CREW CENTRE OF EXPERTISE FOR WATERS

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The James Hutton Institute

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CREW CENTRE OF EXPERTISE FOR WATERS

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Contents

Appendix I Regulatory framework controlling catchment-based FIO sources

Appendix I.1 Regulatory framework for controlling catchment-based sources of faecal pollution

River Basin Management Planning (RBMP).

The Water Environment and Water Services (Scotland) Act 2003 (as amended), aka WEWS Act, which transposes the Water Framework Directive (WFD) (2000/60/EC) to national law, requires SEPA to assess and address the pressures impacting the water quality in BWPA and SWPA. The aim is to achieve good classification status (or, if this is not possible, to reduce pressures) by 2027, as part of the plan developed under the RBMP process (SEPA 2015a; b). The plans are produced every six years by SEPA on behalf of Scottish Government (SG) and summarise: the state of the water environment; pressures affecting the water environment where it is in less than good condition; actions to protect and improve the water environment; and the objectives or outcomes following implementation. In this context, pressures posing a risk of faecal contamination to BWPA and SWPA must be detected and controlled.

Controlled Activities Regulations.

Rural diffuse pollution and direct effluent discharges to the water environment (i.e. rivers, lochs, transitional/ estuarine waters, coastal waters, groundwater, and groundwater-based wetlands) are controlled under the Water Environment (Controlled Activities) (Scotland) Regulations 2011 (as amended), also known as CAR. These regulations arose from the WEWS Act and cover a range of polluting activities including discharges to the water environment and diffuse pollution from agricultural management. SEPA issue authorisation, if appropriate for such activities. The type of authorisation required depends on the environmental risk of the proposed activity. In the context of microbiological pollution, there are three levels of control. The first level refers to the Diffuse Pollution General Binding Rules (DP GBR), which provide statutory controls over diffuse pollution from agricultural activities. The second level requires registration for activities which individually may pose a low pollution risk but cumulatively may pose a risk to the water environment, such as in the case of wastewater discharges to watercourses and to soakaways from septic tank systems (STS)[1] serving a population equivalent (p.e.)[2] of less than or equal to 15. The third level of control involves granting a licence for site-specific controls, particularly if constraints upon the activity are to be imposed, as in the case of Granted Point Sources (GraPS), which include: outflows of wastewater treatment works (WwTW); combined sewage overflows (CSO); stormtank overflows (STO); stormwater drains; emergency overflows (EO); and discharges (including those to soakaways) from STS serving more than 15 p.e.

The priority catchment approach.

The Rural Diffuse Pollution Plan (RDP Plan) was launched in 2011 and aims to help SEPA to control diffuse pollution sources and to deliver the objectives set under RBMP (DPMAG-SEPA 2017). The RDP Plan includes a "national awareness-raising campaign" and the "priority catchment approach" for catchments where tackling diffuse pollution requires a more focused intervention. The priority catchment approach takes targeted action through a sequential process of assessing pressures, raising awareness, providing advice to land managers on compliance with DP GBR and delivering guidance on options available via the Scotland Rural Development Programme (SRDP) support to improve and protect water quality, beyond compliance with regulations. All waterbody catchments draining to BWPA and SWPA, hereafter reported as BW and SW catchments, respectively, are included in the priority catchment approach (DPMAG-SEPA 2017). With respect to faecal pollution, which is the major cause of non-compliances in BWPA and SWPA, the priority catchment approach targets issues related to poaching from livestock, poorly maintained consented STS and unlicensed sewage discharges (SEPA n.d.b). However, the only tangible information on STS in Scotland are their modelled - but not yet verified – locations.

Appendix I.2 Microbiological surveys

Microbiological surveys within BW and SW catchments.

The BWPA and SWPA regulatory framework also requires undertaking microbiological surveys to identify FIO pressures and delineate the areas influencing the classification results at BWPA (i.e. bathing water profiles) and SWPA (i.e. sanitary profiles). The regulations do

1 A septic tank system (STS) consists of a septic tank and a soakaway, aka drainfield or leachfield. The effluent produced through physical settlement (primary treatment) of wastewater in the septic tank is subsequently overspilled to the soakaway for further treatment. Readers are advised to refer to an earlier CREW report by O'Keefe et al (2015), which reviewed evidence on STS design.

2 For domestic housing, a minimum of 5 p.e. is used for any house with up to and including three bedrooms.

not specify how microbiological surveys and monitoring of catchment-based FIO sources should be conducted to inform RBMP and the priority catchment approach. However, expert guidance is available to help agencies comply with the European Union (EU) regulatory framework (Appendix I.3).

Bathing water profiles

The Bathing waters (Scotland) Regulations 2008, hereafter reported as BWPA Regulations, require SEPA to establish and thereafter keep under review, a bathing water profile for every bathing water (Part 2, par. 6). The minimum contents of a Bathing Water profile (hereafter reported as BW profile) are described in Box 1. Every BW profile must be reviewed at regular intervals ranging from every two years for BWPA classified as "poor" to every four years for BWPA classified as "good". The frequency of reviews depends on BWPA water quality status, the nature and severity of the pollution impacting the BWPA, and the need for re-evaluating the management measures. In case of short-term pollution events, the profile must provide information on their frequency and duration and detail management measures for their elimination and/or control.

Box I.1. Regulatory requirements for bathing water (BW) profiles.

With respect to microbiological pollution every BW profile must

- Describe the physical, geographical and hydrological characteristics of the BWPA and of those waterbodies in its respective catchment area that could be sources of pollution to the BWPA.
- Identify the causes of potential pollution to BWPA and the public health risk.
- Identify the location of a monitoring point where most bathers are expected
- Provide information on causes, frequency and duration of short-term pollution in BWPA.
- Detail the management measures to eliminate the causes and control a short-term pollution event.

Source: Bathing waters (Scotland) Regulations 2008, Part 2, par.6.

The BWPA Regulations do not specify what monitoring techniques and strategies must be applied to identify the causes, sources, variability and duration of faecal pollution within BW catchments.

SWPA: Sanitary surveys

The Water Environment (SWPA: Environmental Objectives etc.) (Scotland) Regulations 2013, hereafter reported as SWPA Regulations, provide specifications for the setting of environmental objectives, the preparation of programmes of measures, and the content of the monitoring programmes. SWPA monitoring must, inter alia, assess risk to achieving the objectives that have been set for each SWPA. For a detailed account of national legislation on SWPA and its implementation see the CREW report produced by Akoumianaki et al (2018).

In parallel, Regulation *(EC) 854/2004* (Annex II)[3] requires competent authorities (e.g. food standard agencies) to undertake a number of tasks prior to granting a classification grade to a Shellfish Production Area (SPA). These tasks are collectively known as sanitary surveys and apply to all SPA sitting within SWPA. Sanitary surveys must account inter alia for all likely sources of faecal pollution of human and animal origin into the SPA, and their seasonal variations in the catchment area ~~and~~ in relation to rainfall, waste-water treatment and area-specific relevant factors (Box 2). In Scotland, Food Standard Scotland (FSS) monitors shellfish E. coli in each SPA sitting within a SWPA and has the responsibility for undertaking sanitary surveys.

Box I.2. Sanitary survey tasks.

A sanitary survey must:

- Make an inventory of the sources of pollution of human and animal origin likely to be a source of contamination for the production area.
- Examine the quantities of organic pollutants in relation to seasonal variations of both human and animal populations in the catchment area, rainfall, waste-water treatment and area-specific relevant factors.
- Determine the characteristics of the circulation of pollutants by virtue of current patterns, bathymetry and the tidal cycle in the production area.
- Establish a shellfish sampling programme based on the examination of established data, and with a number of samples, a geographical distribution of the sampling points and a sampling frequency which must ensure that the results of the analysis are as representative as possible for the area considered.

Source: *Regulation (EC) 854/2004,* Annex II: Chapter II, Part A: par. 6

3 This lays down the requirements for the organisation of official controls for live bivalve molluscs from classified SPA where commercial harvesting of bivalve shellfish is allowed. In Scotland, the competent authority for its implementation is Food Standard Scotland.

Appendix I.3 Guidance on undertaking bathing water profiles.

The European Environment Agency (EEA) produced a document providing guidance on undertaking BW profiles (hereafter reported as the EEA Guide) to support Member States in developing BW profiles (EEA 2009). The EEA Guide represents an informal consensus position on best practice, which is not legally binding.

The tasks prescribed in the EEA-Guide include two steps:

(i) initial surveys (field or desk-based) to gather information on locations and types of all potential FIO sources and help to identify the main FIO sources which have the potential to influence BWPA status;

(ii) a closer examination of the main FIO sources involving monitoring, statistical analyses of historical data and modelling in order to understand temporal variability of FIO delivery to a BWPA and inform further management action.

These steps are detailed below.

Steps to identify the area or zone of influence and FIO variability therein.

1. **Initial surveys** refer to initial field or desktop mapping of all FIO sources with the aim of identifying the main FIO sources, i.e. the FIO sources with the potential to influence water quality in the BWPA. Initial surveys may focus on:
    - The immediate vicinity of the BWPA, i.e. the shoreline.
    - The waterbody catchment(s) adjacent to the coastal/inland BWPA.
    - The whole river catchment from headwaters to the BWPA (i.e. BW catchment), only for situations where the initial survey indicates a risk from multiple types of diffuse pollution sources in all sub-catchments nested in the BW catchment.

2. A "closer examination" of the main FIO sources may involve one or more tasks, depending on available resources and FIO risk. These tasks are summarised below.
    - **Using published, generic figures of FIO concentrations in various types of point-sources.** For example, generic figures of FIO in different types of wastewater effluent discharges to a BWPA or in streams discharging to a BWPA can help to assess which FIO point sources may exert an influence on BWPA status. For small enclosed BWPA influenced by small stream discharges or direct effluent discharges into these streams, generic FIO figures can be introduced to simple spreadsheet models for estimating microbiological fluxes and attenuation during freshwater transport[4].
    - **Investigating FIO monitoring and/or experiments.** This type of monitoring is essential to capture all scales of temporal variability and understand which FIO sources must be controlled to improve BWPA status. Investigating FIO sources and in-stream FIO variability may require one to four years' worth of data. The EC-GW Guide does not mention explicitly where and how investigative monitoring or experiments should take place, mainly because circumstances vary by Member State and catchment. However, it is mentioned that this monitoring can take place over a range of circumstances such as dry vs wet weather, winter vs summer, and day vs night.:
        - At the outlet of waterbody catchments with rural land use.
        - At the stream/river mouth to the coastal area within or adjacent the BWPA.
        - Downstream wastewater effluent discharge points.
    - **Using modelling tools.** Accounting for dilution during FIO transport and bacterial decay from source to the BWPA is essential to assess whether a given FIO point source can really influence a BWPA. A simple approach is to account for T90, which refers to the time required for elimination of 90% of a population of microorganisms in-stream under a range of values of temperature, flow in-stream, tidal current speed, UV radiation, salinity, and stream-length or distance of a source from river mouth. Indicative T90 values for E. coli in freshwaters in temperate regions mentioned in the EC-BW Guide are based on Beaudeau et al., 2001:
        - T90 <4hours for small rivers up to 8-10 km long.
        - T90 >4h for rivers more than 10 km long and increases with stream depth and turbidity.

    For BWPA that are influenced by a complex river network, modelling can be used for estimating microbiological fluxes and attenuation during transfer from sources to a BWPA[5]. FIO transport

4 In Scotland, this may be more relevant to SWPA located in small lochs rather than to BWPA, which are often located in open coastal areas.

5 FIO transport models are not further explored because their need and requirements will be explored in CREW projects that are under development.

models are not explicitly mentioned in the EC-BW Guide. This can be partly attributed to the very few examples of models projecting FIO transport from catchment based FIO sources to the sea at the time of developing the EC-BW Guide. In addition, the EC-BW Guide emphasises assessing FIO sources in the immediate vicinity of the BWPA, whereby understanding FIO transport can be based on statistical approaches and historical monitoring data (see below). It must be also noted that the EC-BW Guide emphasises estimating FIO dilution, dispersion and decay in seawater, highlighting the use of coastal hydrodynamic modelling to account for dispersion and duration of FIO plumes in the sea.

- **Analysis of historical data.** Historical data refer to past events and circumstances pertaining to a particular BWPA. Linking historical data with routine monitoring data at BWPA can usefully contribute to assessing the influence of diffuse and point sources of FIO pollution, a prerequisite being a good understanding of locations and temporal variability of discharges from FIO sources. Linking can be explored through statistical analyses (i.e. correlation, regression, 95th-percentile calculation), or more simply, visualised with graphs. Historical data can include:
    - Meteorological data at BWPA and if needed at the BW catchment (rain, temperature and solar radiation). The EC-BW Guide provides examples of exploring the influence of heavy rains on exceedances of FIO standards at a BWPA by simply comparing BWPA FIO during dry conditions and following heavy rain6.
    - Bacteriological data related to exceedances of bathing water standards, in-stream FIO data, and content of direct discharges to BWPA.
    - Data related to man-made pressures, such as numbers of visitors per bathing season; agricultural activity; point sources.
    - Bathing water characteristics, e.g. water temperature, oceanographic data), which can inform hydrodynamic modelling.
    - Registration of complaints; which may indicate the type of FIO source posing a risk to public health.
    - Any evidence on changes in activities or processes related to discharge of FIO to the BWPA or within the catchment draining to the BWPA.

