GEN-FISH
Genomic Network for Fish Identification, Stress and Health
University of Windsor and Partners

GEN-FISH eDNA Sampling Protocol
OSMOS unit – Backpack System use

Overview: The automated OSMOS eDNA sampler is a portable backpack that allows for high-throughput in-field water collection and filtration. The rate of filtration varies with turbidity and pre-filters can be incorporated as needed. Three 0.5–1 L water samples plus a field blank can be filtered in 20–40 minutes.

A. Materials
See ***Appendix B. Recommended Supplies List for eDNA Sampling with OSMOS*** for ordering details. Quantities per site based on three biological replicates (water samples) plus one negative control (NC/blank) per site. See the ***Packing List*** for an itemized list of all equipment that was sent to you and packing instructions.

1. **OSMOS backpack,** which includes quick connect inlet port for hose and hanging apparatus for tripod bracket
2. **OSMOS batteries and charger**
3. **Sterilized aluminum filter housings**, one per site
4. **Tripod** with backpack hanging bracket attached
5. **Tripod swivel** with opening for securing telescopic pole
6. **Telescopic pole attached to hose**
7. **6 ft outlet hose** for water runoff
8. **Deionized water or sealed bottled water (500 mL)**; minimum of three per site for field control
9. **Sterilized large plastic bag** for field control water
10. **Nitrile gloves,** 1–2 pairs per site.
11. **Sharpies and ethanol-proof markers.**
12. **Labeling tape**

13. **Sterile metal forceps.** These are easily sterilized in the field with ethanol
    i. **Sterile 15 mL tubes filled with 70–95% ethanol** for dipping forceps. Minimum one per sampling day and can be reused
    ii. **Lighter,** such as a barbecue lighter.
14. If using **liquid preservative (ethanol or RNAlater)**:
    i. **Sterile screw-top tubes (skirted or non-skirted, Dnase and Rnase free)**, prefilled and prelabeled $^{2}/_{3}$ with molecular-grade ethanol (preferably 99%, no less than 95%) or RNAlater. Four per site, plus unlabeled backups.
    ii. **Storage boxes** or **falcon tube rack** to hold tubes upright.
15. If using **liquid-free preservative (self-indicating silica beads):**
    i. **Coin envelopes** prelabeled. Four per site, plus extras.
    ii. **Plastic specimen bags,** sterile, prefilled with silica and use to store coin envelopes. Three bags per site (#1 for negative control, #2 for water samples and #3 for keeping together both bags #1 and #2 from a single site).
    iii. **Sampler spoons,** sterile, to fill specimen bags with silica. One per site. One scoop holds ~15 g of silica
    iv. **Self-indicating silica,** prefilled or can fill specimen bags in field. ~150 grams per site. ~30 grams for negative control specimen bag #1, ~90-100 grams for water replicate specimen bag #2. Extra to top up as needed as silica becomes saturated.
16. **ELIMINase or sodium hypochlorite solution;** concentrated bleach contains chlorine concentration of 5.25–8% or 52.5–80 g/L or 52,500–80,000 ppm; for example, one part 5% bleach to nine parts cold water will make a 10% bleach solution and the concentration of chlorine in the solution is 5,000 ppm, which is ideal for high level disinfection. Bleach solutions at 10, 20, or 50 % can be made daily and stored in an opaque container away from light. ~1 L of bleach solution per site or 30 mL ELIMINase. ELIMINase is non-toxic and does not leave behind harmful residues but all ELIMINase waste and bleach must be rinsed and the wastewater stored for safe disposal. If you opt to use bleach you will need to bring enough deionized water to be able to submerge the equipment as well as a large container to store waste bleach for safe disposal after sampling
17. **Paper towels.**
18. **Filter membranes,** four per site, plus backups.

B. Cleaning Procedures (see *Equipment Sanitation Flowchart*)

Given the sensitivity of the eDNA techniques, avoiding contamination of field samples is critical:

- All sampling equipment must be kept separate from other field gear (e.g., nets, clothing, truck bed).
- Samples can be easily contaminated. Anything that has come into contact with fish or bodies of water containing fish (e.g., clothing, gear, vehicle) can be a potential source of contamination.
- Always wear fresh gloves while handling sterile items and samples.
- When in doubt, change your gloves.

