



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-F308

eDNA Water Sample Collection

Standard Operating Procedure

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1. Purpose

This procedure describes site-specific water collection strategies for environmental DNA (eDNA) sampling across the range of aquatic habitats encountered in EA Program projects: wadeable streams, non-wadeable rivers, lakes and reservoirs, ponds and vernal pools, and constructed wetlands. It supplements [SOP-F700 eDNA Field Sampling and Preservation](#), which covers the core filtration, preservation, and contamination- control protocols that apply to all eDNA water samples regardless of habitat type.

The separation recognizes that contamination prevention, filtration, and preservation (SOP-F700) are universal, while the physical act of collecting water varies with habitat. This SOP addresses

the collection step: where to sample within a water body, how many samples to collect, and how to adapt the approach to different hydrological settings (Deiner et al., 2017; Goldberg et al., 2016).

2. Scope

- **Applies to:** Water collection for eDNA analysis at all EA Program aquatic field sites. Once water is collected, filtration and preservation follow [SOP-F700](#).
- **Does not apply to:** Soil eDNA or sediment eDNA sampling (see [SOP-F701](#)); airborne eDNA; tissue-based DNA sampling; lab-side filtration and extraction (see [SOP-L700](#))
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling design, confirm permits
Trained Personnel	Collect eDNA water samples per this SOP and SOP-F700
New Students	Complete SOP-F700 contamination training before participating

4. Definitions

- **Capture efficiency:** The proportion of target eDNA in the water column that is retained on a filter during collection; influenced by filter pore size, clogging, sample volume, and the physical state (dissolved vs. particulate) of the eDNA.
- **Closed-system filtration:** An eDNA collection method in which water passes through a filter enclosed in a sterile capsule (e.g., Sterivex) without contact with the external environment; minimizes contamination risk compared to open filtration.
- **Decontamination:** The removal or inactivation of exogenous DNA from sampling equipment and surfaces using bleach (10% sodium hypochlorite), UV irradiation, or other validated methods; essential between sites to prevent cross-contamination.
- **eDNA (environmental DNA):** Genetic material shed into the environment by organisms through mucus, feces, gametes, skin cells, and other biological material; collected from water without direct capture of the target organism.
- **Field blank:** A bottle of laboratory-grade water opened and handled at the field site under identical conditions to real samples; processed through the full filtration and extraction workflow to detect field contamination; must yield no target amplification.
- **Filter pore size:** The nominal diameter of pores in the filter membrane; 0.45 μm is the standard for eDNA capture from surface water, retaining bacteria, cells, and particulate-bound DNA while allowing water to pass.
- **Longmire's buffer:** A non-freezing preservation solution (containing EDTA, Tris, NaCl, SDS, and sucrose) used to preserve eDNA on filters in the field when immediate freezing is not possible; inactivates nucleases and inhibits microbial degradation.

- **Peristaltic pump:** A battery-operated pump used to draw large volumes of water through a filter in the field without the water contacting the pump mechanism; preferred for high-volume eDNA filtration because it reduces contamination risk.
- **Sterivex filter:** A commercially available enclosed 0.22 µm polysulfone cartridge filter; widely used in eDNA studies because the sealed design minimizes contamination, allows direct addition of preservation buffer, and is compatible with standard DNA extraction kits.
- **Target species detection:** The determination of presence or absence of a specific taxon in a water sample based on amplification of taxon-specific eDNA; sensitivity depends on eDNA shedding rate, water volume filtered, detection method (PCR, ddPCR, metabarcoding), and inhibitor load in the extract.

5. Safety and Hazards

All safety requirements from [SOP-F700](#) and [SOP-F001](#) apply. Additional hazards specific to collection settings:



Additional Hazards by Habitat

- **Non-wadeable rivers and lakes:** Boat access required; drowning risk. PFDs mandatory.
- **Vernal pools:** Often in remote locations with limited access; fragile habitat. Minimize trampling of pool margins.
- **Constructed wetlands:** May contain treated wastewater. Wear doubled gloves and avoid ingestion or skin contact.