6 This approach could not be trialled in Scotland because there were not sufficient in-stream FIO data.

Appendix I.4 Guidance on undertaking sanitary surveys prior to shellfish growing.

A European Union (EU) expert working group has produced A Guide to Good Practice for the Microbiological Monitoring of Bivalve Mollusc Harvesting Areas-Technical Application authored by the European Reference Laboratory (EURL) and CEFAS, hereafter reported as the EURL-CEFAS-SW Guide (EURL-CEFAS 2017). The purpose of the Guide is to assist competent authorities in implementing scientifically based OC programs. The recommendations identify good practice in the application of the sampling plan and sanitary surveys in order to meet the requirements or intent of the *Regulation (EC) 854/2004*. The EURL-CEFAS-SW Guide specifies how to undertake: a desk study to identify pollution sources; a shoreline (field) survey to confirm the findings of the desk study; a bacteriological survey, as part of field investigations; hydrographic surveys; assessment of historical microbiological data, if any; and overall evaluation of existing information. An earlier CREW report by Akoumianaki et al. (2018) reviewed in detail the tasks prescribed in the EURL-CEFAS-SW Guide in the context of SWPA.

Table I.1 The SW Guide's recommendation for the tasks in sanitary surveys. Source: EURL-CEFAS 2017a.	
Sanitary survey task	**Description and purpose**
Desk based study to identify pollution sources	This involves: • Characterisation of the production area • Identification of actual and potential pollution sources related to: o Direct sewage discharges: continuous, rainfall dependent, emergency o Land use o Livestock Other pollution sources such as wildlife and ships and boats
A shoreline survey	This is a field investigation (visual/sampling) to confirm initial findings of the desk-based study and whether all significant sources of contamination have been revealed by the desk-based study.
A bacteriological survey	This is to explore and identify the worst-location and the worst- condition (i.e. rain or tidal stage, worst-season) to account for increased FIO risk
Analysis of historical microbiological data	Where such data is available for the species-area SPA, this analysis should supplement and not override the other elements of the sanitary survey.
Data assessment	This may involve assessment of • the effect of each FIO source to the SPA based on available data and maps • the combined shellfish contamination risk on from all faecal pollution sources • hydrodynamic modelling to predict microbial load in SPA
Report	This should describe (including maps) and interpret all data. Its major output is the microbiological sampling plan.
Data handling and storage	This refers to storing the data in a secure, well-organised and easily accessible, GIS-linked database to enable proper validation and access by all interested parties and subsequent analyses of the data.

APPENDIX II MATERIALS AND METHODS

Appendix II.1 Literature review approach

Both peer-reviewed and grey literature was reviewed. Computerised searches for peer-reviewed literature were performed using web-based search engines such as ScienceDirect (SD 2018); Google Scholar (GS n.d.); Web of Science (WoS n.d.); the legislative database of the Food Agricultural Organisation-FAO, FAOLEX (FAOLEX n.d.); and the Official Home of UK legislation (n.d.). Evidence was also extracted by searching the web sites of the organisations involved in the undertaking of bathing water profiles in bathing waters and of sanitary surveys in shellfish waters.

To answer the questions about microbiological monitoring strategies and "how often" and "where" monitoring should be carried out we searched the following terms (alone and in combination): microbial; bathing water* or bathing water protected area or swimming or recreational; "shellfish water* OR shellfish water protected area; "how often"; where; sampling or monitoring; *microbial source tracking; Escherichia coli* OR Bacter* OR microb* OR faecal indicator; "water framework directive" OR WFD; "sanitary survey* OR sanitary profil* OR bathing water profil*. The search output was screening for their relevance to the aim and objectives of the project and mainly informed the delivery of objectives 3, 4 and 5.

Techniques/technologies

To address questions on new and existing technologies for monitoring FIOs, we searched the following terms (alone and in combination): *Escherichia coli* or *E. coli* or faecal indicator organisms or FIOs; monitoring or detection or methods; technologies or sensor or low-cost sensor; surface water; microbial source tracking. The search outputs were screened for their relevance to inform objectives 1, 2 and 3. In addition, expert advice was garnered from a water research and consultancy company and a company that manufactures science equipment. We asked for advice from WRc and Trace2O.

Appendix II.2 Data used for developing the GIS-based approach

Data used for developing the GIS-based approach to help assess placed-based risk of in-stream FIO contamination are outlined in Table II.2a and Table II.2b and are detailed below.

Location and type of treatment data for all potential FIO point sources from SEPA. We used locations of public or private point sources discharging directly or indirectly via a soakaway to surface waters or groundwater. We included the following types of point sources and effluent discharged.

- Effluent discharges granted by SEPA under Controlled Activity Regulations (CAR) (hereafter reported as "granted point sources"- GraPS) such as sewage effluent from:

- waste water treatment works (WwTW), from primary to tertiary treatment
- combined sewage overflows (CSO), which discharge untreated effluent during stormflows
- emergency overflows (EO), which discharge untreated effluent regardless of flow/rain
- septic tanks serving more than 15 people, which usually discharge effluent after primary or secondary treatment

- Organic effluent from GraPS, which contains material of animal/vegetable origin from food and drink manufacture and exerts a notable Biological Oxygen Demand (BOD); it may also contain sewage.
- Other effluent from GraPS, which may be a mixture organic and inorganic effluent[1] including leachate effluent.
- Trade effluent from GraPS, which refers to any effluent produced in the course of any trade of industry and businesses and carries the untreated water straight to local rivers, lochs and the coast.
- Surface water drainage effluent from GraPS, which runoff from residential land, industrial estates, motorways and roads, mines and construction sites.
- Effluent discharges from modelled locations of domestic septic tanks serving fewer than 15 people.

For modelled locations of domestic septic tanks, and modelled locations of septic tanks serving fewer than 15 people, which are simply registered we used:

- Distance from watercourse (i.e. streams, rivers, lochs/lakes and the coastline adjacent to BWPA and SWPA).
- Soil runoff risk based on the runoff risk map of Scotland (partial cover) by Lilly and Bagaley (2018a). This map was included in the analyses because of uncertainties regarding the state and location of drainfields. For example, locating a drainfield in soils prone to surface runoff compromises the hydraulic performance of the drainfield (e.g. Withers et al 2014), thus increasing the risk of FIO discharges to watercourses following storm events.
  - Low runoff risk refers to soils that can store large volumes of water or can allow water to quickly infiltrate and so surface runoff is limited.
  - Moderate runoff risk refers to soils that have a moderate capacity to store rainfall or to allow water to infiltrate; these soils will reach saturation under some circumstances, leading to runoff.
  - High runoff risk refers to soils that have a limited capacity to store rainfall or to allow water to infiltrate. The soil will quickly saturate, leading to rapid runoff.
- Soil leaching potential based on the risk map depicting soil permeability, and thus risk of groundwater FIO contamination, produced by Lilly and Bagaley 2018b. We included this map in our analyses because in areas with high soil leaching potential FIO in drainfields may migrate downward through the vadose zone (i.e. shallow, unsaturated groundwater) and into groundwater, and eventually discharge into surface waters through the stream bed or seabed when the water table is elevated.
  - Low soil leaching potential refers to soils in which potential pollutants are unlikely to move down through the soil due to low permeability.
  - Intermediate soil leaching potential refers to soils with a moderate ability to retain potential pollutants and which allow some pollutants and liquids to move through the soil. For example, deep, permeable, medium textured soils with high topsoil organic matter contents can possibly transmit non - or weakly - adsorbed pollutants and liquid discharges, such as septic tank effluent to drainfields.
  - High soil leaching potential refers to soils with very little ability to retain potential pollutants and which allow pollutants and liquids to move rapidly down through them to underlying groundwater.
- Density i.e. number of septic tanks within a square kilometre. A common finding in the literature is that areas with high septic tank density are most susceptible to groundwater and surface water contamination from septic system FIO discharges (Lusk et al 2017 and literature cited therein). The US Environment Protection Agency (EPA) defines high density as a density greater than 40 septic systems per square mile (Katz et al., 2010; Borchardt et al., 2003 cited in Lusk et al 2017). We calculated density per square kilometre:
  - Low density refers to a density of septic tank systems up to 4 / km^2.
  - Intermediate density refers to a density of septic tank systems in the range of 5-19 / km^2.
  - High density to a density of septic tank systems equal to or higher than 20 / km^2.

We also used GIS spatial data

- Waterbody catchment boundaries in relation to BWPA and SWPA.
- Land use data from LCM-2007 maps.
- Output of SCIMAP-FIO for livestock. This helped

1 Inorganic effluent does not exert a notable BOD, and, therefore, it was excluded from this exercise.

to assess areas at risk from high in-stream FIO due to agricultural land use. We included the output for Channel Erosion Accumulation risk which shows where FIO from livestock accumulate in-stream faster than the stream water flow can dilute them.

- Wildlife. It must be noted that little evidence is readily available.
- OS river network and shorelines

Appendix II-Table II.2a Summary of data used for developing a GIS-based approach to help assess place-based risk of in-stream FIO contamination and thereafter prioritise monitoring towards sites posing greatest risk of FIO delivery from catchment to BWPA and SWPA. For explanation of the regulatory framework see Section 3.2.

Type of data	Description - Use	Justification	Source
Point sources	Locations of Granted Point Sources (GraPS) • waste water treatment works (WwTW) providing primary, secondary or tertiary treatment and discharging domestic/ organic / other / trade effluent. • combined sewage overflows (CSO), • emergency overflows (EO), • septic tank systems (STS) serving more than 15 people, providing primary to tertiary treatment and discharging domestic/organic / other / trade effluent.	Sources of FIO	SEPA
	Modelled locations domestic septic tank systems (STS) serving < 15 people		
Distance of domestic septic tanks from watercourses	We included four distance classes: Near: 0-10m; 10-50m Far: 50 -100m; >100m	Proxy of FIO contamination risk of streams, lakes and coastal waters from domestic septic tanks through runoff and leaching	Calculated (see Appendix II.3)
Soil runoff risk	• Low runoff risk when surface runoff is limited. • Moderate runoff risk when soils reach saturation under some circumstances, leading to runoff. • High runoff risk when soils quickly saturate, leading to rapid runoff.	Proxy of where FIO from domestic septic tanks can contaminate streams and coasts through runoff	Scotland's soils (2018)
Soil leaching potential	• Low soil leaching potential in soils characterised by low permeability. • Intermediate soil leaching potential when soils allow some pollutants and liquids to move through. • High soil leaching potential when soils allow pollutants and liquids to move rapidly down	Proxy of where FIO from domestic septic tanks can contaminate groundwater	Scotland's soils (2018)
Density of septic tanks within a square kilometre	We included the following classes: • Low density: 4 domestic septic tanks per km^2 • Intermediate density: 5-20 domestic septic tanks per km^2 • High density: >20 domestic septic tanks per km^2	Proxy of where FIO loading in an area poses a FIO contamination risk	Calculated (see Appendix II.3)
Output of SCIMAP-FIO for livestock	We included the output for *Channel erosion accumulation risk* which shows where FIO from livestock accumulate in-stream faster than the stream water flow can dilute them.	Proxy of sites with high in-stream FIO from diffuse agricultural pollution	SCIMAP
Land use data	We used Broad Habitats from LCM-2007 maps.	% of LU-LC at a waterbody scale is a proxy of FIO exported from a catchment (Kay et al 2008a).	CEH

Appendix II-Table II.2b Ordinary Survey-OS and catchment and protected area boundary data.		
Data description	**Use**	**Source**
OS river network and shorelines	Reflects the river network which acts as a corridor delivering FIO from catchment based-sources to the coast	Open access
Baseline waterbody catchments	Reflects and integrates point and diffuse pollution sources land use. • Coastal waterbody catchments refer to baseline waterbody catchments adjacent to the shoreline and to the receiving BWPA or SWPA • Upstream waterbody catchments refer to baseline waterbody catchments upstream of coastal catchment	SEPA
BW catchment boundaries	Reflects and integrates land use and management issues potentially influencing BWPA	SEPA
SW catchment boundaries	Reflects and integrates land use and management issues potentially influencing BWPA	SEPA
BWPA boundary	Shows where regulatory monitoring to assess compliance with bathing water standards take place.	SEPA
SWPA boundary	Shows where water quality should be protected to enable shellfish harvesting	SEPA
SWPA monitoring point	Shows where within a shellfish production area there is the highest risk of shellfish E.coli contamination	FSS
Areas designated for birds		Open access

Appendix II.3 Description of GIS-based method

Assessing place-based risk

The following steps were undertaken to develop the GIS-based approach to assess place-based FIO risk:

1. We used readily available datasets and modelled data (see Appendix II.2) and combined selected data layers to assess risk of in-stream FIO contamination based on literature review findings (Section 3.1). SEPA provided spatial data on GraPS and the modelled locations of septic tank systems for 11 BWPA and 5 SWPA (hereafter reported as trial catchments) to support the development of the GIS-based approach. In consultation with SEPA and because of limited resources we focused on one BW catchment (Nairn) and one SW catchment (Loch Ryan).
2. For the risk from GraPS we considered two risk indicators: Location in relation to BWPA and SWPA, and type of treatment.
    - Location: GraPS located in a baseline waterbody catchment immediately adjacent to the BWPA or the SWPA (i.e. coastal waterbody) assumed to be posing a higher risk to BWPA or SWPA than GraPS located in waterbody catchments further upstream.
    - Type of wastewater treatment: effluent from untreated, primary or secondary GraPS was assumed to be posing a higher risk to BWPA or SWPA than effluent from tertiary treatment.
    - Decision on place-based risk to prioritise FIO monitoring at sites where:
        - **Highest priority:** GraPS discharging continuously or intermittently effluent that is untreated, or after primary or secondary treatment and are located within coastal baseline waterbodies.
        - **Intermediate priority:** GraPS discharging continuously or intermittently effluent that is untreated, or after primary or secondary treatment and are located within waterbodies upstream of coastal waterbodies.
        - **Low priority:** GraPS discharging effluent after tertiary treatment in baseline waterbodies upstream of coastal waterbody catchments.
3. For the risk from septic tanks systems, we considered the following risk indicators: soil leaching, runoff, distance of modelled location of septic tanks from watercourses and shoreline and density per square kilometre. Details are provided in Appendix II.2.
    - Decision on place-based risk to prioritise FIO monitoring at sites where:
        - **Highest priority:** Septic tanks are located near the watercourse or shoreline on soils high/intermediate leaching potential and high/intermediate runoff potential and belong to clusters of more than 20 septic tanks per square kilometre.
        - **Intermediate priority:** septic tanks located within 10m of watercourses or the shoreline, and septic tanks within 50m of soils with high or intermediate leaching potential and high runoff risk.
        - **Low priority:** septic tanks located at distances >50m from watercourses and the shoreline, not belonging to clusters of >20 septic tanks

/km2 and not located on soils with high leaching potential and high runoff risk.

4. For risk from pet-related sources of FIO we looked at locations of built-up areas in relation to the river network and the BWPA, assuming that built-up areas adjacent to the river network and the shoreline pose a higher risk than those located further away.
5. For risk from wildlife, we could not identify any reliable risk indicators. Apart from location of designated areas for birds and the type of soils in these areas.
    - **High priority**: Designated areas containing soils with high/intermediate SLP or RR located in coastal baseline waterbodies.

GIS analyses - Data collation steps

1. Dissolve WaterCourseLink on 'fictitious' fields to get crom_streams_diss (15 individual 'reaches')
2. Buffer reaches to 50m, round end, no dissolve - crom_streams_50m
3. Use Spatial Join with crom_streams_50m and septic tank file to get count of septics within 50m of each reach. Alias JOIN_COUNT field as 'septics' - crom_streams_50m_septics
   See https://support.esri.com/en/technical-article/000008599
4. For SCIMAP outputs, the outputs do not follow the 'real' stream network, rather the modelled one. Create new set of buffers @ 500m distance, with the flat option - crom_streams_500m_for_SCIMAP
5. Spatial Join between crom_streams_500m_for_SCIMAP and SCIMAP_FIO, this time using JOIN_ONE_TO_MANY and INTERSECT options - crom_streams_SCIMAP. This produces a file where each SCIMAP risk point is appended to the 500m buffer around each reach. Export this file to Excel and work out average of risk points in order to get an overall 'class' for the reach.
6. Spatial Join the Granted point sources shapefile, as above for septics - crom_streams_50m_septics_GP
7. Spatial Join the coast_500m file to get reaches close to the coast. Use ONE_TO_ONE and INTERSECT for the join type - crom_streams_50m_septics_GP_coast
8. INTERSECT the leaching risk file. Export as with the SCIMAP data, summarise in Excel, work out the dominant leaching class in the reach.
9. INTERSECT the crom_streams_50m_septics_GP_coast file with the Cromarty urban_areas file. Export the table to Excel and work out the total area of each reach covered by urban areas.
10. Import all the Excel tables into Arc and join to the crom_streams_50m_septics_GP_coast file using the ORIG_ID field; create a calculated field to work out the area of urban as a % of each reach area.
11. Export the whole dataset to a new one to 'fix' the joins permanently
12. We now have an attribute table with a row for each 'reach' plus the following:
    - no of septic tanks within 50m
    - no of granted point sources within 50m (and what type it is)
    - avg SCIMAP-FIO risk rating in a 500m buffer around the reach
    - the percentage urban area within 50m
    - whether the reach is within 500m of the coast
    - the dominant soil leaching risk potential in the 50m buffer around the reach

Additional GIS analyses

13. Septic tank density.
    - Create a 50m fishnet for the catchment
    - Add a column to the septic file called Count and populate it with a value of 1 for each point
    - Use Point to Raster to convert the septic tank locations to a raster. Choose 50m cell size, COUNT as the cell assignment type, and set the fishnet as the processing extent in environments. This gives a 50m raster with the number of tanks in each cell as the value field
    - Use Spatial Analyst > Neighborhood > Focal Statistics with a 20 x 20 rectangle neighborhood (so everything with 1km of the cell), and SUM as the statistics type (and check ignore no data)
    - Convert this raster to a polygon (simplify polygons)
    - Add a column to the shapefile table and calculate a class that corresponds to each of the 3 classes: <5, 5 - 20 and >20.
    - Dissolve the polygons based on this class, then symbolise according to High/Med/Low
14. Septic tank proximity to watercourses
    - Spatial Join baseline_WBs with septic tank locations for each range band (<10, 10-50, 50-100, >100m). Make sure to use ONE_TO_MANY join type
    - Export to Excel, then pivot to count no occurring

15. Septic tanks on soils with high leaching potential/ runoff
    - Spatial Join septic tank locations with SLP/runoff layer
    - Spatial Join baseline_WBs with septic tank_SLP/ runoff layer. Make sure to use ONE_TO_MANY join type
    - Export to Excel, then pivot to count no occurring
16. Land Cover
    - See Appendix II in Akoumianaki et al. 2018.
17. Granted point sources
    - filter out anything that isn't an effluent from GraPS layer (e.g. cooling water)
    - Spatial join baseline_WBs with GraPS layer. Make sure to use ONE_TO_MANY join type
    - Export to Excel; list each source rather than counting all occurrences
18. Length of rivers
    - Spatial Join baseline_WBs with OS_Mastermap layer
    - export to Excel, pivot and sum lengths
19. Distance to sea
    - Dissolve OS mastermap Water layer on permanence field to get Nairn_OSWater_diss
    - Edit to extend the lowest part of the network so it reaches the river 'mouth'; add a point
    - Intersect this layer with baseline catchment layer - this produces a multipoint file with a number of exraneous points. See here for how to convert to a simple point file: https://support.esri.com/en/technical-article/000007983
    - Edit out all the unneeded point to give a rough approximation of where the outflow of each catchment is (need to manually remove a number of points where the river and catchment boundaries have crossed because the boundaries were generated from a DEM).
    - With the 'clean' file of points, clip the river network to break it into 'reaches'
    - Sum the length of each reach (cumulative) to get distance of each catchment outflow to the mouth

Appendix III Generic FIO export coefficient per land use/land cover type.

Table III.1 FIO export coefficients (cfu/km2/h) per land use under base-flow and high-flow conditions based on data from 205 sub-catchments in the UK studied by Kay et al (2008a). Base flow refers to dry weather conditions and runoff ranging from 2.43 to 196 m3 / km2 of catchment / hour, with higher values in winter months; high flow refers to rainfall-response flow and runoff ranging from 7.90 to 1070 m3 / km2 of catchment / hour, with higher values in summer months.
IP: Improved pasture; NG=Rough grazing; WL: Woodland; BU: built-up areas; GM: Geometric mean

Land use	FIO group	Base flow (GM)	High flow (GM)
≥75% IG	Total coliforms	2.9×10^{9}	2.7×10^{11}
	Faecal coliforms	8.3×10^{8}	1.2×10^{11}
	Enterococci	9.6×10^{7}	2.2×10^{10}
≥75% RG	Total coliforms	7.1×10^{8}	5.3×10^{10}
	Faecal coliforms	2.5×10^{8}	2.5×10^{10}
	Enterococci	3.3×10^{7}	3.6×10^{9}
≥75% WL	Total coliforms	3.1×10^{8}	1.4×10^{10}
	Faecal coliforms	2.0×10^{7}	3.3×10^{9}
	Enterococci	8.5×10^{6}	3.8×10^{8}
<2.5% BU (Rural)	Total coliforms	9.3×10^{8}	6.1×10^{10}
	Faecal coliforms	4.2×10^{8}	2.6×10^{10}
	Enterococci	4.9×10^{7}	4.7×10^{9}
2.5<BU<9.9%(Semi urban)	Total coliforms	4.2×10^{9}	1.5×10^{11}
	Faecal coliforms	1.2×10^{9}	4.6×10^{10}
	Enterococci	1.5×10^{8}	1.1×10^{9}
≥10% BU (Urban)	Total coliforms	8.5×10^{9}	4.1×10^{11}
	Faecal coliforms	2.8×10^{9}	1.3×10^{11}
	Enterococci	4.0×10^{8}	2.7×10^{10}

Appendix IV Review of monitoring technologies and techniques

Table IV.1A Phase 1: Catchment surveys to identify area of influence and main FIO sources. The majority of the technologies can be used for routine FIO monitoring.

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Membrane filtration techniques	Current technology. Lab-based. Standard methods for isolation and enumeration of coliform bacteria, E. coli, and E. coli O157 in the lab.	Library independent	Easy	Yes	In-stream Bathing Shellfish Drinking Research	Yes	Lab: filtration equipment, autoclave, media, laminar flow cabinet, class II safety cabinet; consumables. Field: standard sampling equipment.	24-48h	Low/ not for use in the field	High - requires basic microbiology lab with Class II cabinet	~ £2.50	Generally reliable as it is a standard method. High sensitivity as only viable cells will grow. Little interference if using selective media.	1, 2
Coliscan Easygel® system	Current technology. Lab-based. Measures E. coli and coliforms through β-d-glucuronidase activity. Add a test sample to the medium, pour it into a petri dish and incubate it at room temperature or at a higher controlled temperature (35°C is suggested).	Library independent	Easy	No	In-stream Bathing Shellfish Drinking Research	Yes – purchase from the US	Lab: incubator (but can grow at room temp) Field: standard sampling equipment.	18-24h if incubating. 48h if leaving at room temp. >48h if sampling remote areas.	Low/ not for use in the field	Low	$2.10	Evidence of false negatives (Fricker et al., 2008). Interference low as only viable cells will grow.	1, 2

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Colilert	Current technology. Lab-based. MPN of *E. coli* and total coliforms through β-d-glucuronidase activity using UV induced fluorescence from chromogenic substrate Indoxyl-β-glucuronide (IBDG) for *E. coli* detection, and colour change due to enzymatic cleavage of the fluorogenic substrate, 4-methylumbelliferyl-β-galactopyranoside (MUG) to detect total coliforms.	Library independent	Easy	No	In-stream Bathing Shellfish Drinking Research	Yes	Lab: tray sealer, incubator, consumables. Field: standard sampling equipment.	18-22h	Low/ not for use in the field	£4000 for the tray sealer. Other costs high, requires basic microbiology lab.	£4.36	Evidence of false negatives (Fricker et al., 2008). Evidence of false positives occurring with Aeromonas. Regulators e.g. SW don't use this anymore due to issues with reliability. Interference low as only viable cells will grow.	1, 2
MI Agar	Current technology. Lab-based. Colony counts of *E. coli, Enterobacter* and total bacteria under both ambient and UV light (requires both lights for positive ID). Based on β-d-glucuronidase activity.	Library independent	Easy	No	In-stream Bathing Shellfish Drinking Research	Yes – purchase from the US	Lab: hotplate stirrer, autoclave, incubator, filtration unit, laminar flow cabinet, class II safety cabinet, consumables. Field: standard sampling equipment.	20-24h.	Low/ not for use in the field	High - requires basic microbiology lab with Class II cabinet	£18.80	Complies with USEPA methods, but evidence of false negatives (Fricker et al., 2008). Interference low as only viable cells will grow.	1, 2