- Keep contaminated, used, or dirty field materials bagged and separate from clean equipment to minimize chances of contamination.
- If fish collection, sampling, or electrofishing is also to be conducted, **collect biological water samples for eDNA before other sampling or measuring water chemistry.**
- Ideally, water samples should be collected downstream from the edge of the water body, without entering the water. This will prevent the sediments from being disturbed (which can increase the amount of clay and sand collected in the water) and will also reduce the possibility of contamination from boots that can carry over DNA from site to site. If you need to enter the water, **always stay downstream of the water collection locale, and do not touch the inside of the water collection bottles.**

C. Field sampling preparation

1. Prepare field sampling bags for each site in a freshly sanitized area, free of contaminant DNA. This is preferably a closed indoor space away from sources of contaminants such as: fish DNA, areas where dissections occur, or where PCRs are conducted. Use gloves and follow the flowchart instructions provided in the ***Equipment Sanitation Flowchart.***
2. If you have access to a PCR workstation with UV light irradiation equipment, field sampling bags can be UV treated and then used.
3. Wearing gloves, wash all aluminum filter housings (including all interior/exterior) with warm soap and water. Wipe down filter housings with 95% ethanol and ELIMINase, and then rinse off. UV-treat filter housings for a minimum of 15 minutes.
4. In the decontaminated UV-hood (using sterile gloves and forceps), place filter membrane in-between the filter screen and the rubber gasket.
5. Prior to reassembly of filter housing, lightly lubricate the O-ring to prevent friction. Using the brush provided, dab a small amount of silicone along the O-ring evenly in 3 or 4 places. Then with a gloved hand gently rub the silicone along the O-ring. NOTE: be careful not to get the silicone onto the inside of the housing, as this will introduce contaminates (see **Figures 1–3**).
6. Reassemble filter housing and place in sterile plastic sandwich bag (one filter housing per bag).
7. All items can be packed into a larger container or tote bag, with extra bags to keep waste (e.g., gloves) that can be disposed of when returned to the lab or field station.
8. Install charged lithium battery into the OSMOS unit (see **Figures 4–10**).

D. Labels

There should be a minimum of two sets of labels for samples per site (i.e., both on the bottles as well as the outside of the bag) to ensure samples are identifiable in case labeling peels or rubs off. Bottles can be labelled ahead of time to save time in field, if desired (we recommend this). Additional information, such as the name or initials of the collector, can be included as well.

Minimum requirements for sample labels for each 2 mL tube, 15 mL tube, or sample container:

- the site name or abbreviation for site
- sub-sample information to distinguish between a blank/negative control and biological water sample

- the replicate number for biological water samples
- date sampled

Any additional information if needed can be given on the label as needed and more details can be provided on metadata sheets

Examples of Labels

- **Sample bag:** The label for each site on the exterior of each bag should include the date, site name, and water body. The label may look something like:

"Site 1 @ Peterson Creek 1Aug2022 AK"

- **Blank/Negative control:** The falcon tube with the negative control can be labelled as:

"Site 1 @ Peterson Creek NC 1Aug2022 AK"

- **Biological water samples:** Falcon tubes with site collected filter can be labelled as:

"Site 1 @ Peterson Creek Rep. 1 1Aug2022 AK"

E. Prepare filter storage containers

Preparing filter storage ahead of time will save time in the field and prevent contamination.

- **Ethanol or RNAlater**
  1. Label the appropriate number of tubes following the conventions above.
  2. Prefill tubes $^{2}/_{3}$ – $^{3}/_{4}$ full with DNA stabilizer.
  3. Store upright in boxes or racks, also labeled.
- **Self-indicating silica**
  1. Label the appropriate number of coin envelopes following the conventions above.
  2. Place three water sample coin envelopes into a plastic specimen bag, and the envelope for control into a secondary plastic bag
  3. Prefill specimen bag for three water samples with ~90–100 g (seven spoon-scoops) of silica
  4. Prefill specimen bag for one control sample with ~30 g (two spoon-scoops) of silica
  5. Store specimen bags in a secondary bag for each site.

F. Sample collection process (see *Appendix A* for figures)

1. Upon arrival at the sampling site, locate your desired field sampling location: even/level surface, ground conducive for securing tripod, etc.
2. Prepare the tripod by first unclipping the strap that holds the legs together. Extend the tripod legs by turning the thumbscrews in a counter-clockwise direction and extend the leg assembly. Retighten the thumbscrew by turning in a clockwise direction (see **Figure 11**).

