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# Developing Scotland's Shellfish Water monitoring programme - Appendices

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**Abbreviations**

| | |
|---|---|
| CEFAS | Centre for Environment Fisheries and Aquaculture Science |
| EA | Environment Agency |
| FSA | Food Standard Agency |
| FSS | Food Standard Scotland |
| SAMS | Scottish Association for Marine Science |
| SEPA | Scottish Environment Protection Agency |
| SG | Scottish Government |

# Contents

# Appendix I - Technical and scientific terms and definitions

Terms are listed in alphabetical order.
(References are cited in Appendix References)

**Accumulation efficiency:** Concentration of microbial contaminants, such as faecal indicator organisms (FIOs) and other microorganisms, accumulated by filter feeding bivalves.

**Accumulation factor:** The ratio of concentration of FIOs in shellfish and in overlying water.

**Annual Classification for SPAs by FSS in Scotland:** An official classification based on results of *E. coli* in shellfish collected during the most recent year and a minimum of 10 monthly samples, taken at least a month apart. It follows granting of provisional classification (FSS protocol 2017).

**Bacteriological survey as part of a sanitary survey for SPAs**: Short-term monitoring undertaken in order to help identify the position(s) for sampling site(s) for the classification monitoring programme. This will usually be undertaken at a larger number of points than will be used in the ongoing programme (CEFAS n.d.).

**Bacteriophages** refer to bacterial viruses and are ubiquitous in the environment. For water quality testing and to model human enteric viruses, most interest in somatic coliphages, male-specific RNA coliphages (F-RNA coliphages) and phages infecting Bacteroides fragilis (Ashbolt 2001).

***Bacteroides fragilis*** is a species of anaerobic, bile-resistant, non-spore-forming, gram-negative bacteria, regarded as the most virulent *Bacteroides* species; it is the most abundant bacteria in human gut but one of the rarest bacteria in faecal material (Wexler 2007).

**Bathymetry and Hydrodynamics:** By reference to *Regulation (EC) 854/2004* (Annex II, par. 6), it refers to the determination of the characteristics of the circulation of pollutants by virtue of current patterns, bathymetry and the tidal cycle in the production area, i.e. to the use of data from hydrographic surveys and the output of hydrodynamic and particle transport models.

**Bivalve (Marine) lamellibranch molluscs:** Benthic **filter-feeding** molluscs with two valves living buried in or at the surface of soft bottom habitats or attached on hard substrates. The bivalve body is enclosed in two hinged valves. When the valves are open, bivalves siphon the overlying water to their gills for breathing and feeding. Particles contained in the overlying water are trapped on a mucus sheet secreted by their gills and transported to the mouth, where they are sorted by size. Accepted larger particles such as phytoplankton, detritus aggregates and associated microorganisms and suspended sediment are passed to the digestive system, where they are broken down and absorbed (Rupert et al 2004). Rejected particles (e.g. fine mineral grains of low nutritional quality) are moved past the gills and ejected as pseudofaeces. Waste material is siphoned off as faecal pellets.

**Burrowing (or infaunal) filter feeding bivalves:** These species burrow just beneath or deep into soft (muddy or sandy) sediments in intertidal or subtidal areas to escape predation pressure on the surface while simultaneously taking advantage of suspended material in the overlying water (Rupert et al 2004).

**Class "A" of Shellfish Production Areas (SPAs):** Bivalve mollusc species harvested from SPAs classified as "A" may be placed on the market directly for human consumption (*Regulation EC 854/2004* and EC 853/2004).

**Class "B" of Shellfish Production Areas (SPAs):** Bivalve mollusc shellfish species harvested from that SPAs classified as "B" must undergo depuration in purification centre or a relay area or appropriate treatment before being placed on the market for human consumption (*Regulation (EC) 854/2004 and (EC) 853/2004*).

**Class "C" of Shellfish Production Areas (SPAs):** Bivalve mollusc species harvested form SPAs classified as "C" must be subject to relaying for at least two months or heat treatment before being place on the market for human consumption (*Regulation (EC) 854/2004 and (EC) 853/2004*).

**Classification of Shellfish Water Protection Areas (SWPAs) in Scotland:** The process whereby SEPA grants an area-based classification grade ("Good", "Fair" or "Insufficient") to assess and classify the quality of each SWPA by reference to specified shellfish water quality standards, and to evaluate whether River Basin Management Planning (RBMP) objectives for the protection and improvement of shellfish water quality are met, or not.

**Classified Production Areas (or classified SPAs): By** reference to the *Regulation (EC) 854/2004*, which applies to Scotland and the EU, it means the areas where live bivalve molluscs can be collected and placed on the market for human consumption in agreement with the health standards and criteria applying for the classification of these areas. In Scotland, shellfish *E. coli* data come from previously or currently classified SPAs by FSS.

**Coliforms (or coliform bacteria):** Gram negative, facultatively anaerobic rod-shaped bacteria which ferment lactose to produce acid and gas at 37°C. Members of this group normally inhabit the intestine of warm-blooded animals but may also be found in the environment (e.g. on plant material and soil). They include **Total coliforms** and **faecal coliforms** (Ashbolt et al 2001).

**Combined Sewage outflow** (CSO) means a system for allowing the discharge of sewage (usually dilute crude) from a sewer system following heavy rainfall. This diverts high flows away from the sewers or treatment works further down the sewerage system and thus avoids overloading of works and flooding of properties, etc.

**Competent authority:** The central authority of a EU Member State competent for the organisation of official control monitoring programmes of shellfish *E. coli* or any other authority to which that competence has been conferred (Aquaculture Scotland-Glossary n.d.); for Scotland, this refers to FSS.

**Depuration** or **Purification:** The process of reducing the pathogenic organisms that may be present in shellfish by using a controlled aquatic environment as the treatment process (NSSP 2015).

**Desktop (or desk-based) Survey (or Desk-study):** A survey that collates existing information on the area from government agencies, internet searches, published reports, hydrographic surveys, research projects, and from the harvesters themselves (CEFAs n.d.). A desktop survey includes the following: Details of the shellfishery; Sewage discharge information; Land use in the area; Animal populations; Shipping/boating activity; Meteorological data; Historical bacteriological monitoring data; Bathymetry and hydrodynamics (EURL-CEFAS 2017a).

**Direct impact**: In relation to pollution, the pollution source has an immediate effect on the growing area. This term has been used by NZFSA (2006).

***E. coli* sample:** It means (i) a sample collected as part of official control monitoring programmes in Scotland and internationally from shellfish flesh or intravalvular liquid (FIL); and (ii) a sample collected from water (marine, estuarine-transitional, in-stream or from effluent) during a sanitary survey.

**Effluent (or sewage)** means a liquid that is or has been in a sewer. It consists of waterborne waste from domestic, trade and industrial sources together with rainfall from subsoil and surface water.

**Emergency Outflow** means a system for allowing the discharge of sewage (usually crude) from a sewer system or sewage treatment works in the case of equipment failure.

**Enteric viruses:** A group of unrelated viruses that have a common characteristic of being transmitted via the faecal-oral route. The group includes *Norovirus* (NoV) and hepatitis A virus.

**Enterococci (Intestinal)** refers to a subset of faecal streptococci that grow at pH 9.6, 10° and 45°C and in 6.5% NaCl. Nearly all are members of the genus Enterococcus (Ashbolt 2001).

**Escherichia coli (*E. coli*)** means a thermophilic faecal coliform which also forms indole from tryptophan at 44°C± 0.2°C within 24 hours, but also defined now as coliforms able to produce β-glucuronidase (although taxonomically up to 10% of environmental *E. coli* may not). It is considered the most appropriate group of coliforms to indicate faecal pollution from warm-blooded animals, and it is the most commonly used **Faecal Indicator Organism** (Ashbolt et al 2001).

**Established (or Full) classification of SPAs:** An official type of classification based on results from an extensive number of sampling occasions and a sanitary survey to ensure that potential seasonal and annual variability has been fully covered (*Regulation (EC) 854/2004*; EURL-CEFAS 2017a; NSSP2015; NZFSA 2006). In Scotland, a minimum of 24 samples collected during the last three years is required to grant established classification (FSS protocol 2017).