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Chromocult Coliform Agar	Current technology. Lab-based. Colony counts of *E. coli* and coliform bacteria based on β-d-glucuronidase activity.	Library independent	Easy	Yes	In-stream Bathing Shellfish Drinking Research	Yes	Lab: autoclave, incubator, filtration unit, laminar flow cabinet, class II safety cabinet, consumables. Field: standard sampling equipment.	23-25h	Low/ not for use in the field	High - requires basic microbiology lab with Class II cabinet	£3.50	Evidence of false negatives (Fricker et al., 2008). Interference low as only viable cells will grow.	1, 2
Membrane lactose glucuronide agar (MLGA)	Current technology. Lab-based. Colony counts of *E. coli* and coliform bacteria based on β-d-glucuronidase activity.	Library independent	Easy	Yes	In-stream Bathing Shellfish Drinking Research	Yes	Lab: autoclave, incubator, filtration unit, laminar flow cabinet, class II safety cabinet, consumables. Field: standard sampling equipment.	18-24h	Low/ not for use in the field	High - requires basic microbiology lab with Class II cabinet	£6.70	Evidence of false negatives (Fricker et al., 2008). Interference low as only viable cells will grow.	1, 2
AquaFlex (by Trace2O)	Current technology. Field-based. A mobile lab that tests for *E. coli, Enterobacteriaceae* and total coliforms as well as staphylococci, yeasts, moulds, *Pseudomonas,* lactobacilli, *Listeria,* and lactic acid bacteria via plate counts.	Library independent	Easy	No	In-stream Bathing Shellfish Drinking Research	Yes	It includes filtration unit, media, consumables, incubator and disinfection. Possibly requires standard sampling equipment.	24h	Low/ need access to a power supply	£1395 for the lab	£4.65	Some evidence of false negatives associated with the use of membrane lauryl sulphate broth (Dufour et al., 1981), which is supplied with the lab. Interference low as only viable cells will grow.	1, 2

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Compartment bag Test (by Aquagenx)	Current technology. Field-based. A mobile lab that tests for *E. coli* through MPN by colour-match test. For use in low resource, rural or disaster areas.	Library independent	Easy	No	In-stream Bathing Shellfish Drinking Research	Yes	Possibly requires standard sampling equipment.	24-48h (depends on incubation temperature)	Low/ easy to carry out in the field	None	$10	Reported sensitivity and specificity at 94.9% and 96.6% respectively (Stauber et al., 2014). Interference low as only viable cells will grow.	1, 2
Colitag (by Palintest)	Current technology. Field-based. A mobile lab that tests for *E. coli* and total coliforms by presence/absence or by MPN.	Library independent	Easy	No	In-stream Bathing Shellfish Drinking Research	Yes	None – includes incubator, sampling vessels, MPN plates, sealing tool, comparator and UV lamp.	16-48h	Low/ easy to carry out in the field	£2262 for the lab	Cost of refill pack	USEPA approved method, but no info on specificity or sensitivity. Interference low as only viable cells will grow.	1, 2
aquaCHECK365 (Brightwater Diagnostics) (same as Aquatest by the University of Bristol)	Emerging technology. Field-based. Measures *E. coli*, enterococci and other bacteria via MPN. Contains a standard sample collection container, a diagnostic lid and a system unit that incorporates a UV reader to provide clear results to a non-technical user. Emerging technology.	Library independent	Easy	No	In-stream Bathing Shellfish Drinking	Yes – comes to market in 2019	Lab: None Field: standard sampling equipment.	18-24h	Low/ no info on logistics in the field	£605 for the device	£8-10 depending on media	No info on specificity. Interference low as only viable cells will grow. Can detect a single bacterium in 100 ml sample. Has a range of 0-920 CFU/100 ml. Lab trials show that results are comparative to ISO standard methods.	1, 2

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Temperature sensing	Current technology. Field-based. Water temperature measurements can identify point sources of relatively cooler or warmer water into a stream, although the use of temperature is likely limited to only the most major of temperature differences (17). Need to test upstream and downstream of a point source.	Library independent	Easy	No	In-stream	Yes	None	>1h	Low/ easy to use in the field.	Up to £100 for probe	None	Low sensitivity as it will only detect major temperature differences (Hyer, 2007). Interference may come from daily and seasonal temperature changes.	1, 2
Conductivity	Current technology. Field-based. Identifies point sources of wastewater with relatively higher conductivity into a stream. Need to test upstream and downstream of a point source.	Library independent	Easy	No	In-stream	Yes	None	>1h	Low/ easy to use in the field	Up to £200 for probe	None	Found to correlate with FIOs (Nnane et al., 2011). Interference from meterological conditions (Nnane et al., 2011).	1, 2

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Turbidity	Current technology. Field-based. Identifies sources of (waste) water with relatively higher turbidity into a stream.	Library independent	Easy	No	In-stream Drinking Research	Yes	None	>1h	Low/ easy to use in the field	Up to £400 for probe	None	Some studies show significant correlation with FIOs and is suggested as a good surrogate for FIOs (e.g. Nnane et al., 2011). However, SEPA's unpublished data show lack of significant correlation	1, 2
Bacti-Wader (Chelsea Technologies Group Ltd)*	Emerging technology. Field-based. Detects UV fluorescence emitted within the tryptophan emission wavelengths.	Library independent	Easy	No (or minimal)	In-stream Bathing Shellfish Drinking Research	Yes	None	?	Low/Issues with logistics in the field (S. Campbell, personal communication).	? Cost of device	None	correlation with FIOs in dilutions of river water and sewage (R 2=0.72-0.81). Issues with sensitivity and interference (S. Campbell, personal communication)	1, 2
MWK 1.0 lab testing kit (by Glacierclean Technologies Inc)	Emerging technology. Field-based. Apply sample to a syringe with membrane filter – filter changes colour if *E. coli* is present (i.e. base on enzymatic reaction). Results (qualitative) can be transmitted using mobile phone app.	Library independent	Easy	No	In-stream Bathing Shellfish Drinking	Yes – purchase from Canada via material transfer agreement	Lab: None Field: standard sampling equipment	5 min - 1h (depends on E. coli concentration)	Low/ easy to use in the field.	None	$20 CAD	Detection limit: 400 CFU/ml (Gunda et al., 2016). No info on specificity or interference.	1

* Potential to progress to routine monitoring with more research.

Table IV.1B Phase 2: Monitoring variability of FIO within catchments

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)Emerging technology	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Faecal sterol, acid bile markers	Current technology. Lab-based. GC-MS/LCMS based determination of source- specific steroid compounds. Indicator of human faecal pollution; cumulative load of faecal pollution; source differentiation.	Library-independent	Difficult	Yes	In-stream Bathing Shellfish Drinking Research	Bespoke analysis available	Lab: rotary evaporator, nitrogen gas stream, filtration and extraction equipment GC-MS or LC-MS. Field: standard sampling equipment	Several days	Med-high/ not for use in the field.	None if analyses outsourced; otherwise cost of GC-MS/LC-MS.	~ £100*	Individual compounds not reliable. Ratios much more reliable but can change depending on extrinsic factors. Seem to agree with FIOs. May be affected by temperature.	1, 2, 3

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)Emerging technology	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Chemical indicators	Current technology. Lab-based. Chemical indicators (nitrogen, caffeine, anionic surfactant, fluoride, and fluorescence whitening agent) measured as proxies for human waste. Particulate nitrogen measured using elemental analyser. Caffeine analysed by GC/ MS. Fluorescence whitening agent measured by fluorescence spectrophotometer. Other measurements according to: APHA. Indicator of human waste pollution.	Library-independent	Difficult	Yes	Research	Yes	Lab: instrumentation – see description. Field: standard sampling equipment	24 - >48h	Low/ not for use in the field.	High	No info	Low sensitivity as dilution effects make it difficult to detect these chemicals. High specificity. No info on interference.	1, 2
Ammonia	Current technology. Lab or field- based. Ammonia can be measured using cuvette tests in the lab (e.g. Hach). Ammonia tests are also available as test strips that can be used in the field. Indicator of both human (20) and animal waste pollution (21).	Library-independent	Easy	No	In-stream Bathing Shellfish Drinking Research	Yes	Lab: instrumentation. Field: standard sampling equipment or test strips.	?	Low/ test strips easy to use in the field	High if doing cuvette test. Low if using test strips.	£3.27 for cuvette test. £0.53 for test strips.	Highly sensitive, but tests can vary. High specificity. No info on interference.	1, 2

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)Emerging technology	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Paper-Origami DNA microfluidics	Emerging technology. Lab-based. Detects FIOs using paper microfluidic device and fluorescence.	Library-independent	Med-Difficult	Yes	Research	Yes (but device needs to be made)	Lab: filtration unit, bead beater, centrifuge, vortex, reagents and consumables, microfluidic device, UV torch. Field: standard sampling equipment.	<24h	Low/ not been tested in the field	High - requires basic microbiology lab with Class II cabinet	£7.36 DNA extraction kit; No info for isothermal amplification kit.	Sensitivity and specificity reported to be high (Yang et al., 2018), but needs to be tested on FIOs. No info on interference. Detection: <1pg (115-274 copies of genomic DNA).	1, 2
RNA biosensor 1	Emerging technology. Lab-based. DNA probe-coated magnetic beads in combination with the electrochemical monitoring of the oxidation state of guanine nucleotides allows direct detection of bacterial RNA. Could be used on RNA extracts or cell extracts.	Library-independent	Med-Difficult	Yes	Drinking Research	No (Probe would need to be made)	If used in the lab: filtration unit, bead beater, centrifuge, vortex (to extract RNA), reagents and consumables. Field: standard sampling equipment.	4h using RNA.	Low/ could be used in the field but requires development	Low if used in the field. High if used in the lab as requires basic molecular biology lab.	£4.58 - RNA extraction	Shows high specificity (LaGier et al., 2005). No info on sensitivity. No info on interference.	1, 2

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)Emerging technology	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
RNA biosensor 2	Emerging technology. Lab-based. The biosensor is a membrane-based DNA/RNA hybridization system using liposome amplification.	Library-independent	Med - Difficult	Yes	Drinking Research	No (probe would need to be made)	Lab: filtration unit, bead beater, centrifuge, vortex, reagents and consumables. Field: standard sampling equipment.	8h (but probe only takes 10-15min)	Low/ could be used in the field but requires development	Low if used in the field. High if used in the lab.	£6.17 - RNA extraction; No info for RNA amplification	Reported to be highly sensitive and specific (Baeumner et al., 2003). However, requires further validation with field samples. No info on interference. Detection limit: 40 E. coli cfu/ ml (Baeumner et al., 2003).	1,2
Flow cytometry and FACS (fluorescence activated cell sorting)	Emerging technology (old technology used in a novel way). Lab-based. Usually in lab but new developments of online FCS. Light scatter and fluorescence from single cells, fluorescence based sorting. Modules can be added to specifically detect FIOs.	Library-independent	Med-difficult	Yes	In-stream Bathing Shellfish Drinking Research	Yes	Lab: Flow Cytometer; FACS machine. Field: standard sampling equipment.	1.5h	Low-med for FCS; med-high for FACS/ not for use in the field.	High (cost of FCS/FACS) - ~ £50K FCS and £100K for FACS	?	Depends on application and matrix. Discrepancies in viable counts FCS vs. culture. Interference from particulates in turbid samples (gives false positives). SW are aiming to get this method accredited for TVCs.	1, 2, 3

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)Emerging technology	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
DNA-based methods - QPCR	Current technology. Lab-based. QPCR of biomarkers (e.g. *Bacteroides*, mitochondria, viruses).	Library-independent	Difficult - – labour intensive and time consuming	Yes	Research	Yes (but need to develop the assay)	Lab: filtration unit, bead beater, centrifuge, vortex, Qubit/ nanodrop, QPCR plates, QPCR machine, water bath, reagents and consumables. Field: standard sampling equipment.	24->48h	Med/not for use in the field	High - requires basic molecular biology lab	£7.36 DNA extraction kit; £0.37 Qubit; £2.49 mastermix. Also requires cloning kit.	Assays need validation. Sensitivity and specificity varies according to assay (Harwood et al., 2014). Interference from PCR inhibitors. Assay will detect both live and dead cells. Detection: usually 102 gene copies (Pagaling, personal comm).	1, 2, 3
DNA-based methods - microarray	Emerging technology. Lab-based. Custom microarray targeting pathogens (viruses, bacteria, protozoa), microbial source tracking (MST) markers, and antibiotic resistance genes.	Library-independent	Difficult – requires facility to make the microarray and then read results	Yes	Research	Yes (but need to develop and make the microarray)	Lab: filtration unit, bead beater, centrifuge, vortex, Qubit/ nanodrop, reagents and consumables, custom microarray, microarray analysis. Field: standard sampling equipment.	24->48h	Med/ not for use in the field	High - requires basic molecular biology lab	£7.36 DNA extraction kit; £0.37 Qubit; £6.12 whole genome amplification kit.	Sensitivity is 21-33% (requires high concentrations of DNA) and specificity is 83-90% (Li et al., 2015). Interference from PCR inhibitors. Assay will detect both live and dead cells.	1, 2, 3

