APPENDIX

CONTENTS

ADDITIONAL INFORMATION FOR *E. coli* ASSESSMENTS

Incubators

An incubator is necessary to keep your *E. coli* plates between 33 and 37 °C. You can build your own incubator using the instructions below or purchase an incubator.

Build your own incubator

1. You will receive a Styrofoam chilly bin in your SHMAK kit. If it leaks, line it with a plastic liner.
2. Fill the chilly bin with tap water up to about 5 cm from the top. Using slightly warm water (<35 °C) will reduce the time it takes for the temperature to stabilise.
3. Cut a hole in the lid about the diameter of the aquarium heater. Position the hole near one end of the lid so there is enough space inside to float the sandwich box. But make sure the aquarium heater will not touch the side of the chilly bin.
4. Slide the heater through this hole, place the lid on the chilly bin and turn on the heater. Leave it for 6 hours or overnight for the water temperature to stabilise.
5. Before you go to the field, check that the temperature is between 33 and 37 °C. Check again just before you place your sample inside. If the incubator temperature is higher or lower than this, carefully adjust the heater up or down using the purple knob on top, and re-check the incubator after about 2 hours.

Purchase an incubator

Most poultry egg incubators are ideal for growing *E. coli* as they have an adjustable thermostat allowing you to select the appropriate temperature (~35 °C). Poultry egg incubators can be purchased from a poultry or farm supply store or on Trade Me.

Note – some poultry egg incubators incorporate an antimicrobial additive embedded within the plastic itself which prevents the growth of harmful bacteria. Before purchasing an incubator read the product specifications to ensure no additives have been used.

For more information, consult the *Petrifilm Interpretation Guide* https://multimedia.3m.com/mws/media/2362460/petrifilm-ecoli-coliform-interpretation-guide.pdf

E. coli assessment – Petrifilm

The field manual provides instructions for using MCM *E. coli* plates (Ngaio Diagnostics). However, other Select *E. coli* Count (SEC) plates are available. A common SEC plate is the 3M™ Petrifilm™ *E. coli*/ Coliform plate. As some of the steps for using this method differ slightly from the MCM plates outlined in the manual, additional instructions are provided below if you decide to use these plates.

Storage

Once opened, Petrifilm plates should be kept in the freezer and stored in a tightly sealed package to prevent condensation from building up on the plate.

Direct Plate Method

With Petrifilm *E. coli* plates, the bacteria grow on a pink gel containing the growth nutrients (with MCM *E. coli* plates, the growth nutrients are embedded in a white fabric). After adding your sample to the pink gel and rolling the top flap back down, leave the Petrifilm plate undisturbed for at least one minute to allow the gel to absorb the sample.

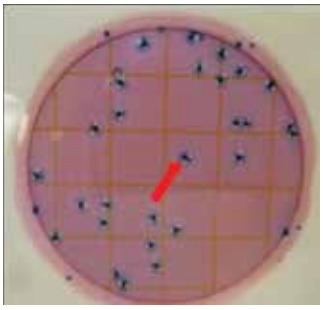
Filtering Method

The Petrifilm plate needs to be pre-wet prior to adding the filter to the plate. Pre-wet the Petrifilm gel using 1 mL of sterile water and a sterile pipette. **Leave the plate for at least 30 minutes to absorb the water.**

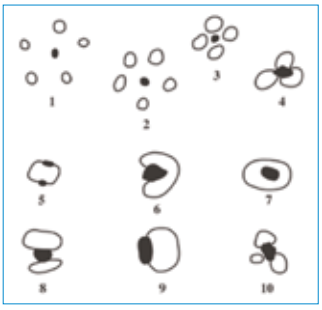
When lifting the clear film to add the filter to the plate, the pink gel will stay attached to the top flap. Place the filter paper on the plate face up. When you roll the film back down, the pink gel will cover the filter paper.

Identifying Colonies

The blue colonies with a gas bubble are confirmed *E. coli* colonies. About 95% of *E. coli* colonies produce gas. The different types of gas bubbles associated with *E. coli* colonies are illustrated in the diagram. You might also see red colonies on the gel. These are other kinds of faecal colonies. Do not count these.



A petrifilm plate is composed of a pink gel in which *E.coli* (blue) colonies and other faecal coliforms (red) can grow.



The different shapes of gas bubbles associated with *E.coli* colonies.