3. You will notice a bracket along the top of the tripod. This is where the OSMOS unit will be hung during operations. Place the tripod in such a way that the hanging bracket is facing AWAY from your water source. Secure the tripod by stepping onto the pedals of the legs to securely plant them into the soil.
4. Attach the tripod swivel to the treaded bolt at the top of the tripod. NOTE: the swivel does not make use of the threads (see **Figure 12**).
5. Thread the hose end of the pole through the opening of the tripod and pivot pole clamp that will secure the pole. Be sure to insert the hose from the water side of the pivot assembly. Carefully continue to thread the hose through the opening.
6. Insert the end of the pole into the opening and secure by turning the lever clockwise.
7. Open the OSMOS unit and turn the operating switch to the ON position. You will hear a series of beeps as the unit powers up.
8. Close the lid on the OSMOS and SECURE THE LATCHES.
9. Connect the end of the inlet hose to the quick connect inlet port on the bottom of the OSMOS backpack (see **Figure 13**).
10. Connect the outlet hose to the OSMOS runoff located in the bottom of the unit. You will see the inlet quick connect facing in one direction and a brass hose barb facing in the other direction. The outlet hose is simply pushing onto the brass hose barb. Make sure the end of the hose will discharge in a safe location (i.e., downstream of your sample site).
11. Hang the OSMOS from the red rubberized handle at the top of the backpack onto the hanging bracket of the tripod.
12. Prior to handling the filter housing, put on new nitrile gloves. If sampling is occurring during colder temperatures, place extra-large nitrile gloves over insulated finger gloves to prevent freezing of hands.
13. Carefully remove the sterilized filter housing assembly from the individual plastic bag (this should have been assembled ahead of time. For pre-priming, a filter membrane is not required; for the negative control and biological water samples, a filter membrane is required).
14. Attach the check valve and filter housing assembly to the end of the telescopic pole. Using both hands, hold the filter housing and pull back on the quick connect collar on the check valve. Insert the housing into the collar and release the collar. MAKE SURE THAT THE FILTER HOUSING ASSEMBLY IS SECURELY ATTACHED. You will hear a distinct click and the filter housing assembly will sit perpendicular to the quick connect collar surface. If improperly attached, the filter housing will appear tilted. It may take more than one attempt to successfully attach the filter housing assembly.
15. Loosen the extension locks on the pole and telescope the pole to the desired length to reach the water source.
16. Using the swivel lock handle, hold the pole with one hand while turning the swivel lock counter-clockwise to loosen it. With the swivel lock loosened, you will now be able to gently lower the pole angle and filter housing into the water (natural or bottle). Before the pole reaches the bottom of the waterbody (natural or bottle), turn the swivel lock clockwise to lock the vertical position of the pole into place.

17. **Pre-Prime the OSMOS:** In the settings menu, set the hose length to zero feet (0.0 ft) and adjust any other setting accordingly (see steps #19–23 below). Start the pump to collect **clean tap water (no filter).** Stop the pump using the BACK button once water is seen flowing steady out of the drain line. With the system full of clean water, a filter can now be added, and sample collection can begin.
18. Filter your negative control by pouring 1.5 L of water from the sealed bottles into one of the large, sterilized plastic bags. Place the filter housing unit into the plastic bag and make sure to keep the water inlet surface submerged throughout the running of the negative control.
    19. The first screen on the OSMOS will display "SETTINGS….CHANGE CONTINUE". Press the "ENT" button to go through the settings.
    20. The next screen will allow you to set the volume of water to filter. The default is 2.0 L. You can change this value by pressing the UP or DOWN buttons.
    21. The next screen allows you to set the number of pre-filters that you are using. Use the UP and DOWN arrows to set this number and press the "ENT" button to continue. In most cases with clear water you will not be using pre-filters, so you can leave this value as ZERO.
    22. Ensure that the hose length is set to zero feet (0.0 ft).
    23. The remaining screens have correct default settings for standard use; simply select "ENT" for the remaining prompts.
24. The final screen will allow you to start your run by pressing "ENT".
25. The pump will run automatically until the pre-set filtration amount has been processed. At this time a very loud and persistent beep will be heard. Remove the filter housing assembly from the sample and note that you will be prompted to invert the filter housing. (see **Figure 14**). Please ignore this step by pressing the "ENT" button followed by a press of the BACK button to terminate the current session.
26. The sample is now complete and the system will remain full of filtered water, primed for the next sample. The display screen will show relevant sampling values for your records.
27. Retract the pole and carefully disconnect the filter housing from the pole by pulling back the quick connect collar. Hold the filter housing assembly with your other hand while disconnecting the quick release (see **Figure 15**).
28. Separate the final stage of the filter housing assembly from the pre-filter stage/inlet stage.
29. **Sterilize Forceps**: Dip your metal forceps into your 15 mL tube of ethanol, holding the pincers of the forceps facing down so ethanol drips off the tips of the forceps and does not run down to the portion you're holding. Then flame them with your lighter to ensure sterility. Using these flamed sterile forceps, carefully remove the rubber gasket and remove the filter membrane from the filter screen (see **Figure 16–17**).
30. Place the filter membrane into the appropriate preservation medium (vial with ethanol/RNAlater or envelope with silica beads).
31. Flame/re-sterilize your forceps and place a new filter membrane into the filter housing. Put the rubber gasket over filter membrane and reassemble the filter housing for use (see **Figure 18**).