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)Emerging technology	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Bactiquant Water (by mycometer)	Emerging technology. Lab or field-based. Measures total bacterial activity (including planktonic bacteria, particle associated bacteria, anaerobes as well as aerobes), so it does not distinguish between FIOs and other bacteria. Water samples are filtered through a 0.22 µm filter which is saturated with a surplus of enzyme substrate, to release a fluorescent compound.	Library-independent	Easy	No	Bathing Drinking	Yes – purchase from the US	Lab: None. Field: standard sampling equipment	<1h	Low/ easy to use in the field	? (cost of device)	None	USEPA verified and reported to be highly reproducible (see website). Technology is specific for a number of bacteria covering all major taxonomic groups (see website). It is sensitive enough that it is comparable with traditional cultivation-based methods (see website).	1, 2
BactoSense	Emerging technology. Continous monitoring. In-line automated flow cytometer – detects light scatter to measure total microbial cell count or intact cell count; web interface for viewing results remotely.	Library-independent	Medium	Yes	Drinking	Yes	None	20 min	Low/ no info on logistics in the field	? Cost of device	? Cost of refill cartridges	Not yet sufficient for real time monitoring of specific targets i.e. FIOs. Sensitivity: $1x10^3$ – $1x10^6$ cells/ml. Detection limit: $1x10^2$ cells/ml. No info on specificity or interference, but presumably affected by turbidity.	1, 2

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)Emerging technology	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
BACTcontrol (MicroLAN)	Emerging technology. Continuous monitoring. Measures the specific enzymatic activities of β-galactosidase (coliforms), β-glucuronidase (*E. coli*) and alkaline phosphatase (total activity, biomass).	Library-independent	Difficult	Yes	Drinking	Yes – purchase from the Netherlands	None	1-2h	Low/ instrument compact**	High (cost of installation and training)	? Cost of refill cartridges	Interference caused by turbidity. More testing is required to understand the limit of detection in different water types. However, it is robust in relatively clean waters (Puigdomenech et al., 2017).	1, 2
Microbial Bioanalyser (Photonic Biosystems)	Emerging technology. Field-based. Measures metabolic activity of coliforms. Originally designed by US Department of Defence (DoD) for troops to test drinking water quality. Portable. Can be qualitative, semi-quantitative or quantitative.	Library-independent	Easy	No	Bathing Drinking	No	None	30 mins (8h for a single bacterium)	Low/ no info on logistics in the field	? Cost of device.	?	High as has IP and used by DoD. No info on specificity or interference. Detection: single cell	1, 2

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)Emerging technology	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
BACMON (GRUNDFOS)	Emerging technology. Continuous monitoring. Samples directly from the water line and delivers results within minutes for you to access on your mobile device or PC. Measures total bacteria (including VBNC) and non-bacteria particles by visual inspection.	Library-independent	Easy	No	In-stream Drinking Bathing Shellfish Research	Yes – purchase from Denmark	None	Minutes	Low/ no info on logistics in the field	? (cost of device))	?	Not yet sufficient for real time monitoring of specific targets i.e. FIOs. Interference from bubbles, biofilm and fouling taken into account (Olesen et al., 2018). Reported to have high sensitivity (Olesen et al., 2018).	1, 2

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)Emerging technology	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
ColiMinder CMI-01 (VWM Solutions)	Emerging technology. Continuous monitoring. Tests for *E. coli*, enterococci and total bacteria (enzymatic reaction) using fluorescence.	Library-independent	Easy	No	In-stream Bathing Shellfish Drinking Research	Yes – purchase from Austria	None	15 mins	Low/ no info on logistics in the field	€39K	€ 2.60	Sensitivity reported to be high, but GLUC was not found to correlate well with *E. coli* counts (Ender et al., 2017). Interference from non-coliform bacteria, algae and other substances was found to be negligible in cases where there were high concentration of target bacteria (Ender et al., 2017). Interference from turbidity is compensated for (according to manufacturer).	1, 2
Speedy Breedy	Emerging technology. Continuous monitoring. Measures bacterial respiration of *E. coli*, coliforms, and other pathogens (via selective media) and measures pressure transients.	Library-independent	Easy	No	Drinking	Yes	Lab: Speedy Breedy device. Field: standard sampling equipment.	24h	Low/ not for use in the field	? (cost of Speedy) Breedy)	?	Reliably used to detect spoilage of beer (Michel et al., 2016). Possibly requires further validation with field samples. No info on interference.	1, 2

* for commercial processing of samples at JHI. Likely to reduce with increasing sample numbers. **(cont.) automation is satisfactory, maintenance is reasonable (20 min) and interface is user-friendly. However, filter clogs very easily when used with turbid waters. It also is prone to leaking (Puigdomenech et al., 2017).

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)Emerging technology	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Membrane filtration techniques	See Table 1A.												
Coliscan Easygel® system	See Table 1A.												
Colilert	See Table 1A.												
MI Agar	See Table 1A.												
Chromocult Coliform Agar	See Table 1A.												
Membrane lactose glucuronide agar (MLGA)	See Table 1A.												
AquaFlex (by Trace2O)	See Table 1A.												
Compartment bag Test (by Aquagenx)	See Table 1A.												
Colitag (by Palintest)	See Table 1A.												
aquaCHECK365 (Brightwater Diagnostics) (same as Aquatest by the University of Bristol)	See Table 1A.												

Table IV.1C Phase 3: Confirmatory/hypothesis-driven testing of FIO sources

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
Biomarkers - Antibiotic resistance	Current technology. Lab-based. Selective cultivation of FIOs, followed by antibiotic resistance patterns (ARPs) of FIO isolates (e.g. enterococci, *E. coli*).	Library-dependent	Difficult – labour intensive and time consuming	Yes	Research	Yes	Lab: antibiotics, selective growth media, autoclave, incubator, filtration unit, laminar flow cabinet, class II safety cabinet, consumables. Field: standard sampling equipment.	>48h	Med (time consuming)/ not for use in the field	High - requires basic microbiology lab with Class II cabinet	Depends on selective media	Low (needs more validation). Technique may be geographically specific. Interference low as only viable cells will grow.	3
DNA-based methods - ribotyping	Current technology. Lab-based. Selective cultivation of FIOs, followed by ribotyping of whole genomes.	Library-dependent	Difficult – labour intensive and time consuming	Yes	Research	Yes	Lab: filtration unit, filters, incubator, centrifuge, vortex, reagents and consumables, Qubit/nanodrop, gel electrophoresis or southern blot with probes. Field: standard sampling equipment.	>48h	Med/ not for use in the field	High - requires basic microbiology lab with Class II cabinet	£1.00 – cultivation; £1.75 - DNA extraction kit; £0.37 Qubit; £ variable for restriction enzymes	Reported to be highly reproducible, but technique is geographically specific (Meays et al., 2004). Interference low as only viable cells will grow.	3
DNA-based methods – Pulse filed gel electrophoresis (PFGE)	Current technology. Lab-based. Selective cultivation of FIOs, followed by DNA fingerprinting of isolates visualised by gel electrophoresis.	Library-dependent	Difficult – labour intensive and time consuming	Yes	Research	Yes	Lab: filtration unit, incubator, centrifuge, vortex, Qubit/nanodrop, gel electrophoresis, reagents and consumables. Field: standard sampling equipment.	>48h	Med/ not for use in the field	High - requires basic microbiology lab with Class II cabinet	£1.00 – cultivation; £1.75 - DNA extraction kit; £0.37 Qubit; £1.50 – PCR; £ variable for restriction enzymes	Extremely sensitive, but may be too sensitive to broadly discriminate (Meays et al., 2004). Interference low as only viable cells will grow.	3

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
DNA-based methods – denaturing gradient gel electrophoresis (DGGE)	Current technology. Lab-based. Selective cultivation of FIOs, followed by electrophoresis of PCR products, which allows discrimination of sequences based on melting temperature.	Library-dependent	Difficult – labour intensive and time consuming	Yes	Research	Yes	Lab: filtration unit, incubator, centrifuge, vortex, Qubit/ nanodrop, PCR machine, DGGE tank, reagents and consumables, DGGE analysis software. Field: standard sampling equipment.	>48h	Med/ not for use in the field	High - requires basic microbiology lab with Class II cabinet	£1.00 – cultivation; £1.75 - DNA extraction kit; £0.37 Qubit; £1.50 – PCR	Low reliability for environmental isolates (Meays et al., 2004). Interference low as only viable cells will grow.	3
DNA-based methods – repetitive DNA sequences (Rep-PCR)	Current technology. Lab-based. Selective cultivation of FIOs, followed by PCR amplification of palindromic sequences and visualised by electrophoresis.	Library-dependent	Difficult – labour intensive and time consuming	Yes	Research	Yes	Lab: filtration unit, incubator, centrifuge, vortex, Qubit/nanodrop, PCR machine, gel, electrophoresis, reagents and consumables. Field: standard sampling equipment.	>48h	Med/ not for use in the field	High - requires basic microbiology lab with Class II cabinet	£1.00 – cultivation; £1.75 - DNA extraction kit; £0.37 Qubit; £1.50 – PCR	Reproducibility is a concern (Meays et al., 2004). Interference low as only viable cells will grow.	3
DNA-based methods – length heterogeneity PCR (LH-PCR)	Current technology. Lab-based. Separation of PCR products for genetic markers based on length.	Library-dependent	Difficult – labour intensive and time consuming	Yes	Research	Yes	Lab: filtration unit, bead beater, centrifuge, vortex, Qubit/nanodrop, PCR machine, gel, electrophoresis, reagents and consumables. Field: standard sampling equipment.	>48h	Med/ not for use in the field	High - requires basic molecular biology lab	£7.36 DNA extraction kit; £0.37 Qubit; £1.50 – PCR	Sensitivity and specificity variable as it is dependent on PCR primers (Bernhard and Field, 2000). Interference from PCR inhibitors. Assay will detect both live and dead cells.	3

Technology	Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)	Library-dependent or library-independent	Ease of use	Expertise /training needed	Relevance to regulatory framework/ management policy	Available in market/ Scotland	Equipment needed	Time to process samples	Planning hours/ logistics in the field	Set-up cost	Cost per sample	Reliability (sensitivity, specificity, interference)	Phase
DNA-based methods – Terminal restriction fragment length polymorphism (T-RFLP)	Current technology. Lab-based. Separation of the terminal fragment of PCR amplicons based on length.	Library-dependent	Difficult – labour intensive and time consuming	Yes	Research	Yes (but need to develop the assay)	Lab: filtration unit, bead beater, centrifuge, vortex, Qubit/nanodrop, PCR machine, sequencer, reagents and consumables, TRFLP analysis software. Field: standard sampling equipment.	>48h	Med/ not for use in the field	High - requires basic molecular biology lab	£7.36 DNA extraction kit; £0.37 Qubit; £1.50 – PCR; £ variable for restriction enzymes. Also requires fluorescently labelled primers.	Specificity variable as it is dependent on PCR primers (Harwood et al., 2014). Interference from PCR inhibitors. Assay will detect both live and dead cells. Detection limit 10^3 – 10^4 gene copies (Pagaling, personal comm).	3
Faecal sterol, acid bile markers	See Table 1B.												
Flow cytometry and FACS (fluorescence activated cell sorting)	See Table 1B.												
DNA-based methods - QPCR	See Table 1B.												
DNA-based methods - microarray	See Table 1B.												

Table IV Content	
Table Header	**Key**
Description; Current or Emerging technology; category (lab or field-based or continuous monitoring)	Brief description of technology, what it measures; whether it is a current or emerging technology; category based on where the technology can be implemented. Continuous monitoring indicates technologies that can be left in situ.
Ease of use	Easy/Medium/Difficult
Expertise or training needed	Yes/No (No indicates nothing more than standard field sampling, good laboratory practice and basic understanding of aseptic technique)
Regulatory framework/ management policy to which technology is relevant	In-stream Bathing Shellfish Drinking Research
Available in the market/in Scotland	Yes/No
Equipment needed	Key laboratory or field equipment required (not exhaustive)
Response of technology to storage	<=24hrs <=48hrs Specify
Planning Hours	Low/Med/High
Set-up cost	Where available (for key equipment; not exhaustive)
Cost per sample	Where available
Reliability	Any biases/known aspects of reliability/unreliability compared with standard techniques
Relevance to strategy purpose	E.g. suitable for initial screening, routine monitoring, source tracking
Blue shading	Denotes the technology which we have identified as having the most potential for the criteria set out in the project aims. Please note that this does not preclude those technologies for other purposes.

Table IV.2 Effects of storage time on FIO quantification	
Category of Technology	**Effect of Storage Time**
Lab-based using cultivation-based methods, DNA-based methods, flow cytometry or other biomarkers	- Results from stored samples will be affected by die-off. -For any techniques based on DNA/RNA; storage is likely to result in loss of genetic material over time. RNA is likely to be lost rapidly. DNA loss depends on cell integrity which will be species dependent (e.g. Bacteroides do not survive for long periods in water, therefore DNA will be released, and free DNA tends to be broken down rapidly. -For flow cytometry, storage may influence cell integrity but depends on matrix composition and is likely to be similar to die-off effects for culture-based approaches. -Stanols/sterols known to change over time but are unaffected by disinfection.
Field-based – mobile labs or probes, including those using cultivation-based methods	Would theoretically be affected by die-off but as these technologies are intended for use in the field this there should be no storage time.
Continuous monitoring	In situ monitoring therefore generally no storage, however some platforms also allow samples to be stored for collection in which case storage time is likely to lead to effects on cell integrity/die-off.