Fish identification and DIY equipment

The NIWA website contains information to show the distribution of all New Zealand's native fish and help predict what fish you might expect in your stream based on their habitat preferences.

http://www.niwa.co.nz/freshwater-and-estuaries/nzffd/NIWA-fish-atlas/fish-finder

http://www.niwa.co.nz/sites/default/files/where_do_fish_want_to_live.pdf

More information on spotlighting protocols can be found in the *New Zealand Freshwater Fish Sampling Protocols, Part 1 – Wadeable Rivers & Streams (Joy, David, Lake; 2013)*

Fish identification

A certain amount of skill and experience is needed to identify the adults of common fish species (e.g. īnanga, banded kōkopu, shortjaw kōkopu, giant kōkopu and kōaro; redfin bully; salmonids), especially if you wish to identify them without catching them. Juveniles are usually harder to identify than adults. To identify them to species you will need to catch them. Alternatively, identify them to genus (e.g. *Galaxias, Anguilla, Gobiomorphus*) and record them as juveniles.

Identification resources are available from the NIWA website and from the Mountains to Sea Conservation Trust's (MTSCT) Whitebait Connection programme (see links below). Some of these identification resources can be printed and taken to the field with you.

http://www.niwa.co.nz/freshwater-and-estuaries/nzffd/NIWA-fish-atlas

https://www.whitebaitconnection.co.nz/teaching-resources/teachers-and-coordinators-resources.html

Equipment

Nets. It is easier to use two nets to capture fish, one behind the fish and one in front to guide it into the net. Your kick net will make a good "guiding net" but you will want a slightly larger net to capture the fish. A long-handled "dip net" is recommended (about 300 mm in diameter, 500 mm deep, and 3-5 mm mesh size). The size of the net can be a little different from these measurements, but the mesh size must be less than 5 mm to ensure that small whitebait don't slip through. Nets can be found at most fishing and hunting stores and even butterfly catching nets will suffice. If you have a net but the mesh size is too large, you can order mesh from Sefar Filter Specialists Ltd. (Auckland) and bring it to a canvas maker.

Fish measuring tray. You need a plain small white tray with a plastic ruler glued in the bottom. You can pick up a plastic tray from any plastics shop and a 30 cm clear ruler at any stationery shop. Superglue is suitable for gluing the ruler into the tray. Measure the total length of the fish to the nearest millimetre from the tip of its snout to the longest part of the tail.

ADDITIONAL SHMAK EQUIPMENT

There are some items not included with the kit that you will need to source yourself. This includes items that you will probably already have at home or can easily purchase from hardware or homeware stores.

Clipboard and pencil	For writing on your data sheets
Bucket 2 L or larger	To collect water for the clarity tube
2 buckets (4 L or larger)	To collect fish and stones for macroinvertebrates (stone method) and to clean debris from macroinvertebrate sample
Rectangular tray with ruler	To measure fish
Batteries	Spare batteries for conductivity meter and phosphate checker
Waratah (Y-post)	To secure temperature logger in streambed
Sledge hammer	To hammer the waratah into the streambed
Wire or cable tie	To secure temperature logger to waratah
Wire cutters	To remove temperature logger from waratah
Mobile phone	For uploading monitoring data, site photos, as a stopwatch, GPS coordinates, downloading temperature logger data
Camera	To take site photos (or use a mobile phone)
GPS	To record location of sampling site or ends of sampling reach
Bottled/distilled water	To dilute your water sample (if nitrate is too high)
Phillips head screw driver	To change the batteries of the phosphate colorimeter
Lint-free cloth or tissue	To clean phosphate vials before inserting in colorimeter
Stopwatch	Necessary for the phosphate test (can use your mobile phone)
Bathyscope	Provides a larger field of view for the periphyton assessment
Marker pen	To mark gridlines on your white tray prior to sorting your macroinvertebrates. Label sample containers and *E. coli* plates
Bottle with squirt-type top	To wash invertebrates off your kick net or sieve
Isopropyl alcohol	To preserve macroinvertebrates if sorting at home
Spotlight	For your fish assessment, 30W bulb is recommended
Dip net	To collect fish (see below)
Fish measuring tray	To measure fish (see below)
Orange	To measure water velocity
Rubbish bags	To remove the rubbish during your rubbish assessment
Pick-up claw tool	To collect rubbish without using your hands
Flagging tape	To mark transects or the boundaries of an assessment area
Aluminium foil	To wrap your *E. coli* water sample and reduce light exposure
Ice packs	To keep your *E. coli* water sample cold
Jar lid	To keep the ziplock bag from pressing on the *E. coli* plate
Bleach	Decontaminate your equipment after use
Kitchen scale	To weigh rubbish
Gumboots/waders	To keep your feet dry and protected in the stream
Warm clothes	Be prepared as weather change quickly
Sun hat and sunscreen	To protect yourself from sun exposure
Water and snacks	Prevent dehydration and keep you motivated
First aid kit	Keep a first aid kit handy at all times
Hand sanitiser	To sanitise your hands until you can wash them
Insect repellent	Sandflies love moving water and you!

