents can cause crazing on the sensor and affect the sensor response. Avoid touching the sensor surface when clean to minimize the potential of scratching the sensor.

1. Wash the sensor with detergent solution at Station 1
   a. Submerge the sensor in the “SOAP” bucket
   b. Use a Kimwipe to clean the exterior of the sensor
      1) Remove all visible soils and organic materials
2. Rinse the sensor with tap water at Station 1
   a. Use the wash bottle filled with tap water
   b. Submerge the sensor in the “SOAP RINSE” bucket
   c. Working over the bucket, spray the sensor with tap water to remove any remaining detergent
3. If mineral deposits are visible, use vinegar to soften them and repeat Steps 1-2
4. Wash the sensor with 2% bleach solution
   a. Fill a wash bottle with bleach solution and label it appropriately
   b. Working over a “BLEACH”-labeled catch basin, spray the sensor with bleach solution to disinfect
5. Rinse the sensor with deionized water
   a. Using the wash bottle filled with deionized water
   b. Working over a catch basin, spray the sensor with water to remove bleach solution
6. Allow the sensor to air dry
7. Package and ship sensors according to procedures outlined in RD[17].

B.3 Equipment

A 2% bleach solution will be used to disinfect equipment at all sites if the status of invasive species is unknown, or no data are available. If specific invasive species have been identified at the site, higher concentration bleach or quaternary ammonia disinfectant will be used following Table 4. Any additional procedures required by the NEON site host will be implemented on a site-by-site basis.

Table 4. Common invasive species and cleaning methods for equipment (Parsons 2008, USFS 2010)

	Cleaning Method				
	Wash and remove mud and debris	**Bleach**	**Hot water**	**Drying (direct sunlight is best)**	**Quaternary ammonium**
Unknown	Yes	2% for 10 min	50-60 °C for 5 min	Dry for 24 hr	N/A
Whirling disease	Yes	1% for 10 min	90 °C for 10 min	Dry for 24 hr	≥4.4% for 10-15 min
Chytrid fungus	Yes	7% for 10 min	60 °C for 5 min	Dry for 3 hr	0.15% for 30 seconds
New Zealand mudsnails	Yes	Not effective	50 °C for 5 min	Dry for 48 hr	≥4.6% for 10 min
Zebra and Quagga mussels	Yes, pressure wash	0.5% rinse	≥60 °C water	Dry for 3-5 days	≥3% for 10 minutes
Didymosphenia geminata	Yes	2% for 1 min	60 °C for 1 min	Dry for 48 hr	2% for 1 min
Eurasian watermilfoil	Yes	N/A	N/A	N/A	N/A
Drawback to method	None	Hard on equipment	Could be difficult to get water hot enough	Depends on ambient humidity	Chemistry sample contamination

B.4 Bleach Cleaning

1. If invasive species are known to exist at the site, treat following the guidelines in Table 4. If no invasive has been previously identified at the site, or if no information is known about the site, treat using a 2% bleach solution.
2. At the Domain Support Facility, mix bleach solution (NOTE: bleach solution is not an effective treatment for New Zealand mudsnails, see SOP B.5) in compression sprayer or soaking tub. Bleach cleaning must occur at the Domain Support Facility so bleach does not run into the body

of water. You may either spray the equipment using a compression sprayer, or soak in a plastic tub.

a. Use 2% bleach if invasive status of the site is unknown (50 mL bleach per 4 L tap water).
b. Use 10% bleach if chytrid fungi are suspected at the site.

3. Label sprayer or tub "2% bleach solution" (or other appropriate percentage).
4. Use the pressure sprayer or soaking tub and bleach mixture to clean all equipment and waders that came in contact with water.
   a. Use the sprayer on equipment that will not easily fit in the soaking tub.
   b. Equipment such as boots and waders are best cleaned using the soaking tub as it is a more thorough method. See Table 4 for soaking times.
5. Follow the bleach wash with a hot water (hottest tap water is fine, 50-60 ^{o}C) rinse or soak. This rinses bleach from the equipment, and also adds another layer of decontamination.
   a. Check the temperature of hot water using a handheld thermometer. Soaking time depends on whether water temperature is 50 or 60 ^{o}C, follow guidelines in Table 4.

6. Air DRY all equipment thoroughly.
   a. Allow 48 hours drying time at humidity <70%. Dry in direct sunlight as much as possible.
      1) If technicians are planning to sample two sites in one day and it will not be possible to dry equipment between sites, careful chemical decontamination of all equipment must occur.
   b. Pay special attention to the laces and eyelets of lace-up wading boots, these areas tend to stay damp longer than other equipment. Bootfoot waders are preferred as they are easier to clean.
7. Dispose of bleach wastewater at Domain Support Facility down the drains as indicated by chemical hygiene plan (AD[03]).