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Appendix V Trial results

This is a demonstration of what the toolbox approach to catchment surveys (Phase 1 of the monitoring strategy) can include in Scotland and how it can help design Phase 2 and Phase 3 of a catchment FIO monitoring strategy. It is useful to keep in mind that, in line with the broad monitoring guidelines for catchment surveys, we assume that the waterbody catchments adjacent to the coastline and the BWPA or SWPA (coastal waterbody catchments) pose a greater risk to BWPA and SWPA due to faster and direct FIO transport pathways from sources to the receiving coastal waters. Non-coastal waterbody catchments (inland waterbody catchments) being further away from the coastline, especially in larger river catchments, pose a lower risk to BWPA and SWPA due to longer pathways and potentially a greater degree of dilution during transport from catchment-based sources to the coast. It must be noted that this assumption is tested during Phase 1 of the FIO monitoring strategy.

Appendix V.1 Nairn Catchment

Risk from Granted Point sources (GraPS)

Figure VI.1a shows that there is high density of CSO in the coastal waterbody catchments, suggesting that FIO monitoring during Phase 1 and Phase 2 should focus on the impact of CSO in the coastal catchments. Specific monitoring considerations-options [1] are:

- Monitoring in-stream FIO concentration in the coastal waterbody catchments during both dry and wet weather (i.e. applying the hybrid monitoring design).
- Monitoring in the main stem of the Nairn river upstream of CSO (at the confluence of streams and the main stem downstream of primary and secondary GraPS) during dry weather. This can show whether continuous discharges from GraPS providing primary and secondary treatment exert an impact on instream FIO concentrations upstream of the coastal waterbody catchments.

1 Specific recommendations cannot be given because action depends on field screening and actual FIO data.

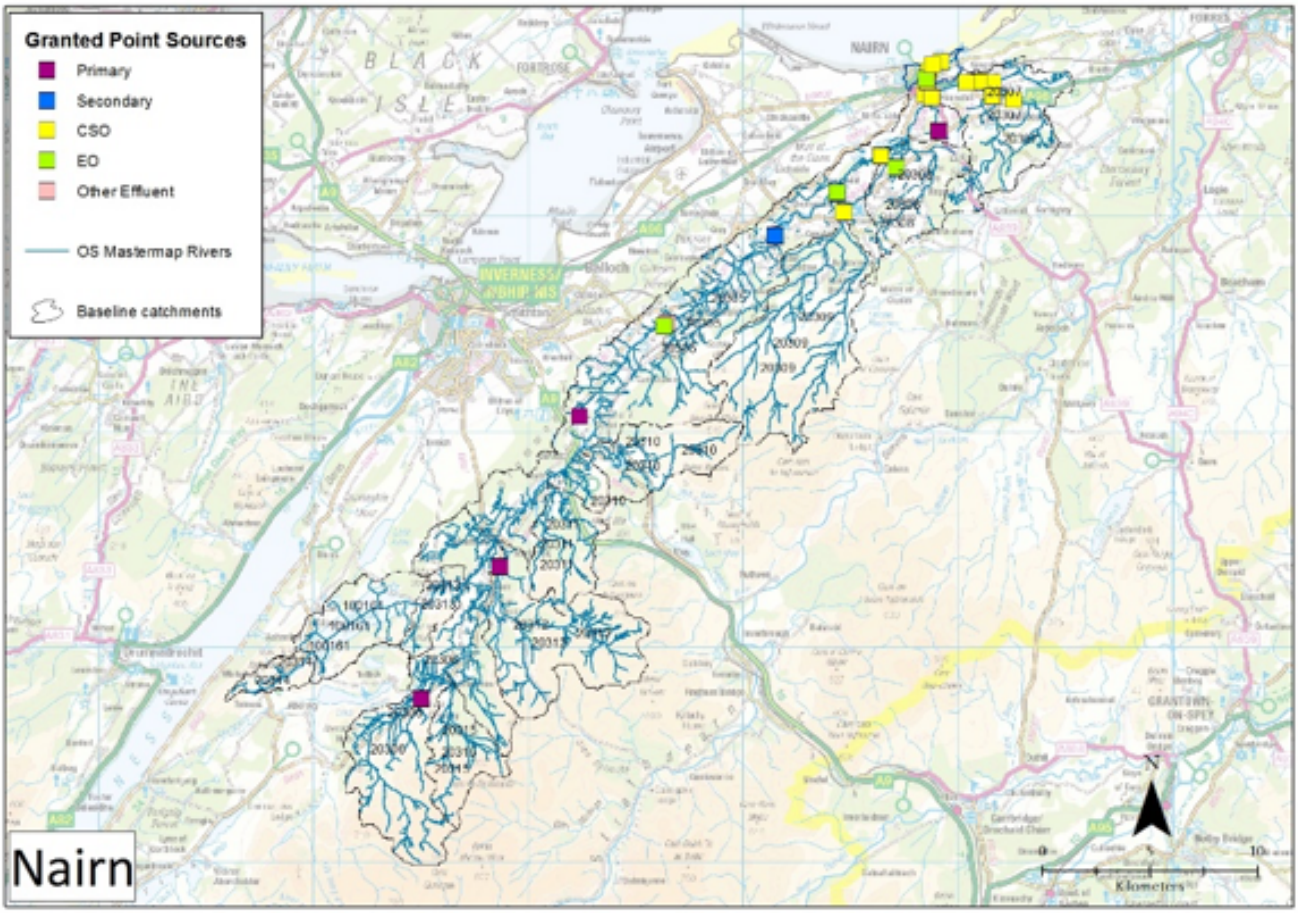

Figure V.1a. Locations of GraPS at the Nairn river catchment. Data sources: SEPA, OS; see also Appendix II.2.

- FIO monitoring in the morning during baseflow. This will enable capturing the maximum in-stream FIO concentrations in relation to continuous discharges from GraPS providing primary and secondary treatment before solar radiation and other factors (see Section 3.1) reduce FIO.
- FIO monitoring before peak flow during stormflow. This will enable understand FIO from the CSO before dilution ("first flush") and whether discharges from rainfall-dependent diffuse FIO sources (see Section 3.1) exert an impact on instream-FIO concentrations. This monitoring may be applied during Phase 1, Phase 2 and Phase 3.
- The "bracketing" approach (See Section 3.3) to monitoring during Phase 2 and 3 in the coastal waterbody catchments under both wet and dry weather. This can help to distinguish between CSO and diffuse pollution FIO sources (as in Figure 2 of the main document) during stormflow and understand background FIO concentrations and predominant sources before CSO discharges.

Risk from septic tank systems (STS)

We examined risk from STS to watercourses in relation to soil runoff risk and soil leaching potential, assuming that there is a higher FIO pollution risk from STS (including discharges to soakaways, due to misconnections and directly to watercourses) when these are located at distances less than 10m from watercourses at areas characterised by high soil runoff risk and or high soil leaching potential. The results show that in the coastal waterbody catchments there are 7 STS within 10m from watercourses, 198 STS on soils characterised by high runoff risk and 324 STS on soils characterised by high leaching potential (Table V.1). It can be also observed that there are high density clusters of STS (i.e. >20 STS/Km^2) on soils characterised by high runoff risk and high leaching potential in the coastal waterbody catchments (Table V.1, Figure V.1b and c). Further, there are many more high-density STS in the coastal than in the inland waterbody catchments (Figure V.1b and c).

These findings strongly indicate that monitoring to detect FIO from STS should initially focus on the coastal waterbody catchments, in line with literature-based monitoring strategy recommendations. Monitoring considerations include:

- Verifying and exploring though field visits the locations and performance characteristics of the 6 STS within 10m from watercourses and the high-density clusters in the coastal waterbody catchments.
- Hourly sampling for a couple of days during wet and dry weather to explore FIO concentrations upstream and downstream ("bracketing") the 6 STS located within 10m from watercourses and the high-density STS clusters for Phase 1 monitoring. This monitoring will show whether and where there are STS-related hotspots within the area of influence.
- Monitoring at the confluence of the inland with the coastal waterbody catchments to explore any FIO influences from STS in the inland waterbody catchments and ensure that the area of influence during both wet and dry weather only includes the coastal waterbody catchments
- Weekly (morning or daily composite samples) monitoring for more than two years downstream of the STS hotspots identified during Phase 1 monitoring to identify their temporal variability (as per Phase 2) and the dominant FIO sources downstream STS clusters (as per Phase 3).

Risk from land use (diffuse pollution sources)

Figure VI.1d shows where FIO from livestock accumulate in-stream faster than the stream water flow can dilute them, therefore this SCIMAP output predicts the sites with

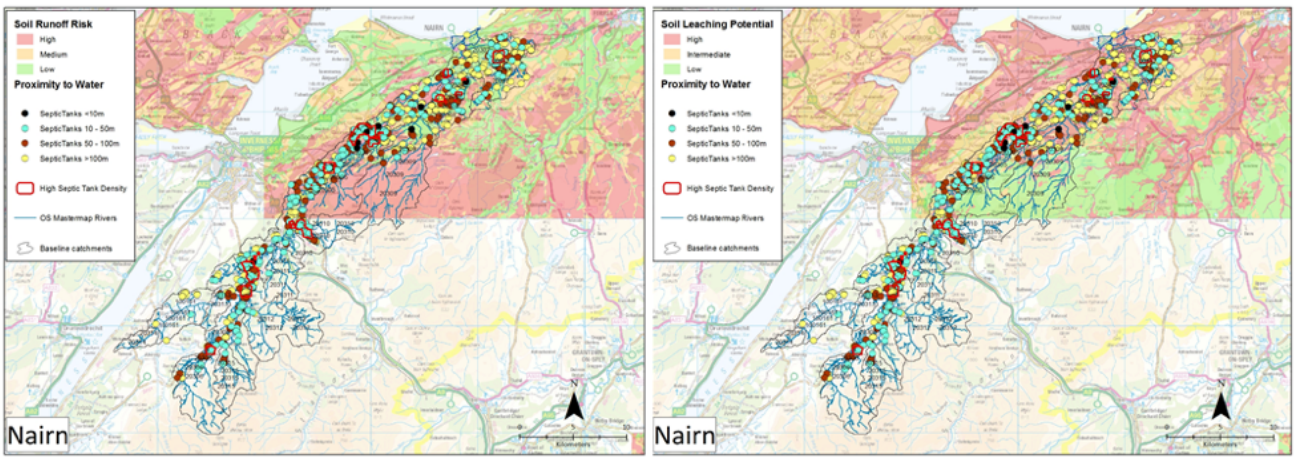

Figure V.6 b and c. Septic tank systems (STS): modelled locations and high density clusters in relation to proximity to watercourses and soil runoff risk (left) and soil leaching potential (right). Data sources: SEPA, OS, SG; see also Appendix II.2.

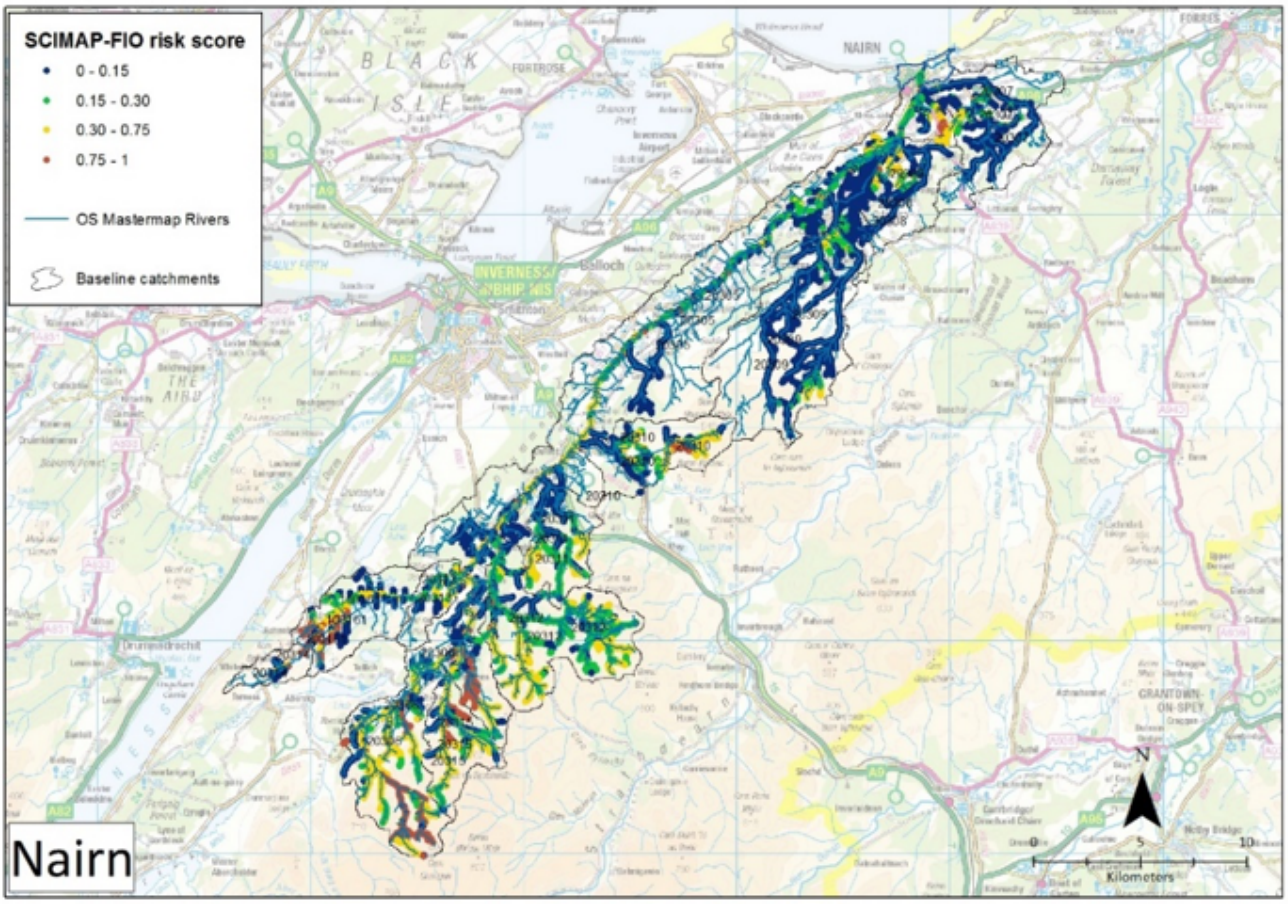

Figure V.1d. SCIMAP-FIO output for the Nairn river catchment. Source: SCIMAP 2019.