GLOSSARY

Accuracy – How close your measurement is to the "true" value. Accuracy can be determined by measuring a sample that has a known value, such as a standard reference sample from a lab, and comparing the measured value to the known (true) value. In the natural environment the true value is not known, so accuracy cannot be assessed.

Aquatic plant – A plant that lives mostly or entirely underwater. Includes periphyton (attached algae) and macrophytes (large aquatic plants, often with roots and leaves).

Aquifer – Underground layers of porous rock or sand through which groundwater flows. This groundwater feeds lakes and rivers. Aquifers are also an important source of well water for towns, cities, farms, and industries.

Base flow – The "normal" flow of a stream, when there is no overland runoff of rain water. Base flow is made up of water that arrives at the stream from shallow and deep underground pathways.

Bias – Where your measurement is consistently higher or lower than the true value.

Benthic macroinvertebrate – Invertebrates are animals with no backbone (e.g. insects, snails, crustaceans). Macro means large enough to be seen by the naked eye. Benthic means living on the bottom of streams and lakes.

Blank – A sample of pure water (e.g. distilled water) that you would expect to give a "zero" measurement, e.g. when testing for dissolved nutrients or bacteria. If it doesn't read zero, you know that something you are doing is contaminating your samples. A "field blank" is added to a sample bottle in the field and picks up any contamination caused by your field and lab methods. A "lab blank" is added to a sample bottle in the lab (or at home). It checks for contamination caused by your lab methods only.

Bug – can mean various things, but in SHMAK it is used as a common name for benthic macroinvertebrates.

Calibrate – adjust a meter (e.g. a conductivity meter) to read the correct value of a known "standard" solution.

Catchment – The area of land from which water flows (by surface or subsurface pathways) into a waterbody. Normally a catchment is bounded by the tops of hills, mountains or ridgelines.

Channel – The physical confines of a stream where water and sediment flow. It is made up of the stream banks and the stream bed. Some channels have water from bank to bank while others have large areas of dry sediment that are only submerged during floods.

Chute – A flow type in a stream where the passage is very narrow (e.g. between two boulders), and water is forced through at higher than normal pressure.

Current velocity – The speed that water travels along a stream channel.

E. coli (or *Escherichia coli*) – A bacterium commonly found in the intestines of warm-blooded animals (mammals and birds). It is used as an indicator of the presence of faecal pollution in freshwater.

Faecal pollution – Contamination of water with animal faeces and potentially disease-causing pathogens.

Field replicates – Two or more measurements or samples collected and analysed from the same site. Replicates can be used to check for either precision or representativeness in sampling.

Flow conditions (also known as State of flow) – How high the streamflow of your stream is today compared to normal (base flow).

Freshwater management unit – A catchment or group of catchments defined by a council to be managed together as a unit under the National Policy Statement for Freshwater Management.

Hard-bottom – See stony-bottom

Impervious – Surfaces such as rooftops, paved areas, and compacted soils where water runs off instead of filtering through. Impervious surfaces increase the rate that water and pollutants in it are delivered to streams.

Incubator – Equipment that creates a warm environment so bacteria added to an *E. coli* plate can multiply and form visible "dots" on the plate. A water bath incubator can be made by filling a chilly bin with water and inserting an aquarium heater.

Indicator – A measurable characteristic or property of fresh water. Includes physical, chemical and biological properties.

Left bank (or true left bank) – The bank of a stream on your left when you are facing downstream.

Macrophyte – An aquatic plant that grows in or near the water and is large enough to be seen by the naked eye. Macrophytes can be either emergent (rooted in the stream bed but growing out of the water), submergent (entirely underwater) or floating (leaves float on the water surface).

Microbe – see microorganism

Microcoleus (previously known as Phormidium) – A type of cyanobacteria (often called "toxic algae") that forms dark brown or black mats on river beds. It can produce toxins that have killed dogs and may cause a health risk to humans swimming (though this is not confirmed).

Microorganism – An organism that can only be seen with the help of a microscope. Often (but not always) single-celled. Common examples are viruses, bacteria, fungi, algae and protozoa.

Order – 1) a way of describing stream size (see Stream order); or 2) a group of organisms, for example mayflies, stoneflies and caddisflies are three common orders of stream insect.

Organism – A living thing, i.e. a plant, animal or microbe.

Parameter – See "indicator".

Pathogen – A bacterium, virus or other microorganism that causes disease.