32. For each biological water sample, select "Continue" (ENT) to run the OSMOS with the previous settings. Submerge the filter housing assembly in the body of water to collect field samples. Gather your desired number of replicate samples (ideally three) and make sure to submerge the filter housing assembly in the same spot for all samples.
33. When finished sampling, remove the filter housing and invert the hose. Start the pump and allow the pump to drain all of the water from the system. Once no water is visible exiting the drain line, press the BACK button to end the session. Disconnect your setup in reverse order from the assembly steps (steps #16–2).
34. Ensure each sample is labeled correctly (see **D. Labels**, above). Remove gloves and place in waste bag.
35. Upon arrival at lab, place samples in -20°C to -30°C freezer for storage.
36. Discard waste from field collection in appropriate disposal.

G. Notes and Precautions

- If you're going to be doing any other sampling in the water, please make sure that you take the biological water samples for eDNA first, followed by any other sampling in the water body. If you're going to be taking any water chemistry parameters, this should take place last so you're not carrying over contaminants from one site to another; cleaning sensitive water parameter equipment may not be possible in field.
- Dress appropriately for the weather. Insulated, water-proof gloves are a must during colder months. Bare hands can easily get frozen/frostbite when dealing with lower water temperatures and handling metal.
- Be cautious when transporting equipment down to water level, as muddy and rocky surfaces can be very slippery. Always have someone assisting you in the field.
- It is helpful to have two separate bin containers, one labelled "clean" and one "dirty", to make sure sterile filter housing assemblies are not confused with used, contaminated ones.
- Since the tripod swivel cannot be secured directly to the threads, it is recommended to have someone hold the tripod and filter housing assembly in position while running sample, in order to maintain constant depth.
- Alternative sanitizing methods for forceps:
    - Dipping in a 50% bleach solution for 1–2 minutes, rinsing with deionized water, and wiped dry
    - Sprayed with ELIMINase and wiped dry.

Storage of Filters (Lab)

- Liquid preservative (ethanol or RNAlater): Filters that are preserved in ethanol can be stored at room temperature, but we suggest storing filters at -20 to -30°C.
- Liquid-free preservative (self-indicating silica beads): Filters that are preserved in silica can be stored at room temperature in a cool, dry location away from light, but we suggest storing at -20 to -30°C.

Appendix A. OSMOS parts, assembly, maintenance, battery installation, and filter membrane removal and replacement.

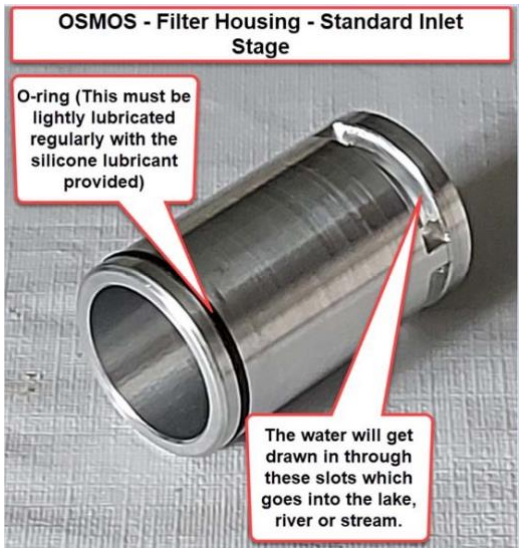

***Figure 1**. Location of O-ring for lubrication.*

***Figure 2**. Application of lubricant.*

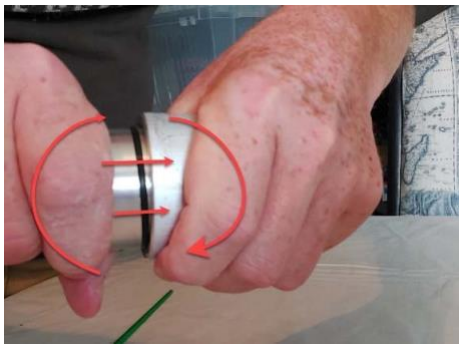

***Figure 3.** Reassembling filter housing.*

Figure 4. *Latched OSMOS unit*

Figure 5. *Opened OSMOS unit*

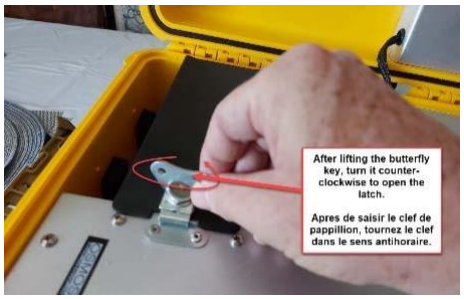

Figure 6. *Location of battery compartment and direction on opening latch*.

