Supplementary file 1. Freshwater mussel mass mortality event response kit

The following tables outline the kit that is recommended for sampling an MME. Each consecutive table provides supplies and equipment needed in addition to the items in the previous table if attempting to do the indicated procedure.

SUPPLEMENTARY TABLE 1

Supplies needed for field sampling when undertaking the core procedure in a lab.

|  |  |
| --- | --- |
| **Sampling supplies** | **Notes** |
| Flotation devices as appropriate |  |
| Mask, snorkel, waterscope/bathyscope, or diving gear | Depending on the depth and environment |
| Wet suit or waders |  |
| Dive bags |  |
| Water shoes/socks |  |
| First aid kit |  |
| GPS |  |
| Marking tool | For writing numbers on shells. A sharp metal object is sufficient.  |
| Waste bags |  |
| Sharps container | For disposal of needles. |
| DO, pH, conductivity, and ammonium meters and probes along with required calibration solutions if available |  |
| Thermometer |  |
| Containers for water sample - alkalinity, hardness, ammonia, molecular | Ensure containers are appropriate for desired analyte. For example, if for molecular work make sure the containers are either sterile or washed with bleach and rinsed with molecular grade or site water. |
| Containers for sediment samples |  |
| Tool for collecting sediment samples | E.g., Ekman grab. |
| Disposable gloves | For avoiding contamination (e.g., when taking water samples) |
| Ethanol or isopropyl alcohol | Cleaning/sterilizing gloved hands and/or tools |
| Paper towels |  |
| Bleach | In addition to ethanol, for sterilization. Also, for sterilizing bottles for water collection if needed.  |
| Filters | Typically, 0.22 µm Sterivex (or similar) filters (Merck, Germany) and any prefilters if needed. |
| Large syringe | At least 50 mL, for filtering water. |
| Sealable plastic bags |  |
| Waterproof paper | For writing notes near the river. Also, could be used for labels in bags with mussels as an alternative/addition to marking mussels.  |
| Buckets |  |
| Cooler with ice and cardboard or heavy cloth | Cardboard or cloth for separating mussels from ice to avoid freezing. Ice prepared from municipal water may contain chlorine, which is toxic to mussels.  |
| Large syringe | At least 50 mL, for filtering water. |
| Camera or phone with camera |  |
| Collectors permit |  |
| Virkon or disinfectant for sampling gear |  |
| Datasheets (on waterproof paper) (Supplementary file 1), clipboard, pens, sharpies, and pencils |  |

SUPPLEMENTARY TABLE 2

Additional supplies needed for in-field non-lethal sampling

|  |  |
| --- | --- |
| **Sampling supplies** | **Notes** |
| Mussel tongs or child-sized nasal speculumStopper to hold valves open | For spreading mussel valves without causing damage. |
| Vials/cryovials | 1.5 mL microcentrifuge vials; other sizes appropriate as available/needed. Use cryovials if intending to use liquid nitrogen. |
| Storage rack/box for microcentrifuge vials |  |
| Lighter | Flame sterilization of metal tools. |
| 25-gauge needles  | If sampling haemolymph in the field. Lower gauge (larger size) needles may be appropriate, depending on mussel size. |
| 1-3 mL syringes | If sampling haemolymph in the field. |
| Disposable, sterile biopsy punctures (3 mm diameter) | Optional, if intending to take biopsies in the field. Alternatively scissors can be used. |
| Scissors | General use pair and biopsy pair. |
| Tweezers/forceps |  |
| Cooler with dry ice (or liquid nitrogen vial) | If samples cannot be taken to a -80⁰C freezer within a few hours. If using liquid nitrogen use cryovials, cryopens for labelling, and canes to immerse the cryovials. |
| Approximately 500 mL of DI or RO water | For non-lethal gonad sampling. |
| Head lamp | Can be useful when drawing hemolymph. |
| Calipers  |  |

SUPPLEMENTARY TABLE 3

Additional supplies needed to conduct the core procedure in the field.

|  |  |
| --- | --- |
| **Supplies** | **Notes** |
| Folding table  | For processing and preserving samples.  |
| Cutting board and/or large petri dishes | For dissecting mussels on. |
| Scalpel(s) |  |
| 50 mL conical tubes |  |
| Jars or whirl-pak bags for storing histological samples in | Size depends on mussel size.  |
| Histological fixative | e.g., 10% neutral buffered formalin. |
| Sturdy latex gloves | For protecting hands when using histological fixatives. |

SUPPLEMENTARY TABLE 4

Additional supplies needed in the lab to conduct the core procedure in the lab instead of the field

|  |  |
| --- | --- |
| **Supplies** | **Notes** |
| Sterile weighing trays |  |
| Scale |  |