high in-stream FIO from diffuse agricultural pollution. We observed that high FIO can be expected at the headwaters of the Nairn river catchment. Less than 3% of the river length within the coastal catchments is at risk from diffuse (livestock) agricultural pollution. However, a small stream contributing to the main stem of the Nairn river in one of the coastal waterbody catchments (ID:20305) is predicted to have high in-stream FIO concentrations across most of its length. The implication for monitoring during Phase 1 is that this small stream can be considered as a "point source" to the main stem and sampled downstream and upstream of the confluence with the main stem with the Nairn river during wet and dry weather (hybrid monitoring). This will help to understand how this small stream responds to rainfall in terms of FIO. The hybrid monitoring will also allow for separating the effects of background FIO due to STS and FIO due to agricultural land runoff. Therefore, this monitoring data must be considered in combination with data from STS in order to inform a better understanding of the separate timing of FIO discharges from different sources (i.e. continuous STS vs land runoff).

Additional considerations-options for Phase 2 (if that waterbody proves to be a FIO hotspot) include weekly (morning or daily composite) sampling or monitoring under the hybrid design upstream of the confluence to capture the FIO temporal variability generated by that "hotspot" waterbody and, potentially by STS hotspots.

Once the timing of livestock and STS FIO is understood, confirmatory Phase 3 monitoring using MST tools can be applied to inform remedial action targeting.

Detecting predominant FIO sources with microbial source tracking at the Nairn Catchment

The National Waters Inventory of Scotland (NWIS) curated at the James Hutton Institute is an archive of surface water collected at end of catchment locations around Scotland. One of the sampling locations was in the Nairn catchment, i.e. Jubilee Bridge (SEPA sample ID: 202313) where several samples were taken over a two-year sampling period. Preliminary analysis of these samples was done using microbial source tracking (MST) tools (i.e. QPCR of host-specific *Bacteroides*). This data showed that over the two-year period, the predominant FIO pollution sources changed between ruminant-specific faecal pollution and human-specific faecal pollution. This indicates that selecting sites and times of monitoring during Phase 3 for MST is crucial for a clear understanding of the dominant FIO sources at a site. This finding also shows that identification of hotspots during Phase 1 and the temporal variability of different FIO sources during Phase 2 is essential for separating the effects of different FIO sources before applying MST tools to test types of sources at each site and time.

Appendix V.2 Loch Ryan Catchment

A description of Loch Ryan SW catchment akin to a desktop sanitary survey is available in an earlier CREW report by Akoumianaki et al 2018. For monitoring considerations related to tidal movements and salinity see Akoumianaki et al 2018.

Risk from Granted Point sources (GraPS)

Figure VI.2a shows that the majority of CSO and stormwater overflows are gathered at the inner (southern) part of Loch Ryan in the vicinity of Stranraer, an area with urban land use. Of concern for the SWPA classification is the presence of WwTW providing primary treatment at Wig Bay (Caravan Park) and the CSO at Cairnryan Port (Table V.1). Loch Ryan SW catchment comprises small coastal waterbody catchments. As a result, CSO and WwTW are located at the coastline or at coastal streams less than 5km long, both situations posing a high FIO pollution risk to the SWPA due to short transport pathways - and therefore lower dilution - from sources to receiving waters. However, the SWPA is at good status and the SPA is classified as Class A (see also Akoumianaki et al 2018). Potentially, the coastal water and tidal circulation in the area enables sufficient mixing and dilution of polluted discharges to Loch Ryan. It is widely accepted that shellfish growing areas must be located in areas devoid of point sources.

The implications for monitoring are to consider the following options:

- Water FIO sampling during wet and dry (before an/or after the rain) weather including sampling during the tourist season at CSO or CSO cluster sites to understand their impact on water quality during and after rain. An earlier study showed elevated shellfish E. coli in samples collected up to 5-7 days after rainfall events in Scotland, indicating prolonged impact of rainfall dependent discharges on shellfish E. coli. Therefore, this sampling can clarify whether this is due to CSO or lingering high water FIO concentrations at SWPA due to the water circulation pattern and diffuse FIO pollution from catchment-based and sediment sources. Once Phase 1 monitoring has revealed the CSO hotspots, then Phase 2 and 3 monitoring can elucidate the contribution of human and animal sources to rainfall-dependent discharges as rainfall events progress and at events with different intensity.
- Water FIO sampling in the vicinity of WwTW providing primary and secondary treatment. For Phase 1, this monitoring can be applied during dry weather and during the tourist season. However, understanding of the contribution of these sources to water FIO in the loch requires wet weather sampling, with more samples during the tourist season (hybrid monitoring).

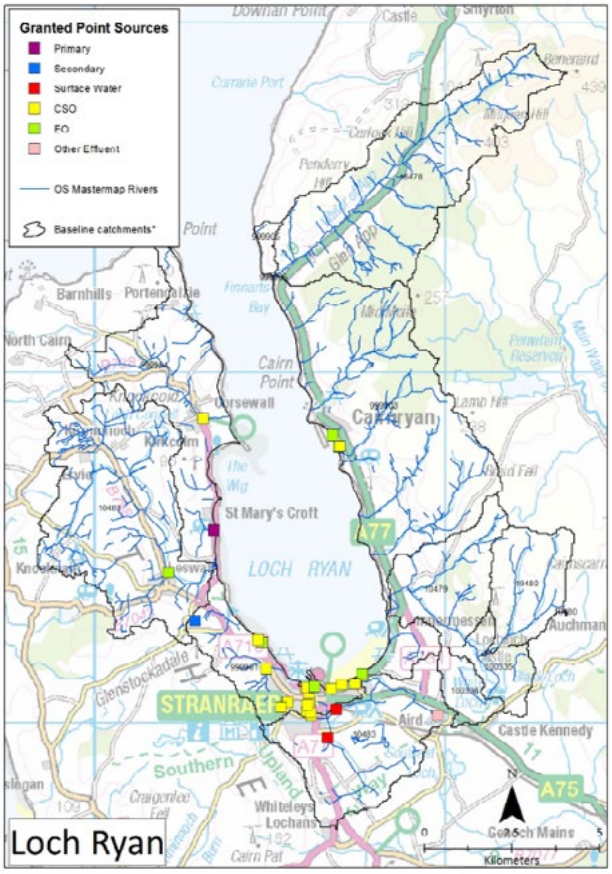

Figure V.2a. GraPS in Loch Ryan SW catchment. Data sources: SEPA, OS; see also Appendix II.2.

Risk from septic tank systems (STS)

We examined risk from STS to watercourses in relation to soil runoff risk and soil leaching potential, assuming that there is a higher FIO pollution risk from STS (including discharges to soakaways, due to misconnections and directly to watercourses) when these are located at distances less than 10m from watercourses at areas characterised by high soil runoff risk and or high soil leaching potential. The results show that there are approximately 400 STS in the small coastal waterbodies comprising the Loch Ryan SW catchment (Table V.1). Of them, there are 7 STS within 10m from watercourses and the coastline (Table V.1). Soil runoff risk and leaching potential were available for a large part of the Loch Ryan SW catchment but not for all of it. It appears that the majority of STS in the southern part of catchment are located on soils characterised by low runoff risk but high leaching potential. By contrast, STS in the south western parts of the catchment are in soils characterised mainly by low leaching potential but high runoff risk. It can be also observed that there is a high-density cluster of STS (i.e. >20 STS/Km2) in the inner part of the loch comprising STS located within 50 to >100m of the coastline on soils characterised by low runoff risk but high leaching potential (Table V.1, Figure V.2b and c).

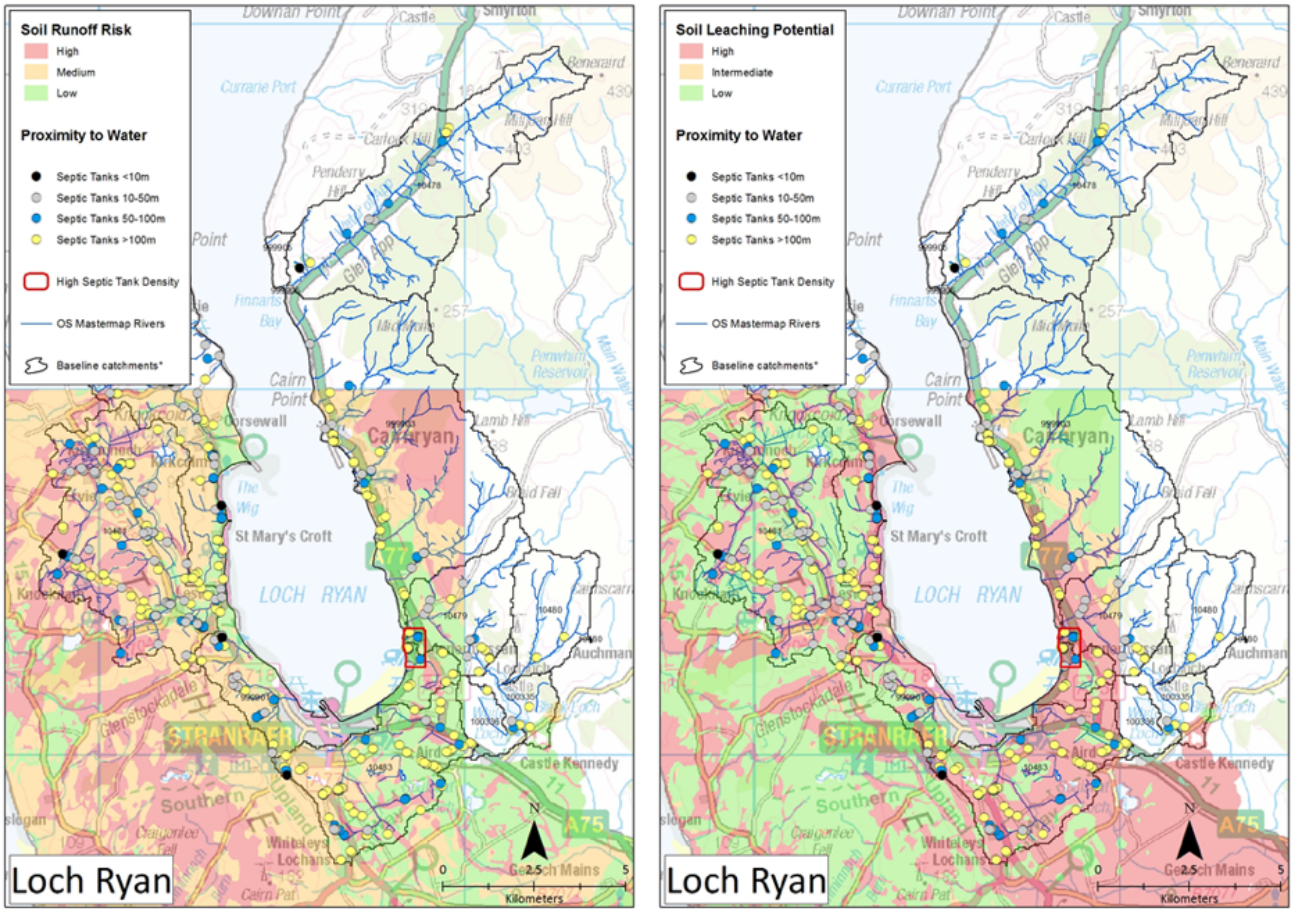

Figure V.2 b and c. Septic tank systems (STS) at the Loch Ryan SW catchment: modelled locations and high-density clusters in relation to proximity to watercourses and soil runoff risk (left) and soil leaching potential (right). Data sources: SEPA, OS, SG; see also Appendix II.2.