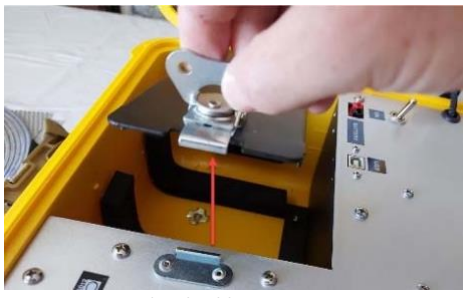

Figure 7. *Unlatched battery compartment.*

***Figure 8**. Fitting of battery into compartment.*

***Figure 9**. Securing of battery latch.*

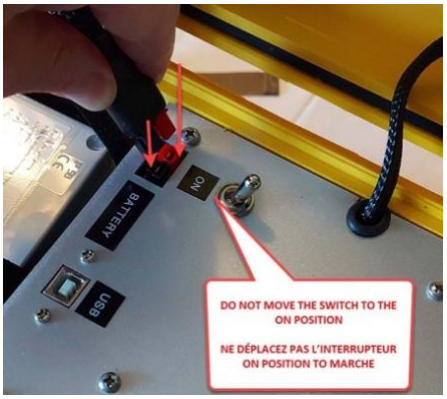

***Figure 10.** Plugging in battery to OSMOS unit.*

Figure 11*. How to extend and adjust tripod legs.*

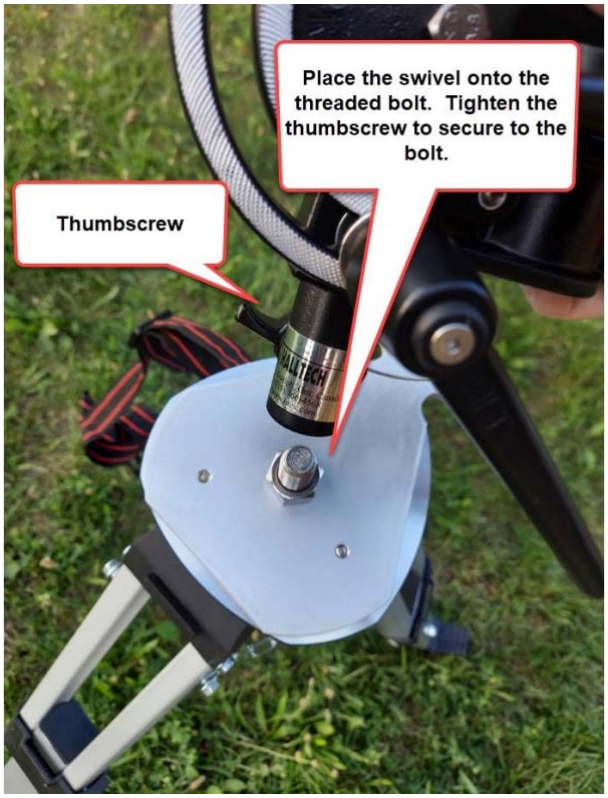

Figure 12. *Attachment of tripod swivel to tripod.*

***Figure 13**. Attachment of the hose to the quick connect inlet port on the OSMOS.*

***Figure 14.** Position of inverted filter housing for purging stage.*

***Figure 15**. Disconnecting filter housing from quick connect collar.*

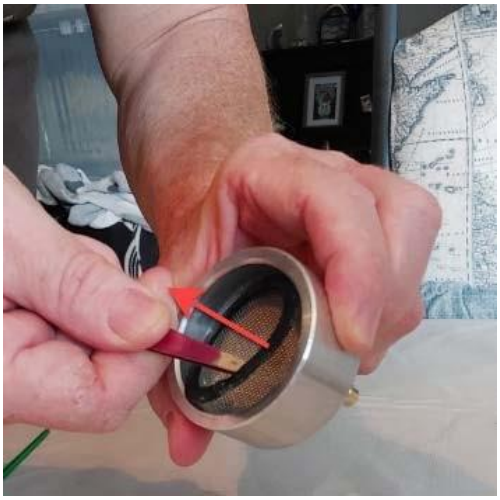

Figure 16. *Removal of rubber gasket with sterile forceps.*

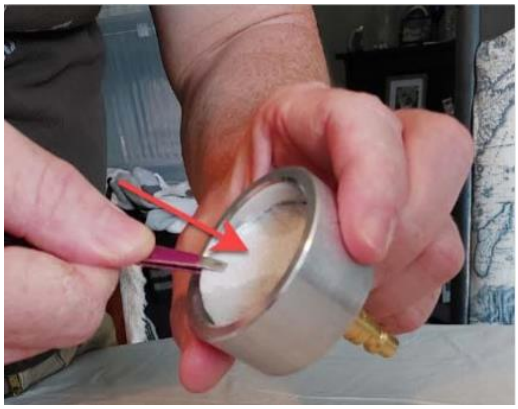

Figure 17. *Placement of new filter membrane.*

Figure 18. *Placement of rubber gasket over new filter membrane.*

Appendix B. Recommended Supplies List for eDNA Sampling with OSMOS (for emergency replacements); materials provided by central lab are in bold.