**Faecal coliforms (FC)**: facultative aerobic, gram-negative, non-spore forming, cytochrome oxidase negative, rod-shaped bacteria that are able to ferment lactose with gas production in the presence of bile salts, or other surface-active agents with similar growth-inhibiting properties, at 44°C ± 0.2°C within 24 hours (Ashbolt 2001; NSSP 2015).

**Faecal contamination:** Contamination of food and water by human and animal faecal bacteria and viruses.

**Faecal Indicator Organisms (FIOs)**. This group of organisms refers to *E. coli*, total coliforms, faecal coliforms, the genera *Enteroccus* and *Streptococcus, Clostridium prefrigens*, bifidobacteria, bacteriophages (including phages infecting *Bacteroides fragilis*[1]) and coliphages (Ashbolt et al 2001).

**Faecal streptococci:** Gram-positive, catalase-negative cocci from selective media (e.g. azide dextrose broth or m Enterococcus agar) that grow on bile aesculin agar and at 45°C, belonging to the genera Enterococcus and Streptococcus possessing the Lancefield group D antigen. (Ashbolt et al 2001).

**Fair quality of Shellfish Water Protection Areas** (SWPAs) means that the 90-percentile of MPN of shellfish *E. coli* is equal to or below 4600 per 100g of shellfish flesh or intravalvular liquid.

**Flesh and intravalvular liquid (FIL)** refers to the muscles, body and organs of a bivalve mollusc together with the liquid contained within the valves when the animal is tightly closed out of the water.

**Flushing time** of an estuary or an embayment can be defined as the time needed to replace its freshwater volume at the rate of the net flow through the estuary, which is given by the river discharge rate.

**Free swimming (or unattached) filter feeding bivalves:** In the context of this report, this refers to scallops, which can lift themselves from the sea bed and swim by jet propulsion to avoid predators (Rupert et al 2004). This is achieved by rapidly contracting their large adductor muscle and clapping the valves together.

**FSS - Food Standards Scotland** Food Standards Scotland (FSS) is the public-sector food body for Scotland. FSS was established by the Food (Scotland) Act 2015 as a non-ministerial office, part of the Scottish Administration, alongside, but separate from, the Scottish Government.

**Gastropod (Marine) molluscs:** Benthic organisms which possess a distinct head, generally with eyes and tentacles, and a broad flat foot and usually are enclosed in a spiral shell (Rupert et al 2004). Edible naturally occurring marine gastropods in Scotland include the common whelk (*Buccinum undatum*) and periwinkle (***Littorina littorea)*** (SG 2015b). The common whelk is a species scavenging or preying on other invertebrates in soft-bottom subtidal habitats (World Reference of Marine Species-WoRMS 2018). In Scotland, whelks are fished, on a limited scale, with baited traps and periwinkles are collected by hand (SG 2015b).

**Gatherer (or Harvester)**: means any natural or legal person who collects live bivalve molluscs by any means from a harvesting area for the purpose of handling and placing on the market (*Regulation (EC) 853/2004*; NSSP 2015).

**Geographical Information System (GIS)** means a computer-based system that combines mapping and data storage functions in order to store, manipulate, analyse, display and interpret spatially referenced data. It is used in sanitary surveys in the USA and New Zealand (NSSP 2015; NZFSA 2006).

**Global Positioning System (GPS)** is a system for determining position on the Earth's surface and specifically the *E. coli* sample's NGR.

**Good quality of Shellfish Water Protection Areas** (SWPAs) means that the 90-percentile of MPN of shellfish *E. coli* is equal to or below 230 per 100g of shellfish FIL.

**Governance** (in the context of aquaculture and fisheries) denotes the rules shaping the management of shellfish waters and the organisations involved as defined by the High Level Panel of Experts (HLPE) on Food Security and Nutrition of the Committee on World Food Security (HLPE 2014). The rules applying to shellfish waters may be formal (i.e. international and national legislative frameworks); informal (e.g. policies, strategies, expert recommendations); or both. The organisations involved may be governmental and non-governmental. The term governance also refers to the way the rules are implemented and enforced/monitored. This is related to the standards and parameters used for classification; procedures to award classification or designate boundaries of protected areas and exclusion zones; and the role of sanitary surveys in awarding classification.

**Growing method:** This refers to: beds on the sea bottom, trestles, ropes or long-lines.

**Harvesting method:** This refers to collection by mechanical methods, such as dredging and trawling, or manually by hand-gathering or diver-held collection.

**Health standards for live bivalve molluscs harvested from SPAs**: Organoleptic characteristics associated with freshness and viability, including shells free of dirt, an adequate response to percussion and normal amounts of intravalvular liquid, and limits for marine biotoxins (*Regulation (EC) 853/2004*).

**Historical bacteriological monitoring data:** This refers to classification data from FSS for SPAs and for Bathing Waters and SWPAs (CEFAS n.d.).

**Hybrid monitoring strategy**: A sampling strategy using both the random and the worst-condition strategies to classify an area. NZFSA (2006) proposes this approach as a feasible alternative to the random or the worst-condition strategies when it is not possible to collect a sufficient number of samples under any of them.

**Hydrodynamic models** refer to numerical models that approximate the flow of seawater, i.e. velocities and water depths as functions of time and space. Output from these models can then be used together with a representation of diffusion processes in the water column to represent the fate and dispersion of bacteria.

**Indirect impact**: In relation to pollution, a secondary impact on a SPA or SWPA or the contaminant may reach the growing area in a roundabout way. This term has been used by NZFSA (2006).

**Initial classification of SPAs means** an official classification based on results from a limited number of sampling occasions (EURL-CEFAS 2017a). This term is not reported by FSS.

**Insufficient quality of Shellfish Water Protection Areas** (SWPAs) means that 90-percentile of MPN of shellfish *E. coli* is above 4600 per 100g of shellfish FIL.

**Inventory of pollution sources:** By reference to *Regulation (EC) 854/2004* (Annex II, par. 6), this refers to recording the location of sources of pollution of human or animal origin likely to be a source of contamination for the production area. Inventorying pollution sources is achieved through desktop and shoreline surveys.

**Land Use for sanitary surveys:** Type of land cover, agricultural uses, forestry, human population, slurry storage and application (CEFAS n.d.)

**Management** (in the context of aquaculture and fisheries) refers to practices and routines intended to generate the information needed to make effective decisions on shellfish harvesting and ensure effective protection from faecal contamination for public health and the shellfish industry (e.g. Rees et al 2010; HLPE 2014). Practices may refer to data collection from sanitary surveys, data handling, analyses and storage, data use for classification, monitoring design, and to relating *E. coli* data to catchment planning and pollution control measures. These practices are part of routines, which may or may not be documented in official control protocols and standard operating procedures.

**Meteorological data:** Precipitation data at the catchment, wind intensity and direction at the vicinity of the shellfishery.

**Method of sampling (it may refer to bivalve harvesting method):** Same as collection method.

**Microbial source tracking (MST):** The science used to determine the source of faecal contamination is termed microbial source tracking and it encompasses molecular biology, microbiology and chemistry.

**Monitoring programme of Shellfish Water Protected Areas (SWPA) established by SEPA:** By reference to SG Directions (2015;2016), this is a monitoring programme that (i) covers monitoring of the quality of the area; (ii) enables a reliable assessment of the shellfish water quality of the area and extent to which each SWPA objective has been or is likely to be achieved (iii) enables a reliable assessment of any risks to the achievement of the SWPA objectives; and (iv) enables the area to be classified by reference to its shellfish water quality.

**MPN (Most Probable Number)** of *Escherichia coli* (*E. coli*) means the most probable number enumerated using a two stage, five-tube, three dilution most probable number test which is consistent with the international standard analytical method ISO/TS 16649-3:2005 (SG Directions 2015; 2016; *Regulation (EC) 854/2004*).

**Non-burrowing (or attached or epifaunal) filter feeding bivalves:** These species have become adapted to life attached to hard surfaces by a byssus such as the common mussels (*Mytilus edulis*); or by cementing one valve to substratum such as the Pacific oysters (*Crassostrea gigas*) and the native oysters (*Ostrea edulis*). They can be found or cultivated in both subtidal or intertidal rocky habitats but may also use their dead shells to develop reefs (Rupert et al 2004).