Periphyton – The community of microbes (algae, cyanobacteria, bacteria and fungi) that are found attached to the surface of submerged stones, wood or aquatic vegetation. It is commonly used to mean only the algae and cyanobacteria.

Pool – Deep areas of the stream where the water flows very slowly and the water surface is smooth. The streambed in pools is often covered by deposits of fine sediment.

Precision – How similar repeated measurements are when collected by the same person. Repeated samples are often called replicates. Precision does not tell you how accurate a measurement is because you don't know the true value.

Quality assurance (QA): The overall plan, including study design, monitoring protocols, training, quality control, and data management, that promotes data quality. QA begins well before you step in the stream.

Quality control (QC): The steps that are in place to control error while you are conducting your monitoring (e.g., collecting replicates, checking field instruments). These steps ensure the monitoring results are representative of the overall condition of the sample area. QC procedures can be internal (done by members of your group) or external (done by outside professionals).

Reach – A length or section of stream that you define for a particular purpose, e.g. for monitoring particular indicators.

Reagent – A chemical or mixture of chemicals added to a water sample to test for a particular substance in the water. The reagent undergoes a chemical reaction with the substance in the water that is being tested.

Replicates – two or more measurements or samples collected and analysed from the same site. Replicates can be used to check for either precision or representativeness in sampling.

Representativeness – How well a measurement represents the overall condition of the stream. It is typically affected by where you sample in your stream. For example, water quality measurements are more representative when they are taken in the main flow of the stream.

Reproducibility – Where two different people or agencies, working independently, get similar results. Reproducibility implies (but does not quite prove) accuracy. Where accuracy cannot be assessed (e.g. most field situations), reproducibility is the next best thing.

Resolution – The ability of a method or instrument to show different levels of an indicator (e.g. small differences in temperature).

Riffle – A shallow, fast-flowing area of the stream where the water surface is broken by ripples or small waves as the water flows over a rough bed.

Right bank (or true right bank) – The bank of a stream on your right when you are facing downstream.

Riparian zone – The margin of a stream (or lake) that includes the banks and the land up to about 20 m from the water's edge. Riparian zones represent the transition between land and water habitats. They are often rich in biodiversity and have a particularly strong influence on the stream or lake. They are often regarded as part of the aquatic ecosystem, although they are above water.

Run – A reach of a stream where the water is obviously flowing but the water surface is smooth or nearly smooth. Runs are usually deeper than riffles.

Sensitivity – The ability of a method or an instrument to detect very low values.

Soft-bottom – a streambed made up mainly of sand or mud.

Species – A group of individuals having common characteristics and capable of reproducing with one another. For example, common bully and upland bully are different species.

Standard solution – A solution containing a precisely known concentration of a chemical or a substance. Standard solutions are often used to calibrate an instrument (e.g. a conductivity meter) or check the accuracy of a chemical test.

State of flow (also known as flow conditions) – How high the streamflow of your stream is today compared to normal (base flow).

Stony-bottom – a streambed made up mainly of large stones, i.e. gravels, cobbles, boulders and/or bedrock

Stormwater – Rainwater that runs off the land (usually paved or compacted surfaces in urban or suburban areas) and often routed into drainage systems in order to prevent flooding.

Stream order – A measure of stream size that is defined by where in the stream network the stream is located. First order streams occur at the top of a catchment and have no tributaries. Where two first order streams come together they form a second order stream. Where two second order streams come together they form a third order stream, and so on. However, when a second order stream is joined by a first-order stream, it remains a second order stream. In New Zealand the largest rivers are eighth-order.

Streamflow – The volume of water per unit time flowing past a point in the stream.

Sub-catchment – If a catchment is defined as the land area that drains to a particular stream, a sub-catchment is the land area that drains to one of its tributaries.

Taxon (plural taxa) – A group of related organisms that are classified together, i.e. they are thought of as a unit. "Low" taxa are grouped together to form higher taxa. For example, several species can be grouped within a genus, several genera can be grouped within a family, several families can be grouped within an order. A taxon could be an informal grouping that is not strictly scientific, e.g. "flat mayflies" in SHMAK.

Tributary – A stream that flows into a larger stream or lake. A tributary does not flow directly into the ocean.

Turbidity – A measure of the relative clarity of water. Water with high turbidity is cloudy (or turbid) while water with low turbidity is clear. Typically measured in nephelometric turbidity units (NTU).

Variable – See indicator.

Wadeable – A stream that is shallow enough to be waded across safely at normal flows. This is normally about knee-deep at moderate current speed, but could be waist-deep in very slow flow.

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