Item	Description	Vendor and Item Number(s)
Battery powered Backpack	Halltech™ OSMOS Sampler	Halltech: OSMOSKIT
4-12' Telescoping Pole	Halltech™ 4-12' telescoping pole	Halltech: OSTLPS
4' Inlet Hose with Quick Connects	Halltech™ 4' Inlet Hose with Quick Connects (Benchtop operation)	Halltech: OSIHQC
Tripod and pivot clamp	Halltech™ Adjustable aluminum Mini Tripod with backpack bracket	Halltech: OSMTP
29V Lithium-Ion Power Pack	Halltech™ 29 V 10Ah Lithium Battery	Halltech: 77521L
Smart Charger	Halltech™ 29.4V 2A li ion charger	Halltech: 77522L
A/C Adapter for Battery Powered Backpack	Halltech™ Bench Top Conversion Kit for OSMOS eDNA Sampler (plugs into 110v outlet)	Halltech: OSBTCK
Rugged Field case with wheels	Halltech™ Pelican Transportation Field Case with Handle & Wheels	Halltech: 1650-021-110
Pelican Telescoping Pole and Tripod Case	Pelican Long Case – Black with foam for transporting the telescoping pole and tripod	Pelican: iM3220
Aluminum re-usable filter housing components	Halltech™ STANDARD INLET STAGE Halltech™ MODIFIED INLET STAGE (Benchtop operation) Halltech™ FINAL STAGE	Halltech: OSIS Halltech: OSHBIS Halltech: OSFS
Glass fiber filters	Cytiva Whatman™ 1.5 µm Binder-Free Glass Microfiber Filters, Grade 934-AH, 47 mm Circles	Fisher Scientific: 09-873DD

Forceps	Fisherbrand™ Filter/Membrane Stainless Steel Forceps	Fisher Scientific: 09-753-50
Single-use nitrile gloves	Fisherbrand™ Powder-Free Nitrile Exam Gloves	Fisher Scientific: 191301597 (variety of sizes available)
Graduated container or cylinder	Fisherbrand™ Polypropylene Graduated Cylinders	Fisher Scientific: 03-007-44
Ethanol-proof markers	Fisherbrand™ Fine Tip Marking Pens	Fisher Scientific: 13-379-6
ELIMINase	Decon™ ELIMINase™ Decontaminant	Fisher Scientific: 04-355-32
FILTER PRESERVATION MATERIAL IN LIQUID PRESERVATIVE (ETHANOL OR RNALATER)—PROVIDED IF INDICATED		
Storage box, size appropriate to tube size.	Fisherbrand™ Cryo/Freezer Boxes (100- tube capacity)	Fisher Scientific: 03-395-465
Sterile tubes (2 mL or 15 mL)	United Scientific Supplies 15 mL Centrifuge Tubes National Scientific™ BioStor™ 2 mL Screw Cap Vials, Skirted	Fisher Scientific (15 mL): S99410 Fisher Scientific (2 mL): 11-844-18
RNAlater	Invitrogen™ RNA*later*™ Stabilization Solution	Fisher Scientific (500 mL): **AM7021**
Ethanol	Ethyl Alcohol Denatured, MilliporeSigma™	Fisher Scientific (4 L): MEX02803
FILTER PRESERVATION MATERIALS IN LIQUID-FREE PRESERVATIVE (SELF-INDICATING SILICA)—PROVIDED IF INDICATED		
Coin envelopes, sterile	Coin Envelopes with Gummed Flaps, 2-1/4" x 3-1/2"	Suggested Vendor: Staples Item: 438346 Model: 530164
Self-indicating silica beads, sterile	Silica Gel, Honeywell Fluka (metal free) 2.5 kg	Fisher Scientific: 6002003
Plastic specimen bags, sterile	Minigrip™ Reclosable White Specimen Bags	Fisher Scientific: 22-310-032
Sterile sampler spoons	Bel-Art™ Sterileware™ Sterile Styrene Sampler Spoons (~15 mL capacity)	Fisher Scientific: 03-990-232

GEN-FISH
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OSMOS Packing List

Item	Quantity	Images	Returned (*check when packed*)
OSMOS Backpack Shipping Case OSMOS Tripod / Pole Case	2	 	☐
OSMOS Backpack Unit	1		☐
OSMOS Lithium Battery	2		☐
OSMOS 110v AV Lithium Battery Conversion Adapter *Benchtop operation*	1		☐
OSMOS Lithium Battery Charger	1		☐
OSMOS Tripod Swivel	1		☐
DepthTrax Depth Finder	1		☐
OSMOS 4’ Modified Inlet Hose (with Quick Release Connectors) *Benchtop operation*	1		☐
OSMOS Tubing for Modified Inlet Stage (bag containing various lengths *Benchtop operation*	1		☐