**Non-point** or **diffuse source** means a source of pollution, that is not point source, including agricultural farm runoff, urban runoff or storm-water, sewage discharge from vessels, dredging operations, forestry practices and other sources which are diffuse and dispersed. Non-point source discharges enter surface waters in a diffuse manner and at intermittent intervals that are generally related to the occurrence of meteorological events (Kay et al 2008a; NZFSA 2006).

**Norovirus** means small, 27-to 32-nm, structured RNA viruses which have been implicated as the most common cause of nonbacterial gastroenteritis outbreaks.

**Official Control Sample collected by FSS:** means a verified shellfish or water sample taken by Food Standards Scotland's sampling officers, for the purposes of Food Standards Scotland's Official Control monitoring programme (Aquaculture Scotland Glossary n.d.).

**Pathogen**: An organism such as bacteria (e.g. *Salmonella*), viruses (e.g. NoV, hepatitis A virus), or parasites (e.g. *Giardia, Cryptosporidium*) that may cause disease in humans.

**Permitted treatment:** The process of subjecting bivalve shellfish, when these have not been submitted for purification or relaying, to any form of treatment with the aim to eliminate pathogens prior to placing on the market for human consumption (*Regulation (EC) 853/2004*; NSSP 2015). By reference to *Regulation (EC) 853/2004*, permitted treatment includes: sterilisation in hermetically sealed containers, immersion in boiling water to raise the internal temperature of the mollusc flesh to not less than 90°C for 90 seconds, pressure cooking or steaming for three to five minutes.

**Point Source** means any discernible, confined and discrete conveyance including any pipe, ditch, channel, tunnel or conduit that carries pollution (NSSP 2015).

**Preliminary classification of SPAs by FSS in Scotland:** This is for areas currently classified for another species or areas that have been declassified within the past 2 years. It may be considered when the area has been subject to a sanitary survey and/or where existing or historic monitoring data allows *E. coli* assessment (FSS protocol 2017).

**Production:** In the context of aquaculture, this means the output from an aquaculture site, e.g. the provision of shellfish for human consumption and the provision of shellfish for on-growing on another aquaculture site (Aquaculture Scotland-Glossary n.d.). The *Regulation (EC) 854/2004* uses the term to refer to commercially harvested live bivalve molluscs from natural or cultivated populations.

**Prohibited Shellfish Production Areas (SPAs):** Harvesting of a specific bivalve mollusc species is not permitted from SPAs graded as "prohibited" (FSS protocol 2017).

**Provisional classification of SPAs by FSS in Scotland:** an official type of initial classification based on a minimum of 10 samples taken at least a week apart. This is currently considered for new areas where there is no existing monitoring data and where no full sanitary survey has been undertaken (see below). It is preceded by a desk-based provisional assessment of pollution sources to identify a provisional RMP, known as provisional RMP (pRMP) assessment (FSS protocol 2017).

**Provisional RMP (pRMP) assessment for SPAs on behalf of FSS** means a written desk-based evaluation of faecal pollution sources in an area to identify a provisional sampling plan (i.e. boundaries of the SPA, RMP, sampling frequency) to facilitate the classification process and allow classification sampling from a provisional RMP to begin as soon as possible (FSS protocol 2017). The provisional sampling plan will be reviewed later in the sanitary survey process as additional information becomes available.

**Rare or unusual or anomalous event**: A "one-off" event that is unlikely to recur such as a sewage treatment works failure, a sewage pipeline break, a one in five years storm event, failure of an animal slurry storage facility or other animal waste disposal practices, or a failure to comply with a sampling protocol standard (EURL-CEFAS 2017a; NZFSA 2006).

**Relay area:** Any sea, estuarine or lagoon area with boundaries clearly marked and indicated by buoys, posts or any other fixed means, and used exclusively for the natural purification of live bivalve molluscs (*Regulation (EC) 853/2004*; EURL-CEFAS 2017a). FSS, by reference to EC 854/2004, must classify and monitor relaying areas (FSS protocol 2017).

**Relaying**: The transfer of live bivalve molluscs to sea, lagoon or estuarine areas for the time necessary as the treatment process to reduce contamination to make them fit for human consumption (*Regulation (EC) 853/2004*; NZFSA 2006).

**Remote areas:** Areas that are not subject to impact from any actual or potential human or animal sources of faecal pollution and where the monitoring data is stable (EURL-CEFAS 2017a; NSSP 2015; NZFSA 2006).

**Representative Monitoring Point (RMP) (also known as "worst-case" or worst-location monitoring point):** A specified geographical location from which samples are taken to represent either a single, or several, wild bivalve mollusc beds or aquaculture sites. The location and number of RMP is based on the outcome of the sanitary survey; a single RMP, should reflect the location at highest risk of faecal pollution within the classified area (EURL-CEFAS 2017a).

**Review or assessment period**: One or more years required to collect a sufficient number of samples for classification every year, as determined by the competent authority (SG Directions 2015, 2016; FSS protocol 2017). In Scotland, and elsewhere, this refers to a period of three years (CEFAS 2017; NSSP 2015; NZFSA 2006). However, the minimum number samples that must be collected varies by country and depends on practical issues.

**Runoff** means water that flows over the ground surface or through the ground directly or indirectly into drains, streams, rivers before reaching a SPA or SWPA.

**Sample National Grid Reference (Sample NGR)**: The National Grid Reference of any *E. coli* sample monitoring point (to an accuracy of 10m).

**Sampler/sampling officer (Authorised):** A person who takes samples of bivalve molluscs from a SPA for the purposes of official control testing under *Regulation (EC) No 854/2004*. A sampling officer is a sampler directly employed by the FSS or other control body delegated responsibility for official control sampling.

**Sampling depth:** Depth of sampling for bivalves grown on ropes. Sampling should target the depth that yields the highest *E. coli* results (EURL-CEFAS 2017a).

**Sampling frequency as part of the Sampling Plan for SPAs:** A frequency that must ensure that the shellfish *E. coli* results are as representative as possible for the area considered (EURL-CEFAS 2017a).

**Sampling Plan for Shellfish Production Areas (SPAs): A** formal record of the intended sampling to be undertaken in SPA with respect to species, position of sampling points and frequency of sampling as part of Food Standards Scotlands' (FSS) monitoring programme (EURL-CEFAS 2017a). The components of the Sampling Plan are identified following the sanitary survey carried out on the SPA and include: SPA boundaries, **Site Identification Number** (SIN), geographical location; the **Representative Monitoring Point** (**RMP);** sampling tolerance; sampling frequency; sampling depth; method of sampling; and authorised sampling officer.
**Sampling tolerance as part of the Sampling Plan for SPAs**: The allowed maximum distance from identified RMP, in metres (EURL-CEFAS 2017a).

**Sanitary Survey on behalf of Food Standards Scotland (FSS): A** written evaluation of the sources of faecal contamination in or near a SPA, together with an assessment of the potential impact of these sources on shellfish microbial contamination within a SPA (Guide). The sanitary survey identifies a Sampling Plan prior to granting classification for the commercial harvesting of a shellfish species from an area (*Regulation (EC) 854/2004*-Annex II, par. 6). FSS undertakes sanitary surveys only after an application for commercial harvesting has been submitted (FSS protocol 2017).

**Sanitary Survey:** In the USA and New Zealand and countries that have signed a MOU with these countries it means the written evaluation report of all environmental factors, including actual and potential pollution sources, which have a bearing on the water quality in a shellfish growing area (NSSP 2015; NZFSA 2006). In addition to identifying the sampling plan it identifies the classification of a production area.

**Scotland's National Marine Plan** (SG 2015): It covers the management of both Scottish inshore waters (out to 12 nautical miles) and offshore waters (12 to 200 nautical miles). It also applies to the exercise of both reserved and devolved functions. It has been prepared in accordance with the *Directive 2014/89/EU* which came into force in July 2014. This Directive introduces a framework for maritime spatial planning and aims to promote the sustainable development of marine areas and the sustainable use of marine resources. It also sets out a number of minimum requirements all of which have been addressed in this plan. In doing so, and in accordance with article 5(3) of the Directive, Marine Scotland have considered a wide range of sectoral uses and activities and have determined how these different objectives are reflected and weighted in the marine plan. Land-sea interactions have also been taken into account as part of the marine planning process.