B.5 Ammonium-Based Cleaning

1. At sites where New Zealand mudsnails have been identified, cleaning with a quaternary ammonia solution will take the place of bleach cleaning.
2. Mix a 3.1% solution of quaternary ammonium disinfectant and tap water (0.5 cup [118 mL] quaternary ammonium disinfectant to 1 gallon [4 L] tap water) in the sprayer or plastic tub.
   a. Mix fresh solution when the soaking liquid becomes cloudy. This reduces its effectiveness. This is only applicable if you are using a tub to soak equipment.
3. Label sprayer or tub "3% QUAT".
4. Dispose of wastewater at Domain Support Facility down drains as indicated by the chemical hygiene plan (AD[03]).

B.6 Boats and Trailers

1. At a commercial car wash, spray the boat and trailer with high pressure hot water (no detergent). This is the best way to remove plant fragments or veligers.

a. If washed at a car wash, proceed to Step 4. If not, continue with Step 2.

2. At the Domain Support Facility, use the pressure sprayer and bleach mixture to spray any surface of the boat and trailer that came in contact with water from the site. See Table 4 for soaking/spraying times.
   a. Dispose of wastewater down drains following the chemical hygiene plan (AD[03]).
3. Follow the bleach wash with a hot water (hottest tap water is fine, 50-60 ^{o}C) wash. This rinses bleach from the equipment, and also provides additional decontamination.
   a. Check the temperature of hot water using a handheld thermometer. Spraying time depends on whether water temperature is 50 or 60 ^{o}C, follow guidelines in Table 4.

4. Air DRY boat, trailer, and all associated equipment thoroughly.
   a. Allow 48 hours drying time at humidity <70%. Dry in direct sunlight as much as possible.

B.7 Ending the Day

1. Refreshing the cleaning kit
   a. Ensure that there is enough bleach, quaternary ammonium, and other consumables available for the next sampling trip.
2. Equipment maintenance, cleaning, and storage
   a. Triple rinse pressure sprayer and soaking tub with tap water to remove residual bleach and debris.
   b. Allow sprayer and tub to dry completely before storage.

8 REFERENCES

Hadland, Randy. Region Manager. YSI, Inc. Personal communication, June 11, 2013.

Invasive Species Advisory Committee (ISAC) 2006. Invasive species definition clarification and guidance white paper. National Invasive Species Council (NISC), U.S. Department of the Interior, Washington, D.C. 11 p.

Moulton, S. R., II, J. G. Kennen, R. M. Goldstein, and J. A. Hambrook. 2002. Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program. Open-File Report 02-150. U.S. Geological Survey, Reston, VA.

NPS. 2006. Guidelines for prevention of introduction and spread of aquatic threats by cleaning an disinfecting fishing and field equipment. Retrieved from http://www.nps.gov/romo/planyourvisit/upload/aquatic_guidelines2006.pdf. June 2013.

Parsons, J. 2008. Minimizing the Effects of Invasive Species through Field Work: Effectiveness of Treatments. Retrieved from http://www.ecy.wa.gov/programs/eap/InvasiveSpecies/AIS-PublicVersion.html. June 2013.

Rutala, W.A., Weber, D.J., HICPAC. 2008. Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008: Center for Disease Control, U.S. Health & Human Services- Report. 158pp. available online at http://cdc.gov/hicpac/Disinfection_Sterilization/toc.html.

Stein, B. A. and S. R. Flack. 1997. 1997 Species Report Card: The State of U.S. Plants and Animals. The Nature Conservancy, Arlington, Virginia.

USEPA. 2007. National Rivers and Streams Assessment: Field Operations Manual. EPA-841-B-07-009. U.S. Environmental Protection Agency, Washington, DC.

USFS. 2010. Aquatic Invasive Species of Concern and Methods of Control. Retrieved from http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5305472.pdf. June 2013.

USFWS. 2012. Frequently asked questions about invasive species. Retrieved from http://www.fws.gov/invasives/faq.html#q20. July 2013.

Wilde, F. D., Skrobialowski, S. C., and Hart, J.S., 2010, Sampling protocol for post-landfall Deepwater Horizon oil release, Gulf of Mexico, 2010: U.S. Geological Survey Open-File Report 2010-1191, 155 p., available online at http://pubs.usgs.gov/of/2010/1191/.

Wilde, F.D. (ed.). 2004. Cleaning of Equipment for water sampling (ver. 2.0): U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9, Chap. A3. Retrieved from http://pubs.water.usgs.gov/twri9A3/, June 2013.