OSMOS 6' Outlet Hose	1	 NOTE: The outlet hose has a piece of green tape on it.	☐
OSMOS Aluminum Filter Housing Assembly (individual components below)	10	 10 in tripod/pole case	☐
OSMOS Filter Housing – Final Stage (with Quick Release; contains a screen and rubber gasket)	10	 10 in tripod/pole case	☐
OSMOS Filter Housing – Pre-Filter Stage (contains a screen and rubber gasket)	10	 10 in tripod/pole case	☐
OSMOS Filter Housing – Standard Inlet Stage	10	 10 in tripod/pole case	☐
OSMOS Filter Housing – Modified Inlet State (with Hose Barb) *Benchtop operation*	10	 Use this inlet stage and cut a piece of the additional hose to length to reach the bottom of a sample bottle. 10 in backpack case	☐
Wrench (not needed for regular operation)	1		☐
Silicone Grease Application Kit (silicone grease, application brushes, small bottle brush)	1		☐
Telescoping Pole with 15' Inlet hose (incorporated into pole)	1		☐
OSMOS Tripod	1		☐

Additional items:

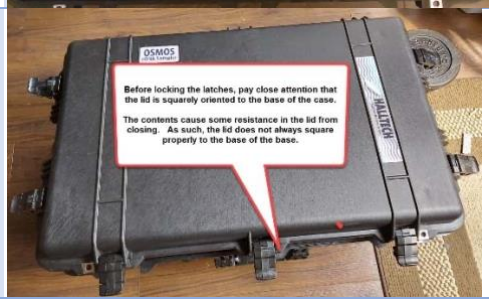
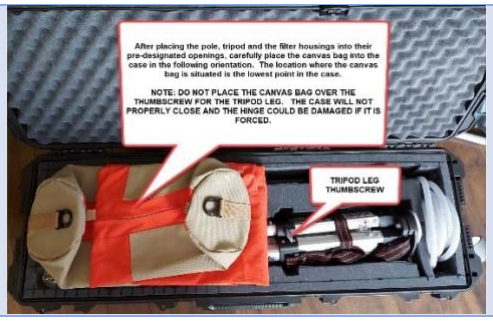
- Tubes pre-filled with self-indicating silica beads OR pre-filled with RNAlater or ethanol.
- Filter membranes
- *NOTE: Please indicate the entire amount(s) of the above preservation materials needed for all summer sampling on the GEN-FISH equipment booking form. Multiple requests will not be accepted, as this negatively impacts our shipping budget and available stock may be limited.*

OSMOS Packing Instructions

The components in the OSMOS Backpack Case fit very tightly in the case. This is done intentionally to minimize the movement of the contents during shipping. Please follow the steps below when packing the OSMOS Backpack Case.

STEP #	DESCRIPTION	IMAGE
1	Begin with the empty case. Insert the battery cable through the battery strap. Place the batteries into the space as shown. Place the battery cable into the small square opening.	
2	Insert the battery charger into the space provided.	
3	Insert the tripod swivel into the space provided.	
4	Insert the DepthTrax Depth Finder into the same space, next to the tripod swivel.	