**Seasonal (Or Part-year) classification of SPAs** by FSS: An official established classification based on results from an extensive number of sampling occasions (i.e. a minimum of 24 samples from the most recent three years) during a specified period of time (season) that results in significantly different shellfish *E. coli* results than other periods (seasons) during a year (FSS protocol 2017).

**Shellfish *E. coli* monitoring**: Sampling of *E. coli* from shellfish flesh or intravalvular liquid.

**Shellfish Harvest (Commercial harvesting):** In Scotland and the EU, by reference to the *Regulation (EC) 854/2004,* shellfish harvest applies to collection of live bivalve mollusc species for commercial purposes, regardless of production method; hereafter this is reported as commercial harvesting. Likewise, in New Zealand, shellfish harvest means commercial harvesting (NZFSA 2006). However, in the USA the term has a broader meaning as it refers to the act of removing bivalve mollusc shellfish from growing areas and its placement in a manmade conveyance or other means of transport (NSSP 2015).

**Shellfish industry** means the persons who, or organisations that, are considered to represent that shellfish industry.

**Shellfish Production (or Harvest) Areas (SPAs):** This term refers to shellfish growing areas where shellfish species are commercially cultivated or harvested from natural beds that contain commercial quantities (Aquaculture Scotland - Glossary n.d.; FSS n.d.; *Regulation (EC) 854/2004*).

**Shellfish Water Protected Area (SWPA) environmental objectives:** The objectives required to comply with Article 4 of the Water Framework Directive-WFD (*Directive 2000/60/EC*) (environmental objectives) and the shellfish water protected area objectives set in the Water Environment and Services (Scotland) Act 2003 (9.7), which refers to such objectives as SEPA considers necessary or desirable to improve or protect that area in order to support shellfish life and growth and to contribute to the high quality of shellfish products suitable for human consumption.

**Shellfish Water Protected Areas (SWPAs):** This term is exclusively used in the context of the Water Framework Directive (2000/60/EC), which repealed the Shellfish Water Directive (*79/923/EEC* and *2006/113/EC*). In the Scottish legislation, Section 5A of the Water Environment and Water Services (Scotland) Act 2003 as amended) and the Aquaculture and Fisheries (Scotland) Act 2013 (asp 7) on the Protection of Shellfish waters provide for the designation of any area of coastal water or transitional water in Scotland as a SWPA where this is considered necessary or desirable for the protection or development of significant shellfish production. Designations aim to ensure the protection or development of economically significant shellfish production through a package of measures integrated within the river basin management planning (RBMP) process (SG 2016).

**Shellfish water quality standards (or criteria)**: The most probable number of *E. coli* per 100g sample of shellfish flesh and intravalvular liquid (SG Directions 2015; 2016).

**Shellfish water quality:** By reference to WFD, and the SG Directions (2015; 2016), this term means the quality of a SWPA, assessed in accordance with the shellfish water quality standards (criteria) for SWPAs.

**Shellfish waters:** This term is reported in the repealed EU Shellfish Water Directives-SWD (*79/923/EEC* and *2006/113/EC*) and applies to those coastal and brackish waters designated by the EU Member States as needing protection or improvement in order to support shellfish (bivalve and gastropod molluscs) life and growth and thus to contribute to the high quality of shellfish products directly edible by man. For historical reasons pertaining to the progress from the inception of the SWD to its repeal by the WFD, in Scotland this term has been used interchangeably with the term shellfish growing area or shellfish growing waters (e.g. SG 2016) The NSSP (2015) in the USA further specifies that shellfish growing area means any site which supports or could support the propagation of bivalves such as oysters, clams, mussels and scallops (except when the final product form is the adductor muscle only) by natural *or* artificial means*.* However, the NZFSA (2006) specifications in New Zealand explicitly define shellfish growing waters as areas used for the harvesting of natural bivalve mollusc beds or the cultivation of bivalves for commercial purposes.

**Shellfish:** A broad term for fisheries comprising crustaceans (such as lobsters and crabs) and molluscs (such as scallops, razorfish cockles, mussels, whelks and periwinkles) (SG 2015b); or, more generally, for bivalve and gastropod mollusc, crustacean and echinoderm foodstuff (*Regulation (EC) 853/2004*). More rarely, it may also refer to freshwater molluscs or crabs and to sea squirts (tunicates) (e.g. Decree of 6 November of 2013). The species referred to by the term Shellfish vary widely and, to some extent, this depends on a country's coastal habitats, cuisine as well as the harvest-method (fishing, cultivation, hand gathering).

**Shellfishery:** A site where shellfish occur. They can be wild shellfisheries, where populations of shellfish occur naturally; or private shellfisheries, which are set up for commercial exploitation of species such as oysters, mussels and clams.

**Shipping/boating activity for sanitary surveys:** Numbers of people per vessel and boats, type of on-board sewage treatment. (EURL-CEFAS 2017a).

**Shoreline Survey as part of the Sanitary Survey process for FSS: A** physical survey of the shoreline and the area adjacent to the shore to confirm the presence of potentially contaminating sources first identified through a desk-based study, and to identify additional potential sources of contamination (CEFAS n.d.). This includes walking the shoreline to identify septic tank outfalls and other potential sources of faecal contamination, collecting samples of both water and shellfish for bacteriological analysis, and identifying the exact location of the shellfishery (CEFAS n.d.).

**Site Identification Number (SIN) of FSS shellfish *E. coli* samples:** The unique reference number used by FSS for a shellfish harvesting area (FSS protocol 2017).

**Source apportionment**: Catchment-scale investigations and monitoring directed to define the complex and highly episodic mix of inputs from both point and diffuse terrestrial sources in order to explain continued non-compliances (Kay et al 2010).

**Species-area (as of SPA) classification**: By reference to *Regulation (EC) 854/2004*, this is a species-specific classification given to a SPA by a competent authority every year once sufficient samples of ***E. coli*** concentrations in shellfish flesh or intravalvular liquid from specimens collected within that specific SPA have been submitted within the official control monitoring programme for SPA classification (Aquaculture Scotland-Glossary n.d., EURL-CEFAS 2017a). Classification grade of SPAs (Class "A", "B", "C") determines the extent of microbiological contamination in shellfish and ensures that shellfish harvested from this SPA meet the food safety criteria laid down in Annex II, Chapter II of *Regulation (EC) 854/2004* as amended by *Regulation (EC) 2015/2285* and the health standards laid down in *Regulation (EC) 853/2004.*

**Species-specific monitoring:** Sampling only for one shellfish species from a single or multiple monitoring points.

**Random or Systematic random sampling strategy (SRS strategy)**: A sampling strategy based on a randomly selected dates of sampling, which may be applied to a commercially harvested area not impacted by point source pollution (modified from NZFSA 2006; NSSP 2015). Generally, this involves monthly or bimonthly monitoring and requires more than 30 samples to cover the range of environmental conditions in a specific area (NSSP 2015; NZFSA 2006).

**Water FIO monitoring:** Sampling of faecal indicator organisms such as Faecal Coliforms, Total Coliforms, Interstinal Enterococci or *E. coli* from the coastal or estuarine water.

**Worst-condition or Adverse Pollution Condition** means a state or situation caused by meteorological, hydrological or seasonal events or point source discharges that has historically resulted in elevated levels of microorganisms in shellfish and/ or water (NSSP 2015; NZFSA 2006). The Guide identifies the following conditions as worst-case: high rainfall, storm events, high river flows (EURL-CEFAS 2017a).

# Appendix II - Methods

**Types and sources of data:** Data analyses were based on already available EXCEL or GIS-linked data collected by FSS; SEPA; SG; the MetOffice (n.d.) (until 2015); Edina AgCensus (n.d.) (until 2015); the Office for National Statistics (n.d.) (2011 Census); and the Centre for Ecology and Hydrology-CEH (Land Use/Land Cover data 2007) (Table II.1). The bulk of shellfish *E. coli* data for the period 1999-2017 and the boundaries of currently classified SPAs (as of 2017) were provided by FSS. SEPA[2] contributed a small number of shellfish *E. coli* data, which were used for the 2014 SWPA classification. The GIS-linked data collected included: boundaries of SWPAs and overlapping SPAs (i.e. SPAs sitting within SWPAs); boundaries of catchments and waterbodies draining to SWPAs and overlapping SPAs (hereafter reported as source-catchments and water bodies); land use data (LCM07 map); locations of public and private effluent discharges; daily rainfall; human resident population, livestock species data; locations of Grey Seal colonies; and locations of bathing water sampling stations. The boundaries of SWPAs were provided by the SG. The boundaries of source-catchments and the septic tank locations were provided by SEPA. Rain, livestock, waste water and population data were freely available online (Table II. 1).