The implications for Phase 1 monitoring is to:

- Verify the locations and performance characteristics of the STS being located within 10m from watercourses and the coastline.
- Monitor water FIO in the vicinity of the high-density STS cluster, at the mouth of the streams with STS being located within 10m from watercourses and the vicinity of the STS within 10 m form the coastline.
- Explore the performance and influence of STS located >10- 100m from the coastline and in streams discharging to the inner part of the loch, where they be accumulating causing legacy FIO problems.
- Apply the hybrid monitoring design but in addition to rainfall, tidal stage must be taken into account, e.g. consider collecting samples during high and low tide to assess how the tide affects seepage from coastal STS soakaways.

STS hotspot identification during Phase 1 can inform Phase 2 monitoring to identify temporal variation in STS-related FIO discharges. Phase 3 can be used in areas where there is a need to confirm or elucidate the effect of STS-related human sources at the SWPA against animal FIO sources, and potentially help to delineate exclusion zones for shellfish harvesting, if necessary.

Risk from land use (diffuse pollution sources)

Figure V.2d shows where FIO from livestock accumulate in-stream faster than the stream water flow can dilute them, therefore this SCIMAP output predicts the sites with high in-stream FIO from diffuse agricultural pollution. We observed that high and moderate FIO concentrations can be expected throughout the small streams discharging at the south eastern parts of the Loch Ryan SW catchment as well as at the north eastern part of the catchment. The implication for water FIO monitoring during Phase 1 is that the small streams predicted to have high in-stream FIO concentrations can be considered as "point sources" to the coast and sampled at their mouth to Loch Ryan. The effect of rain, tide and wildlife (including gulls) must be considered during short-term monitoring to assess whether streams draining agricultural land are FIO hotspots to the SWPA.

Additional considerations-options for Phase 2 include weekly (morning or daily composite) sampling or monitoring under the hybrid design and accounting for the influence of coastal current and tidal circulation. Once the timing of FIO discharges from GraPS, STS and livestock, has been investigated and evidence gaps have been identified, confirmatory Phase 3 monitoring using MST tools can be applied to enhance understanding and to inform remedial action targeting.

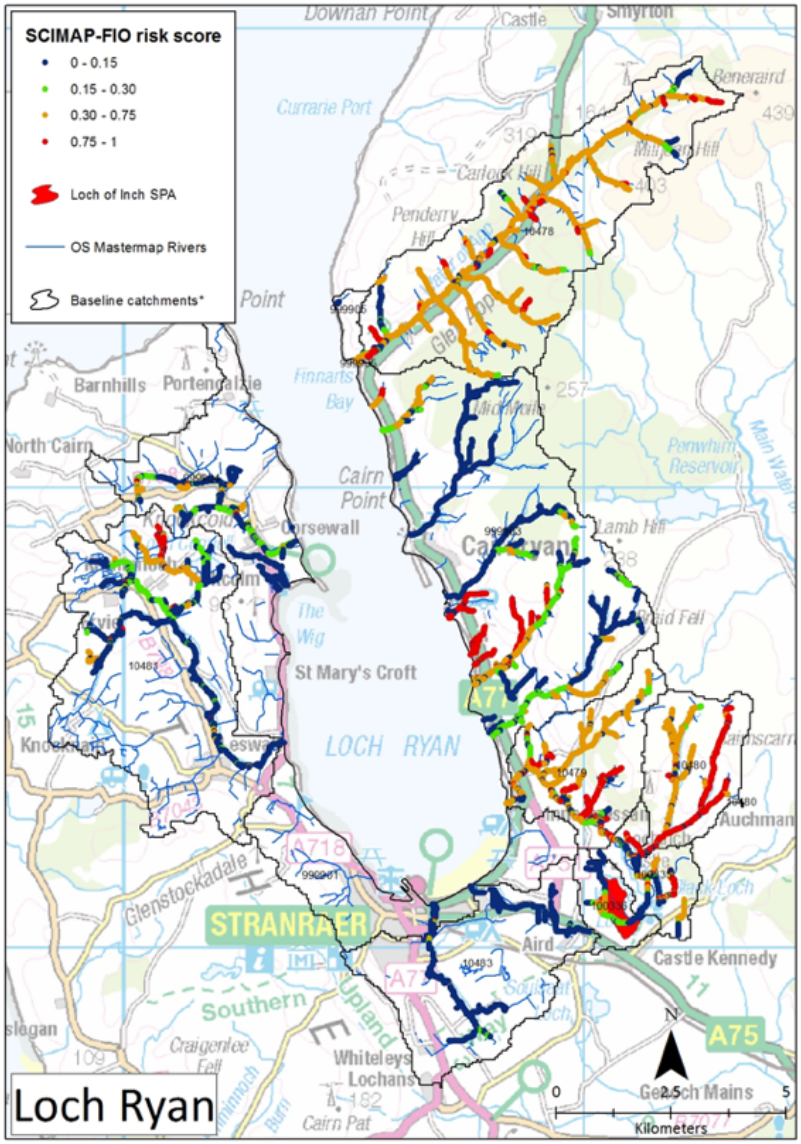

Figure V.2d. SCIMAP-FIO output for the Loch Ryan SW catchment. Source: SCIMAP 2019.

Appendix VI Effect of storage time on efficacy of technique

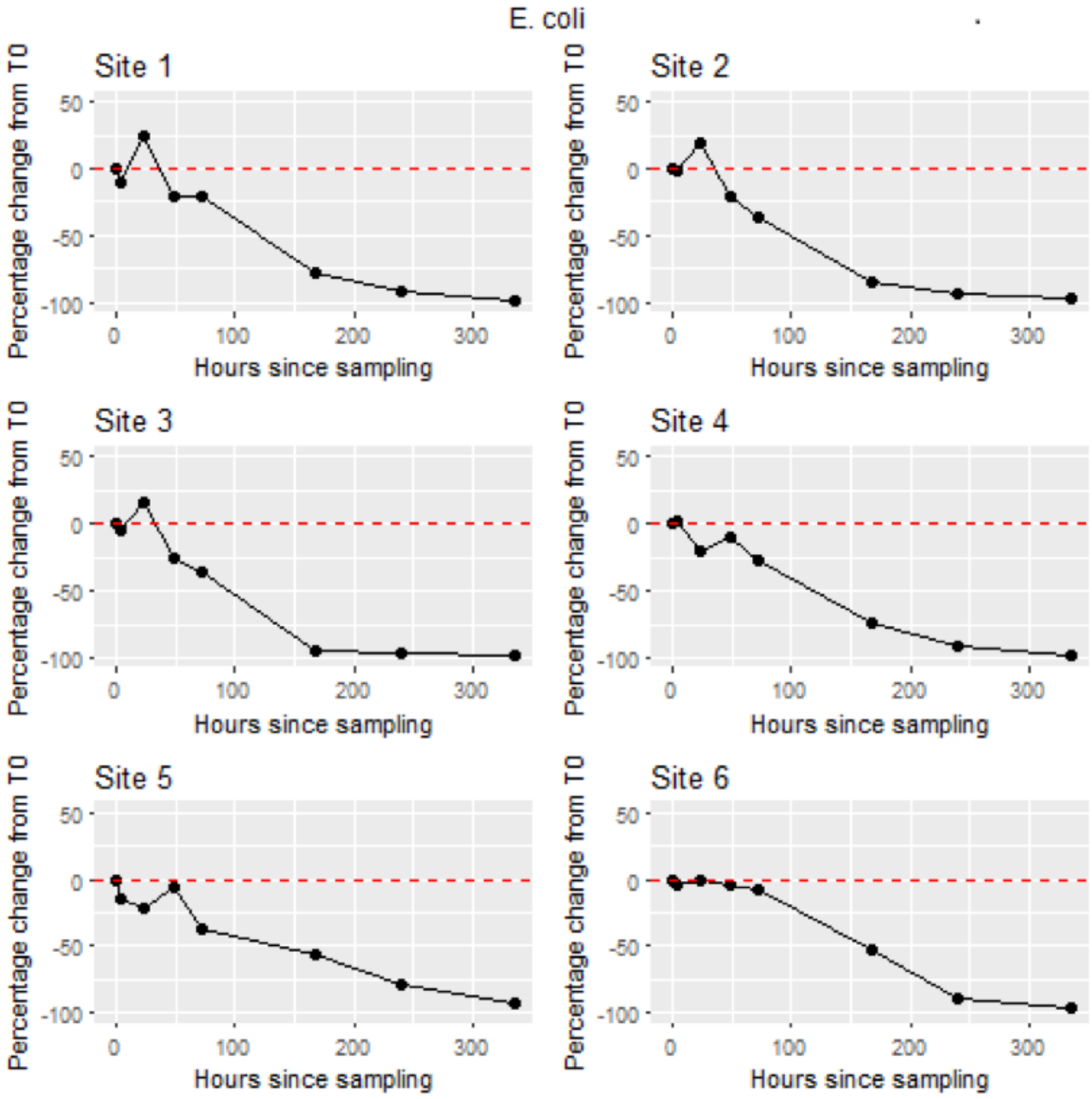

Figure VI. 1. Percentage change in E. coli concentrations during sample storage. Source: Avery, unpublished data.

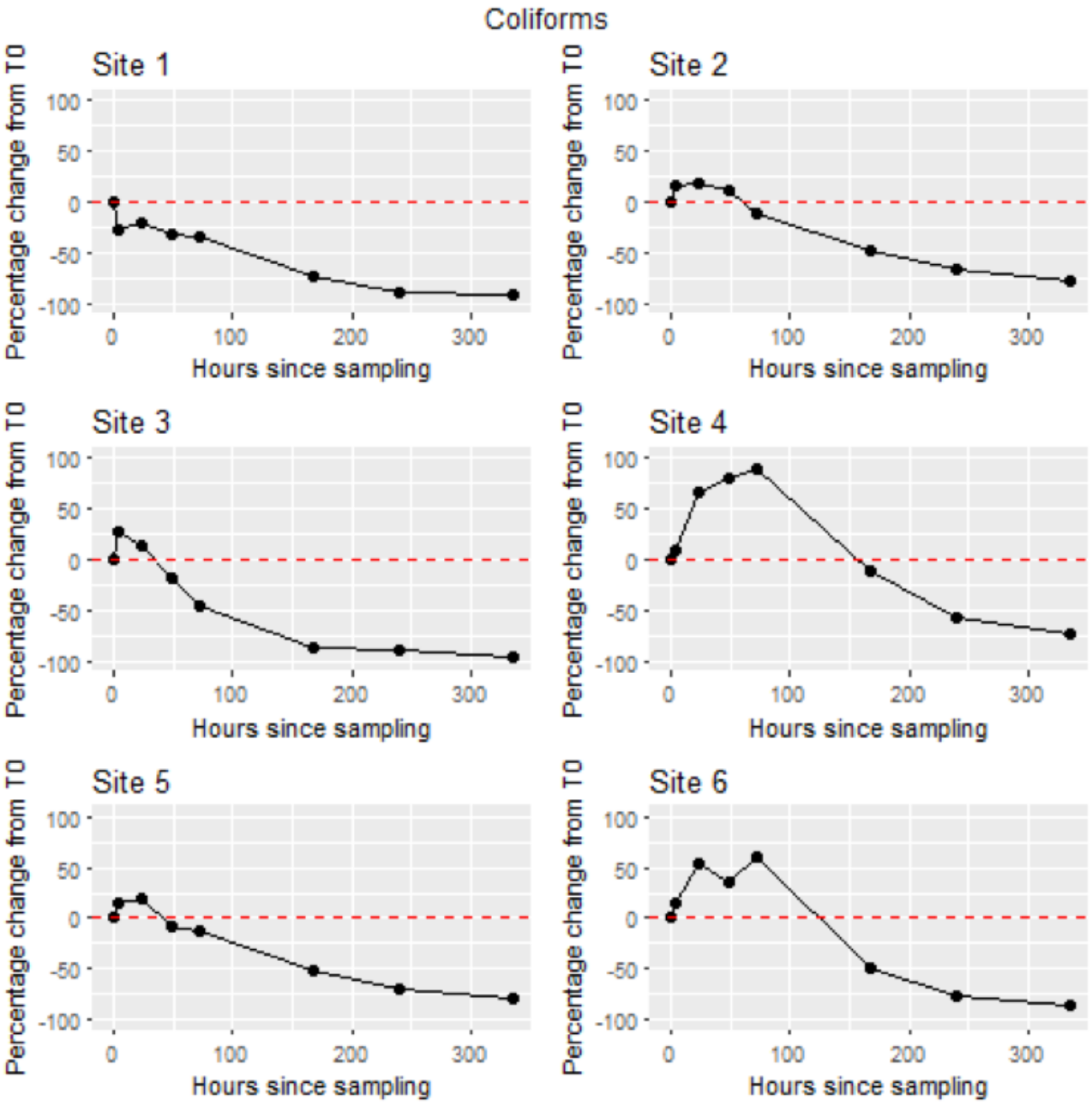

Figure VI.2. Percentage change in Total Coliform concentrations during sample storage. Source: Avery, unpublished data.

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