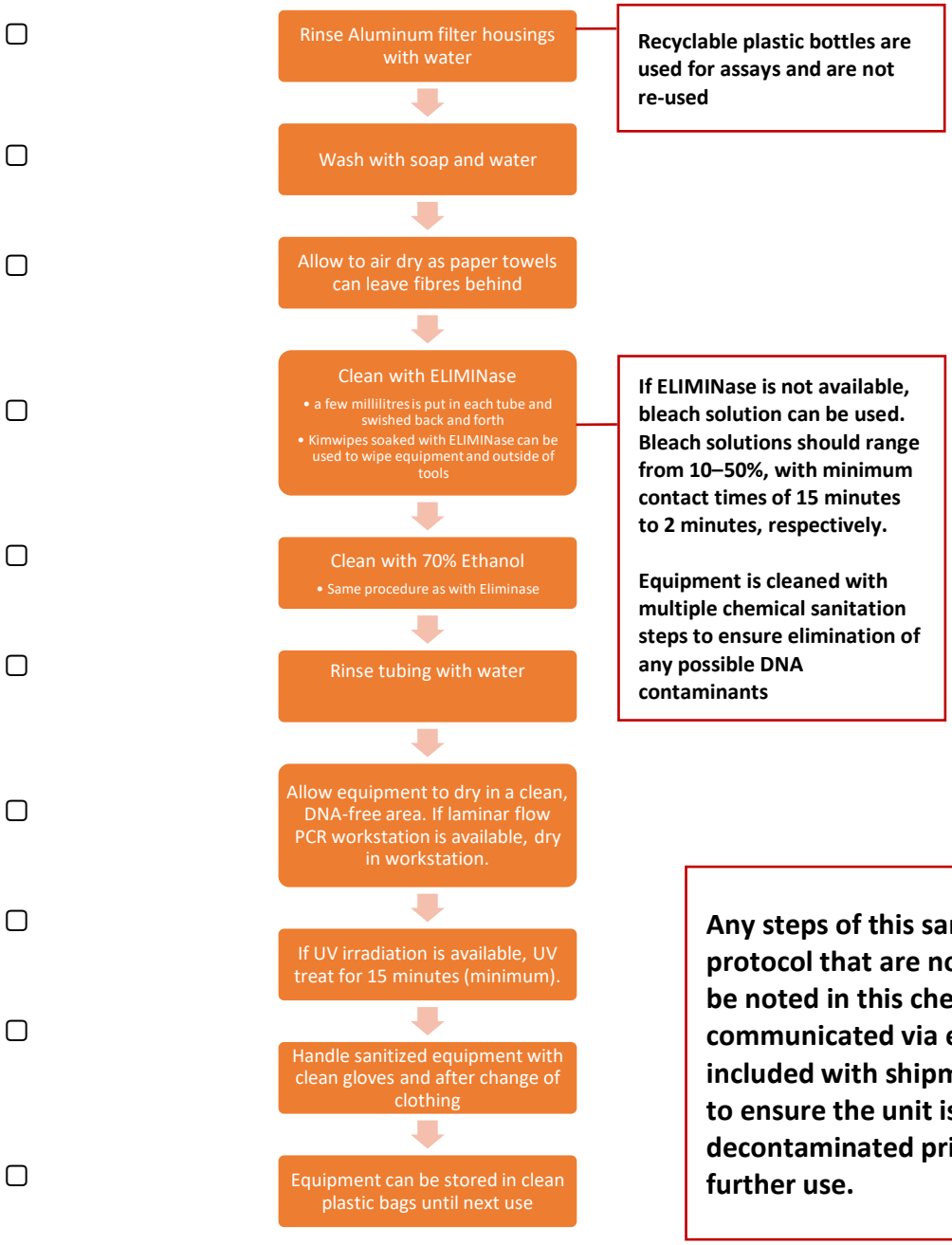
STEP #	DESCRIPTION	IMAGE
5	Along the hinge side of the case, you will see partial holes. Place 10 of the MODIFIED aluminum filter housing assemblies into these holes. Each assembly should contain the final stage, the pre- filter stage, and the modified inlet stage. Place each filter housing in with the final stage facing down and the flat (bottom) side of the inlet stage facing up.	
6	Place the foam backer between the aluminum filter housings and the back side of the case. You may have to gently move the filter housings to get the foam backer in place. Make sure that it is pressed down against the foam. NOTE: The top edge of the foam backer should be roughly even with the tops of the aluminum filter housings.	
7	Gently place the OSMOS backpack into the large space provided. Make sure that the handle is swiveled upward so that it is above the foam inside of the case. The red backpack handle should be oriented toward the right. Once in place, you may have to gently press the backpack down to put it into position.	
8	Place the filter housing foam retaining piece on top of the aluminum filter housings. NOTE: This piece must be used in order to secure the filter housings in place during transport.	
9	Coil the outlet hose and place it into the opening to the right of the OSMOS backpack. CAUTION: You will notice a small green light on the top of the backpack. As you place the hose into this opening, be careful to not damage the bulb.	

STEP #	DESCRIPTION	IMAGE
10	Place the Silicone Grease Application Kit into the opening next to the outlet hose.	
11	Close the lid carefully, ensuring that nothing is sticking out of the case. NOTE: There will be a bit of resistance from the backpack harness against the case lid. Ensure that the lid is squarely closed and lines up with the latches. Begin latching with the centre latch and work your way to the sides. Secure all 5 latches.	
Packing the Tripod/Pole Case Packing the Tripod/Pole Case is far less complicated than the backpack case, as there are not multiple layers of equipment. Everything has a clearly designated space.		
1	Place the pole, tripod, and STANDARD filter housings into their pre-designated openings. The last piece that is to be placed is the tripod/pole canvas bag. It is critically important that the bag be placed into the case in the manner shown. It must be at the point where it does not go beyond the tripod thumbscrew. If this happens, the amount of material extending past the top edge of the case will be too great and the case will not close. If it is forced, the hinges could be damaged.	
2	Carefully close the 6 latches on the tripod/pole case.	

GEN-FISH
Genomic Network for Fish Identification, Stress and Health
University of Windsor and Partners

Equipment Sanitation Flowchart

Procedure developed by the Docker lab (University of Manitoba) to be completed before and after sampling and before returning OSMOS unit. All cleaning steps must be performed while wearing gloves and in a space free of active fish dissections, PCR, or DNA amplification. *Check when each step is complete. Be prepared to submit this checklist upon return of equipment.*



Any steps of this sanitation protocol that are not met must be noted in this checklist, communicated via email, and included with shipment of unit to ensure the unit is properly decontaminated prior to further use.