**Shellfish *E. coli* data from currently classified SPAs - Validation:** The locations of shellfish *E. coli* data were validated through map visualisations and checks of the national grid reference (NGR). Data with correct NGR were geocoded. Then, shellfish *E. coli* data collected from 1999 to 2017 with a NGR within currently designated SWPAS were selected for the analyses. This helped to identify the currently classified SPAs sitting within currently designated SWPAs. Only currently classified SPAs sitting within SWPAs were used for the analyses because the boundaries and NGR of previously (pre-2017) classified SPAs were not available in a shapefile format and due to a great degree of discrepancies between the location of shellfish *E. coli* samples and their assigned SPA.

**Indicators of catchment *E. coli* sources (catchment indicators):** The source-catchments were assumed to be a potential, direct or indirect, source of *E. coli* contamination for their corresponding SWPAs. Therefore, livestock, waste water effluent, population, and rain data were used as indicators of the effect of catchment-based sources of *E. coli*. Livestock data indicated agricultural sources; sewage effluent, septic tanks and population indicated human sources of *E. coli*; and rain indicated both livestock and human sources of *E. coli* transported via rain driven land runoff and stream discharges. The indicator data were aggregated on catchment scale for each SWPA source-catchment.

***Catchment İndicator: Daily Precipitation data***

The dataset used was extracted from UK MetOffice Gridded Precipitation Data (5 km X 5 km Raster Cells): daily precipitation (1961-2015). UK MetOffice interpolated station data to a regular grid based on normalised rainfall values. Quality control of this interpolation method has been performed visually by checking for inconsistencies in the gridded dataset (Perry and Hollis 2005a; b; UK MetOffice n.d.)[3]. The UK Metoffice gridded precipitation dataset is a standard product used in many hydrological and other applications.

[2] SEPA provided environmental and shellfish *E. coli* data collected under the Shellfish Water Directive (SWD) before its repeal by WFD (see Section 3.1 and Annex III), and more recently in selected SWPAs (e.g. Loch Ryan). However, data from Loch Ryan were not used because of the temporally limited record, which could not be related to the catchment indicator data range (1999-2015).

[3] The description by UK MetOffice (n.d.) refers to 1961-1990 only; however the methodology has been applied by UK MetOffice to compile the raster data up to 2015.

Table II.1 Types and sources of data used in the analyses

| Table 1. Summary of data types, sources and challenges. | | | | | |
|---|---|---|---|---|---|
| Type of data | Temporal range | Source | Format | Accessibility | Data issues/Pre-analysis processing |
| Shellfish *E. coli* concentration | 1999-2017<br><br>2011-2013 and 2016-2017 | FSS<br><br>SEPA | EXCEL data | Quantitative/ Restricted | No georeferencing and discrepancies in NGR recording / Validation though map visualisation and georeferencing of correct NGRs. |
| Source-catchment boundaries | Depends on SWPA designations | SEPA | Polygon vector data | Qualitative/ Upon Request | Boundaries of catchments draining to SWPAs and SPAs sitting within SWPAs (hereafter reported as source-catchments) (polygon vector data). SWPA source-catchments were identified by SEPA as part of the RBMP process. However, SPA source-catchments have not been identified by FSS or SEPA. SPAs intersecting SWPAs do not match 100% to SWPA source-catchments. |
| SPA boundaries | 2017<br><br>(currently designated) | FSS | Polygon vector data | Qualitative/ Upon Request from FSS | SIN of boundaries and SIN of shellfish *E. coli* samples did not always coincide/ Identification and selection of correct SPA samples |
| SWPA boundaries | 2017<br><br>(currently designated) | SG | Polygon vector data | Qualitative / Upon Request from SG | SWPA 85 was not available; it was represented by the polygon for SPA: Loch Ryan , which covers only the south, inner area of the loch. |
| Land Use/Land Cover LCM07 map | 2007 | CEH | 25*25m raster data | Open Access from Land Use/Land Cover data (2007) | A more recent open access version (LCM15) was available but at a coarser resolution (5X5KM). The LCM15 25X25m is not open access. |
| Surface waterbody boundaries | Not relevant | SEPA | Polygon vector data | Open Access from SEPA (2017) | |
| Private Septic Tank locations | 2016 | SEPA | point vector data | Qualitative/ Upon Request from SEPA | Locations are modelled and address base points not on public sewer network but there is no information on associated FIO discharges or number of malfunctioning Septic Tanks and any temporal variation/ Calculation of number of Septic Tanks per source-catchment as an indication of risk to be analysed in the context of rainfall |
| Waste Water effluent locations | 2016 | SEPA | point vector data | Qualitative/ Open Access from SEPA (2017) | This refers to locations of: Sewage and Trade effluent, Combined Sewage Outflows (CSOs) and Emergency outflows, and public Septic Tank effluent. Population served and frequency of discharges is available There is no information on associated FIO discharges / Calculation of number of location per source-catchment as an indication of risk to be analysed in the context of rainfall |
| Daily precipitation (mm) per 2X2 Km | 1999-2015 | MetOffice | | Quantitative/ Open Access from MetOffice (n.d.) | This is modelled for areas 2x2 km but smaller coastal areas have not been modelled / Estimation of weighted average per source-catchment and use of nearest neighbouring catchment data |
| Population | 2011 census | Office for National Statistics | | Quantitative/ Open Access from National Statistics data for population (n.d.) | This is given on number of individuals per parish. / Clipping the parish data to include only source-catchment data |
| Livestock number of livestock per 5X5 km. | 1999-2015 | Edina AgCensus | | Quantitative/ Open Access from Edina AgCensus (n.d.) | Estimation of livestock density per source-catchment (Annex 2) |

| Table II.2. Catchments not covered by the UK MetOffice gridded precipitation data and nearest catchment used for deriving source-catchment daily precipitation data for this report. | | | | |
|---|---|---|---|---|
| Shapefile | Catchment no data coverage | Number | Nearest catchment | Number |
| ShellfishAreaCatchments_WigtownBay | East Tarbert | 15 | Loch Tarbert | 61 |
| ShellfishAreaCatchments_WigtownBay | Hawkness | 10 | Uyea Sound | 79 |
| Clipped_coastal_10Nov | Gigha | 14 | Knapdale | 39 |
| Clipped_coastal_10Nov | Oronsay | 16 | Colonsay | 53 |
| Clipped_coastal_10Nov | Eilean Mor | 36 | Loch Fyne | 65 |
| Clipped_coastal_10Nov | Pabay | 58 | Scalpay | 61 |
| Clipped_coastal_10Nov | Eilean Ban | 62 | Sounds | 43 |

The method to derive precipitation for shellfish catchments included the following steps:

- Intersection of catchments and MetOffice raster cells.

- Estimation of area weighted average of Daily precipitation per catchment s (for those parts of the catchments covered by the UK MetOffice dataset). The catchments and the raster cells have been intersected whereby some raster cells would be entirely located in the catchment, others just to a proportion. The areas of each cell in each respective catchment have been calculated and summarized in a table using a geospatial software. For each day, the daily precipitation of each raster cell has been multiplied by the area of each raster cell [km²] in the catchment. The values of all raster cells have been added and divided by the total catchment area [km²]. Finally, the values have been rounded to a precision of two digits. The equation describing these calculations is:

$$P_{catchment} = \frac{\sum_{i=1}^{n\ cells} P_{cell} * Area_{cell}}{Area_{catchment}}$$

Where,
$P_{catchment}$= Area weighted average or daily precipitation;
$P_{cell}$=Precipitation in each 5*5 Km raster cell
$Area_{cell}$ = 5*5 km
$Area_{catchment}$= the area of each SWPA source-catchment (this data came from SEPA).

For these calculations a script/ programme has been written in a programming language called R. The programming language & software environment R are open-source. This script:

- Reads in the table containing all raster cells that are located in the catchments, and which area of the respective catchment they cover

- Reads in the gridded precipitation values from ASCII files for each raster cell, assigns the raster cells to the respective catchments and automatically calculates the daily precipitation for each catchment

- Stores the daily precipitation of all catchment in a table.