6. Equipment and Materials

All sterile sampling supplies, preservatives, and field documentation are listed in [SOP-F700](#) Section 5. Additional items for specific habitats:

Item	Habitat	Notes
Extension pole with sterile bottle clamp	Non-wadeable streams, bridges	For mid-channel collection without entering the water
Van Dorn or Kemmerer bottle (dedicated to eDNA, pre-sterilized)	Lakes, deep pools	For discrete-depth collection
Peristaltic pump with sterile tubing	Any depth-specific application	Single-use tubing per sample
Telescoping boat hook	Boat-based sampling	For retrieving floating bottles

7. Procedure

7.1. General Principles (All Habitats)

These principles supplement [SOP-F700](#):

- **Sample where the target organism is most likely detected.** eDNA concentration varies spatially: it is higher near organism activity centers (spawning areas, resting pools, feeding zones) and downstream of occupied habitat in lotic systems (Jane et al., 2015).
- **Sample volume:** Collect at least 1 L per replicate (the volume actually filtered may be less if the filter clogs). Plan for 3 replicates per site unless the study design specifies otherwise.
- **Sampling order:** Within a day, sample sites from least likely to most likely occupied (downstream to upstream in a connected system, or reference sites before impacted sites) to minimize carry-over contamination (Goldberg et al., 2016).

7.2. Wadeable Streams

1. Approach the sampling point from downstream
2. Face upstream; submerge the sterile bottle to 10–20 cm below the surface, tilted slightly into the current
3. Fill to the 1 L mark and cap immediately
4. For a reach-level composite: collect 250 mL from each of 4 microhabitats (pool tail, riffle margin, run, and backwater) and combine into one bottle
5. Proceed to filtration per SOP-F700 Section 6.4

7.3. Non-Wadeable Rivers and Bridges

1. From a bridge or bank, lower a sterile bottle attached to an extension pole or a pre-sterilized Van Dorn sampler to mid-depth
2. If using an extension pole: open the bottle at depth, fill by current flow, and cap using a pull-string mechanism or retrieve and cap immediately
3. Record the approximate depth and distance from shore
4. Decontaminate the extension pole between sites per SOP-F700 Section 6.7

7.4. Lakes and Reservoirs

1. Collect samples from a boat positioned over the target area (e.g., littoral zone for nearshore species, open water for pelagic species)
2. For surface eDNA: submerge a sterile bottle to 15–30 cm below the surface from the upwind side of the boat to avoid contamination from the vessel
3. For depth-specific eDNA (e.g., below the thermocline): deploy a pre-sterilized Van Dorn bottle to the target depth, trip, and retrieve. Transfer to a sterile sample bottle.
4. If sampling multiple depths, work from deepest to shallowest to minimize disturbance
5. Record collection depth, water depth, and location (GPS)

7.5. Ponds and Vernal Pools

1. Approach slowly to minimize sediment disturbance (eDNA can be resuspended from sediment, creating false-positive risk for species no longer present) (Turner et al., 2015)
2. Collect water from 5–10 cm below the surface near the center or deepest point of the pool
3. For very shallow pools (less than 15 cm), tip the bottle on its side to fill without disturbing the bottom

4. If the pool is small (less than 10 m²), a single 1 L grab from the center is acceptable; for larger pools, composite from 3–5 points around the perimeter

7.6. Constructed Wetlands and Treatment Systems

1. Wear doubled nitrile gloves; these systems may contain treated wastewater, elevated pathogen loads, or chemical residues
2. Collect from the outflow or from an open water area; avoid collecting from areas with dense emergent vegetation where filtration may be rapid
3. Collect a field blank at the site using the molecular-grade DI water from the field kit (this is especially important in constructed systems where background DNA loads are high)

7.7. Filtration and Preservation

After collection in any habitat, proceed immediately to filtration and preservation per [SOP-F700](#) Section 6.4. If on-site filtration is not possible, keep samples on ice and filter within 6 hours.

8. Quality Control

All QC requirements from SOP-F700 Section 8 apply. Additional habitat-specific considerations:

- For lake depth samples, collect a field blank at the same depth using the same equipment to detect cross-contamination from the Van Dorn or pump
- For bridge sampling with an extension pole, collect an equipment blank (DI water poured through the pole apparatus) at least once per event
- When sampling connected sites in a watershed, record the order of site visits and the time elapsed between sites

9. References

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10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Between-site decon
F008	Sample Labeling and COC	Sample ID and chain of custody
F300	Surface Water Sampling	General water collection
F700	eDNA Field Sampling and Preservation	Core eDNA protocol (filtration, preservation, contamination control)
L700	DNA Extraction	Lab-side extraction
L706	eDNA Metabarcoding	Full lab workflow

11. Training Record

Before collecting eDNA water samples independently, personnel must:

1. Complete all training requirements in [SOP-F700](#) Section 11
2. Review the habitat-specific collection methods in this SOP for the site(s) to be sampled
3. If boat-based sampling is required, complete boat safety orientation (PFD use, boat handling)
4. Demonstrate proper sterile collection technique (bottle handling, depth sampling) under supervision
5. Be signed off by the PI or field lead