Wong, W.H. and S.L. Gerstenberger. 21011. Quagga muscles in the western United States: monitoring and management. Aquatic Invasions 6: 125-129. doi: 10.3391/ai.2011.6.2.01

APPENDIX A QUICK REFERENCES

Step 1 – Before leaving the site, DRAIN all buckets, coolers, waders, boots, and any other equipment that can hold water.

Step 2 – CLEAN all debris off sensors and equipment, including waders, nets, and brushes by hand. Leave debris at the site (discard on the shoreline or in the water).

Step 3 – CLEAN all debris off boat, motor, and trailer by hand. Leave debris at the site (discard on the shoreline or in the water).

Step 4 – All sensors must be cleaned and decontaminated before being returned to HQ for servicing and/or calibration, or when being moved to a new body of water.

Step 5 – General cleaning methods for decontamination of aquatic sensors (except uPAR)

Sensor Decontamination Procedure				
Step	**Action**	**Method**	**Solution**	**Delivery**
1	Remove debris	Dry brush	N/A	Manual removal
2	Remove soils and organic material	Detergent solution wash	0.2% Liquinox (v/v) or Alconox (w/v)	Wash bucket; wetted Kimwipes and brush as needed
3	Rinse	Rinse	Tap water	Rinse bucket; wash bottle
4	Remove inorganic chemical contaminants, mineral deposits, biofilms	Acid solution wash	HCl 5 % (v/v)	Wash bottle; wetted Kimwipes and brush as needed
5	Rinse	Rinse	Deionized water	Rinse bucket; wash bottle
6	Remove organic chemical contaminants, disinfect surfaces	Alcohol solution wash	Isopropanol 70% (v/v)	Wash bottle
7	Rinse	Rinse	Deionized water	Rinse bucket; wash bottle

Step 6 – Decontaminate the uPAR with detergent solution, tap water and 2% bleach solution.

Step 7 – Decontaminate all equipment with 2% bleach solution if the status of invasive species is unknown, or no data are available. If specific invasive species have been identified at the site, higher concentration bleach or quaternary ammonia disinfectant will be used.

Step 8 – At a commercial car wash, spray the boat and trailer with high pressure hot water.

APPENDIX B REMINDERS

General Decontamination:

- ☑ The basic premise of decontamination for aquatic invasive species is threefold:
  - Drain → Clean → Dry.
- ☑ Felt-soled waders and wading boots are prohibited for NEON field staff, NEON designated visitors, and subcontracted personnel. Bootfoot waders are preferred.

Sensor Decontamination:

- ☑ All sensors must be cleaned and decontaminated before being returned to HQ for servicing and/or calibration, or when being moved to a new body of water.
- ☑ Handling hydrochloric acid should be done under a fume hood or at the very least a well-ventilated area. Acid causes burns on contact and safety glasses and gloves are required
- ☑ Do NOT use alcohol, organic solvents, abrasives, or strong detergents to clean the diffuser element on the uPAR sensor.

Equipment Decontamination:

- ☑ A 2% bleach solution will be used to disinfect equipment at all sites if the status of invasive species is unknown, or no data are available.
- ☑ Air DRY all equipment thoroughly.
- ☑ Air DRY all boats and trailers thoroughly.

APPENDIX C SITE-SPECIFIC INFORMATION: AQUATIC SITES AND SPECIES OF CONCERN

Domain	Site	Species of Concern	Best Cleaning Practice
D1	West Branch Bigelow Brook		
D1	Sawmill Brook		
D2	Posey Creek		
D2	*To be determined*		
D3	Ichawaynochaway Creek		
D3	Lake Barco		Not required by site host
D3	Lake Suggs		Not required by site host
D4	Rio Cupeyes		
D4	Rio Guilarte		
D5	Crampton Lake		
D5	*To be determined*		
D6	Kings Creek		
D6	McDowell Creek		
D7	Leconte Creek		
D7	Walker Branch		
D8	Mayfield Creek		
D8	Black Warrior River	Several aquatic plant invasives	Bleach and hot water cleaning
D8	Tombigbee River	Several aquatic plant invasives	Bleach and hot water cleaning
D9	Prairie Lake		
D9	Prairie Pothole		
D10	Arikaree River		
D11	Pringle Creek		
D11	South Pond		
D12	Blacktail Deer Creek		
D12	Bozeman Creek		
D13	Como Creek		
D13	Little Vasquez Creek		
D14	Sycamore Creek		
D15	Red Butte Creek		
D16	McRae Creek		
D16	Planting Creek		
D17	Convict Creek		
D17	Providence Creek		
D18	Oksrukuyik Creek	None	Normal cleaning practices
D18	Toolik Lake	None	Normal cleaning practices
D19	Caribou Creek	None	Normal cleaning practices

*Site-specific equipment required.