The gridded MetOffice data have limited coverage in coastal regions, so that gridded precipitation data are not available for all of the catchments. For these, precipitation data from the nearest neighbouring catchment should be used (Table II.2).

***Catchment İndicator: Livestock counts***

Raw livestock counts (2*2 km) for cattle/sheep/pigs/poultry/goats/horses/deer from 1997 – 2015 (minus years where one or more were not collected) were downloaded as csv files from Edina AgCensus data base. These data were downscaled to get a 'better' idea of where within each 2*2km grid the various livestock may be found in relation to land use data from the LCM07 database. This prevented false positive values due to assumptions of homogeneous distribution of animals (which is not always true) to influence the determination of number of animals. The method has been described in detail in a previous CREW report by Akoumianaki et al (2016).

EDINA have taken the raw data collected during the survey and developed a method of extrapolating (upscaling) and spatially weighting it so it can be represented as 2*2km grids in line with keeping confidential information on farm-scale management secure. The major 'flaw' with using Edina data would be that upscaled data are downscaled again but in a different way based on the assumptions on how the livestock would be distributed (see method developed and described by Akoumianaki et al 2016).

***Other Catchment İndicators: Land Use, Septic Tanks, Population, Point sources, Wildlife***

Percentage land use was assessed on a waterbody scale per surface waterbody and on source –catchment scale to estimate FIO export on the basis of Kay et al (2008a) predictions for areas with ≥75% urban, semi-urban or rural land use. Numbers of locations of septic tanks were integrated into numbers per source-catchments. Population from parishes within source-catchments (partly or entirely) were integrated to give values on source-catchment scale. Point sources and known grey seal colonies were related with a limited number of SWPAs. These data were not used in statistical analyses but were assessed as qualitative information on map-based observations.

**Development of a GIS-linked catchment-shellfish *E. coli* database:** An EXCEL file was created to link the identification numbers (i.d.) of SWPAs and SPAs sitting within SWPAs (as two different columns) with shellfish *E. coli* data for each sampling date and station from 1999 to 2017 and the indicator data for SWPA source-catchments (as separate columns per type of indicator). Other data linked to shellfish *E. coli* samples, such as date, site identification number (SIN) and classification on the date of sampling, were also included. This created a comprehensive GIS-linked database (hereafter reported as the catchment-shellfish *E. coli* database) linking shellfish *E. coli* data and classification in SWPAs and SPAs sitting within with data on indicators of catchment *E. coli* sources. Data (and their locations) collected by SEPA were not included in the database.

**Risk of faecal contamination by land use and type of sources:** The percentage (%) of improved pasture (IP), rough grazing (RG), woodland (WL) and built-up (BU) areas in each source-catchment and waterbody was estimated using the LCM07 raster map to assess faecal indicator organism (FIO) export to SWPAs in relation to land use, as of Kay et al (2008a).

Calculating the percentage of each broad habitat (BH) category of the LCM07 raster map (as a proxy of land use) in SWPA source-catchment and waterbodies helped to estimate the FIO catchment export coefficient to SWPAs using the coefficients presented by Kay (2008a); see also Table IV.1. For each SWPA source-catchment and waterbody, this involved the following steps (A python script was written to automate the generation of the output):

1. Clip all LCM07 data for the area covered by source-catchments and waterbodies.
2. Recalculate polygon area and generate summary table with BH as the field to summarise on and sum on recalculated areas.
3. Add a column (field) to the output table and populate the column with name of the SWPA catchment and waterbody (different shapefiles for catchments and waterbodies).
4. Combine all the tables generated to form a table and retain 3 columns – Catchment or Waterbody name, BH and Area.
5. Pivot the table above into – Row label: Waterbody name; Column label: BH; and Values: sum area. This generates the percentage area for each BH of the catchment or waterbody.
6. Identify sources catchments and waterbodies with percentage of IP, RG and WL over the total source-catchment or waterbody area equal to or above 75% and the percentages of built up areas, as a measure of urban land use (see also Table IV.1).

**Identification of rare rainfall events:** By reference to Kendon et al (2016), "heavy rainfall" and extreme rainfall events were estimated as the rainfall levels above the 95th- and 99th-percentiles of the whole rain data series from all source-catchments during 1999-2015, respectively. The relationship between dates of "heavy" and extreme rainfall and shellfish *E. coli* concentrations above the 700-threshold was also examined to inform understanding of rainstorm on shellfish *E. coli*.

**Statistical analysis-Regression.** The catchment-shellfish *E. coli* database was used for statistical analyses for the potential temporal and spatial effects of indicators of catchment sources of *E. coli* on shellfish *E. coli* concentrations as well as to explore temporal variation in rain and in each shellfish species' *E. coli* concentrations. The *E. coli* data used in the analysis were from locations that are both within a current SPA and within a SWPA. A linear mixed model was fitted to the log-transformed *E. coli* counts. Four species (cockles, mussels, native oysters and Pacific oysters) were examined. Other species were excluded due to insufficient data. Rainfall data were only available for the period 1999-2015 so the analysis covered that period. The model included random effects for the SWPA and the SPA sitting within the SWPA. The possible fixed effects considered were species of shellfish, month of the year, human population, number of septic tanks, rainfall in the two days prior to the sample being taken, rainfall in the period three to seven days prior to the sample being taken, density of livestock (comprising sheep, cattle, pigs, poultry, goats, horses and deer) and a linear trend with year. Human and livestock populations and number of septic tanks were from the coastal catchment whereas rainfall was based on the area consisting of the coastal and any upstream catchment. Sheep, cattle, pigs and poultry separately were fitted separately rather than as total livestock. Goats, horses and deer make up less than 5% of livestock numbers in all catchments so were not included when livestock types were fitted separately.

**Statistical analysis-Principal Component Analysis (PCA):** PCA was carried out on average annual rainfall, average livestock density, catchment area (log transformed), population (log transformed), and number of septic tanks (log (x+1) transformed) for SWPAs.

**Trial desk studies:** SEPA provided a list of 22 SWPAs flagged by harvesters as a priority for the shellfish industry. Of these, four catchments (i.e. Cat Firth, Cromarty Bay, Loch Ryan and Loch Creran) were individually analysed to:

- Assess the existing monitoring design, where monitoring data were available from currently classified areas or sanitary surveys;
- Explore the effect of species, rain and month on classification.

- Explore the feasibility of desk studies based on examination of catchment data, historical shellfish *E. coli* data and their assessment in the context of evidence presented in the pre-2015 sanitary survey reports produced by SEPA.

# Appendix III - Additional information supporting the review of governance frameworks

The *Regulation (EC) 854/2004* stipulates the following official controls (OCs) for bivalve shellfish:

- Classification of SPAs and relaying areas based on compliance with specified shellfish *E. coli* concentrations for each class (*Annex II; A;par. 1-5*). Prior to classification, sanitary surveys must be undertaken (*Annex II; A; par. 6*)
- Monitoring of classified SPAs and relaying areas according to the specified sampling plan (Box 2) to check microbiological quality at regular intervals (*Annex II; B)*.
- Decisions after monitoring (*Annex II; C*).
- Additional monitoring requirements, e.g. for areas where harvesting has been forbidden or subjected to special conditions and post-harvest to verify that the microbiological quality of shellfish does not constitute a hazard to public health (*Annex II; D*).
- Recording and exchange of information, which refers to: establishing and keeping up-to-date a list of currently classified SPAs and relaying areas (including sampling location, boundaries and class); informing on any change in microbiological quality of SPAs; and acting promptly where the official controls show that a SPA must be closed or reclassified or must be re-opened. *( : Annex II; E*).
- Food business operators' own checks *(: Annex II; F*).

The microbiological sampling plan should be based on the following requirements:

(*Annex II: Chapter II.A. par.2*):
"The competent authority must classify production areas from which it authorises the harvesting of live bivalve molluscs as being of one of three categories according to the level of faecal contamination. It may, where appropriate, do so in cooperation with the food business operator. In order to classify production areas, the competent authority must define a review period for sampling data from each production and relaying area in order to determine compliance with the standards referred to in this paragraph and in paragraphs 3, 4 and 5."

(*Annex II: Chapter II.B. par1*):
"Classified relaying and production areas must be periodically monitored to check:

(a) that there is no malpractice with regard to the origin, provenance and destination of live bivalve molluscs;
(b) the microbiological quality of live bivalve molluscs in relation to the production and relaying areas"

(*Annex II: Chapter II.B. par2*):
"…sampling plans must be drawn up providing for such checks to take place at regular intervals, or on a case-by-case basis if harvesting periods are irregular. The geographical distribution of the sampling points and the sampling frequency must ensure that the results of the analysis are as representative as possible for the area considered."

(*Annex II: Chapter II.B. par3*):
"Sampling plans to check the microbiological quality of live bivalve molluscs must take particular account of:

(a) the likely variation in faecal contamination, and
(b) the parameters referred to in paragraph 6 of Part A."

## III.2 Roles of competent authorities

The implementation of the OCs for commercially harvested bivalve shellfish and their enforcement is the responsibility of competent authorities. The type of organizations considered as competent authorities, and therefore what they can do with respect to the OCs varies by country. For example:

- In Scotland, FSS has the overall responsibility for the organisation and undertaking of the OC programmes required by the *Regulation (EC) 854/2004*. FSS implements monitoring and classification; however, sanitary surveys are contracted to other organisations (i.e. CEFAS and SAMS). In addition, local authorities play a role in licensing bivalve shellfish operations and in ensuring the safety of live bivalve shellfish "from farm to fork" in their jurisdictions, even when shellfish *E. coli* results out-with the classification rating are found (FSS protocol 2017); see also Appendix V.1.
- In England and Wales and in Northern Ireland (NI), the food standard agencies of the devolved administrations have competency for the monitoring, classification and undertaking of sanitary surveys in their jurisdictions (FSA-England and Wales protocol 2017; FSA-NI protocol 2017).
- In Ireland, the responsibility for developing and applying OC programmes lies with the Sea-Fisheries Protection Authority (SFPA 2017). Microbiological monitoring is contracted to the Marine Institute and the undertaking of sanitary survey data to the Marine Institute, the Sea Fisheries Board, the Irish Shellfish Association, the Local Authorities and the Food Standard Agency of Ireland (SFPA 2017). The undertaking of sanitary surveys is also supported by the implementation of the Shellfish Directive and the Department of Environment, Health

and Local Governance (SFPA 2017).

- In France, a number of organisations are involved in implementing the *Regulation (EC) 854/2004* on national, regional and local levels, as follows (French Institute for the exploitation of marine resources-IFREMER 2017):
  - o The overall responsibility for tailoring the requirements of the *Regulation (EC) 854/2004* to the context of the French shellfish industry lies with the General Directorate for Food (DGAL) under the Ministry for Food and Agriculture.
- The classification is established by the departmental prefect, which is the competent authority for the microbiological inspections in the *département* via a prefectural order that applies to commercial shellfish harvesting.
  - o The monitoring of SPAs is the responsibility of the government, and IFREMER operates the microbiological monitoring network.
  - o The formulation of specifications for viral monitoring in shellfish, for public health purposes, is the responsibility of the DGAL and IFREMER, through the National Reference Laboratory (NRL).
- In the USA, the prime agency regulating seafood is the US Food and Drug Administration (FDA) (NSSP 2015). OCs are enforced at Federal and State level through the National Shellfish Sanitation Programme (NSSP) and the Interstate Shellfish Sanitation Conference (ISSC). Participants in the NSSP include agencies from producing and non-producing states, FDA, the Environment Protection Agency and the shellfish industry.
  - o State agencies monitor shellfish growing waters to determine that they are safe before harvesting.
  - o FDA routinely audits the classification given at a State's shellfish harvesting waters to verify that none pose a threat to public health.
  - o FDA, State and Federal Law Enforcement officers have responsibility for preventing illegal harvesting from closed waters and for ensuring that all shellfish in interstate commerce are properly labelled.
- In New Zealand, the New Zealand Food Safety Authority (NZFSA 2006), which is a public agency under the Ministry of Health and the Ministry of Agriculture and Forestry Food Assurance Authority, has the overall responsibility for protecting and promoting public health and safety, and for facilitating market access for New Zealand's food and food-related products. Amongst other competencies:
  - o It provides the Minister for Food Safety with policy advice on food and food-related issues.
  - o It sets standards related to food safety and suitability as required by legislation, or market access.
  - o It implements programmes that ensure all safety and suitability requirements are met;
  - o It enforces legislative requirements.

## III.3 The Guide: Recommendations on sanitary surveys, monitoring and classification.

### *III.3.1. Content of the Sanitary survey report*

According to the Guide a sanitary survey report should be prepared based on the information gathered and the assessments made during the sanitary survey. A sanitary survey report should include the following:

- Overview of bivalve shellfishery
- Description of shellfishery
  Location and extent
  Bivalve species
  Aquaculture or wild stocks
  Production area or relay area
  Seasonality of harvest
  Harvesting techniques
  Any controls under other legislation
- Location, size and treatment level of human sources of contamination[1]
- Location and estimated volume/load of agricultural sources of contamination[1]
- Significant wild animal/bird populations[1]
- Records of shoreline surveys
- Hydrography/hydrodynamics
- Analyses of historical microbiological data
- Records of bacteriological survey results
- Assessment of effect on contamination of bivalve molluscs
- Sampling plan

*Guidance on handling and storage of data*

*Storage*

Data from the monitoring programme should be stored in a secure database, which has tables containing the following:
i) Information on the sampling plans
ii) Information relating to the samples
iii) Results of the testing of samples

The following may also be considered for inclusion in the database:
i) Results of the sanitary survey
ii) Information on pollution events
iii) Results of investigations into pollution events and anomalous *E. coli* results

*Security features*
In order to maintain the integrity of the data held within the system, access should be password protected and users are individually assigned read only or write permissions according to organisational requirements.

### III.3.2 Uptake of recommendations by EU Member States

The interpretation and implementation of the requirements of the *Regulation (EC) 854/2004* and the recommendations of the Guide (since 2006) vary by Member State. The degree of discrepancies among Member States remains unexplored. Evidence on the way some OCs are implemented among Member States is illustrated below.

*Review period, i.e. years to review established classification*
The use of the 24 most recent samples collected in a period of three years is applied in: Denmark, France, Germany, Greece, Ireland, Italy, Norway, Portugal, Galicia (Spain), and Scotland (EURL-CEFAS 2017b). FSA in England and Wales and in Northern Ireland uses the three years' worth of data and 30 samples (i.e. a minimum of 10 samples a year) for A classification and 24 samples for B or C classification (EURL-CEFAS 2017b; FSA-England &Wales 2017; FSA NI 2017). Using 24 to 30 samples to review established classification is in line with the Guide's recommendation (Table 2).

*Seasonal classification*
This refers to established classification that reflects consistent seasonal variation. A recent review showed that seasonal classification is currently applied in France, Galicia (Spain), Ireland, Italy, and the UK, with Scotland granting seasonal classification more often than any other EU jurisdiction (EURL-CEFAS 2017b). Italy and the UK, including Scotland, use a three-year dataset for each season; however, Scotland may use a one-year dataset (EURL-CEFAS 2017b).

*Sanitary surveys*
A comparison of standard operational procedures (SOPs) for sanitary surveys in new areas in the UK (e.g. FSS protocol 2017), in France (DGAL 2016) and Ireland (SFPA 2017) shows that these SOPs are generally in line with the recommendations for sanitary surveys mentioned in the Guide. However, the tasks vary by country depending on availability of resources, data and budget limitations. For example, sanitary surveys in the UK including Scotland comprise all the tasks mentioned in the Guide apart from data storage (CEFAS n.d.; Kershaw et al 2012; CEFAS 2018[4]). The most recent Code of Practice applied in Ireland does not mention salinity measurements. In France, sanitary surveys include three major tasks i.e. desk studies, a field visit and microbiological and chemical sampling for a year or longer (IFREMER-REMI 2015).

In addition, the term "new harvesting area" may mean two different things with important practical implications for the implementation of sanitary surveys (Lee 2009). For some Member States the term applies to any new SPA, even if this is located within a broader area that has been surveyed to classify another SPA (for the same or different species). This approach triggered a review of the sanitary survey of the broader area covering both the new and existing shell-fisheries and the identification of a sampling plan specific for each SPA (new and old) using the data from the same sanitary survey (e.g. CEFAS approach to sanitary surveys in Scotland). Other Member States were found to interpret the term "new" to cover only new broad areas and thus have not undertaken sanitary surveys to identify a sampling plan for "new" operations within those broad areas. The most recent Guide published in 2017 has not provided any clarity on this.

### III.4 Caveats related to the implementation of the Regulation (EC) 854/2004

The following questions (Q) can be raised in relation to the requirements of *Regulation (EC) 854/2004.*

<u>Q1: Can SPA classification based on historical data predict risk of faecal contamination in the future?</u> Classification of SPAs under the *Regulation (EC) 854/2004* is designed to deliver an assessment of the risk of faecal contamination based on historical time series of shellfish *E. coli* monitoring data. Thereafter, classification rating, as a post-harvest strategy for the mitigation of faecal contamination (e.g. Lee and Murray 2010), determines the level of treatment which needs to be applied to bivalves prior to sale for consumption. As such, classification is instrumental in protecting consumers from the risk of foodborne disease, but this depends on the ability of historical monitoring data to predict the risk of faecal contamination in the future (e.g. the upcoming year). This risk is determined by a varying number of site-specific environmental (catchment- and marine- based) factors which may lead to fluctuations of classification from year to year, with potential implications not only for public health but also for the sustainability of shellfisheries[5]

The Guide recommends that using at least three years' worth of data from environmentally homogeneous SPAs to grant a classification grade and regularly reviewing this classification grade[6] (i.e. annually or on a rolling basis) is essential to avoid fluctuations in the classification of SPAs.

[4] All sanitary surveys used for the analyses in this report were retrieved from CEFAS (2018).

[5] For example, downgrading may increase the cost of the shellfishery because of required changes in post-harvest treatment; or, lead to loss of employment due to a temporary prohibition of harvesting.

[6] Appendix III.2

Sanitary surveys may also help to identify locations for siting new shellfish farms at a safe distance from human and/or animal faecal sources (e.g. Kershaw et al 2012), which in turn may reduce the risk of fluctuations in classification. That said, **sanitary surveys combined with pathogen monitoring, or microbial source tracking (MST), may yield a better prediction of health risk than classification alone** (Kay et al 2010).

Q2: Is SPA classification based on shellfish *E. coli*, as a FIO, a reliable tool for determining contamination by faecal pathogens? The use of FIOs in FIL is dictated by practical reasons. FIOs are much easier and less costly to detect and enumerate than the pathogens themselves (Lee and Murray 2010). The presence of FIOs (and *E. coli*) does indicate that faecal contamination has occurred (Meays et al 2006). The problem is that small MPN counts of shellfish *E. coli* do not reliably ensure lack of risk of contamination by faecal viruses (EURL-CEFAS 2017a). Therefore, classification under the *Regulation (EC) 854/2004* is reliable only when the sampling plan and the classification programme have accounted for the presence of pathogens (EURL-CEFAS 2017a). **This requires a proper identification of sources of faecal pollution during sanitary surveys and the use of MST methods to enable effective targeting of mitigation measures** (see review by Santo Domingo and Edge 2010).

A report by the European Food Safety Authority (EFSA 2011) suggested that sanitary surveys could potentially include a viral component to enhance their use beyond the current bacterial indicator classification focus. In addition, EFSA has stated that the most effective public health measure to control human NoV infection is to produce shellfish from areas which are not faecally contaminated (EFSA 2012). An international workshop on the application of sanitary surveys also suggested that sanitary surveys in the EU and elsewhere should identify areas unsuitable for harvesting and a management plan encompassing *inter alia* closure criteria, regulator training and a Vibrio control plan (EURL-CEFAS-FDA 2013). The Guide has accounted for the risk from viruses in the recommendation for the identification of the sampling plan during the sanitary surveys.

Q3: Is it feasible to identify "seasonal variations of both human and animal populations in the catchment area, rainfall readings, waste-water treatment, etc" as required by the *Regulation (EC) 854/2004*? A review of the application of sanitary surveys in Europe showed a wide range of reasons that data on "seasonal variation" is not available (Lee 2009). For example, this data may not be required by environmental regulators. Where livestock data is available (e.g. number of animals per farm per season), this information may be accessible to one agency but not to the agency doing the sanitary survey due to confidentiality issues. Lee (2009) proposed that Member States should enable data-sharing procedures and communication between relevant regulatory authorities in their jurisdictions or develop online, open access databases. This has the potential to make the data collected by one authority/agency available to the agencies undertaking the sanitary surveys. The Guide does not provide specific recommendations on acquisition and sharing of data.

Q4: Is it feasible to "determine the characteristics of the circulation of pollutants by virtue of current patterns, bathymetry and the tidal cycle in the production area" as required by the *Regulation (EC) 854/2004* ? Hydrography (i.e. seabed depth, density, salinity ad tidal data) and hydrodynamics (i.e. the study of river, tidal and wind forcing on water circulation in an area) influence transport of faecal contaminants. When entering a coastal environment, faecal microorganisms, both free and bound to suspended sediment particles, undergo physical dilution induced by hydrography and hydrodynamics or die due to exposure to sunlight, irradiation, and salinity and temperature gradients (Crowther et al 2001; Lee and Morgan 2003; Rozen and Belkin 2001). The Guide highlights that knowledge of these processes is important in interpreting the information on sources of pollutants obtained for the sanitary survey.

However, hydrographic data, and thus hydrodynamic studies, may not be available for all coastal areas (Lee 2009). The availability of hydrographic information often depends on the area being of importance to the navy or large merchant ships or to it having been the subject of studies for other purposes, e.g. large sewage improvement schemes (Lee 2009). In addition, where this information is available, it may not be freely accessible or affordable for a competent authority. Kershaw et al (2012), based on a review of the application of sanitary surveys in England and Wales, recommended that a standard approach to hydrographic assessment is to encompass the study of available nautical and tidal charts and a discussion of how wind and density effects may modify circulation in the area. In addition, the Guide recommends hydrodynamic (process-based) modelling to SPAs where:

- Shellfish *E. coli* results do not match the desk-based assessment of pollution sources.
- There is a large bivalve shellfish production.
- Shellfish *E. coli* levels frequently exceed the classification grade granted to that SPA.
- There is a potential link between bivalve shellfish harvested from a SPA and a disease outbreak.

Q5: Is it feasible for a competent authority to obtain information for sewage discharges, including the flow and microbial content? The *Regulation (EC) 854/2004* and the Guide imply that detailed information should be obtained on sewage discharges. However, the collection of this type of data is the responsibility of the environmental regulators and public or private/domestic dischargers (Lee 2009). In practice, data on actual flows and the microbial content of the effluent are only measured for very specific purposes. Lee (2009) suggested that this information should be derived from generic scientific studies such as that by Kay *et al* (2008a) on catchment export coefficients. The Guide does not provide further information on the acquisition of data on sewage discharges. However, it is recognised that significant resources would be required to determine loadings for a number of discharges in an area over a range of conditions.

Q6: Is sampling of worst-case conditions feasible for competent (monitoring) authorities? Worst-case conditions sampling refers to the timing of sampling with respect to factors known to cause significantly higher results at the selected RMP. These may be caused by rainfall, spring/neap tide, low/high tide, wind direction and speed or be related to season (Campos et al 2013a; Lee & Morgan 2003). The factors influencing the selection of worst-case of adverse conditions would be identified using the outcome of the sanitary survey and should also account for safety and convenience issues of sampling officers. However, there would be very few potential dates and times referring to several interacting factors coinciding to produce the highest shellfish *E. coli* results at a particular RMP (Lee 2009). It has been argued that worst-case conditions could be represented spatially instead of temporally, practically suggesting the establishment of multiple RMPs (which represent worst-case locations) to capture the worst-case conditions at different sites of the SPA (Lee 2009). Alternatively, as suggested in the Guide, SPAs should be homogeneous so that a single RMP is representative of the greatest impact from faecal pollution.

Q7: Can sanitary surveys account for the impact of all catchment-based pollution sources? The *Regulation (EC) 854/2004* requires Member States to "examine the quantities of organic pollutants in relation to seasonal variations of both human and animal populations in the catchment area". It is important that this is mentioned in the legislation because knowledge of how pathogens and faecal indicator organisms (FIOs) persist within or become mobilised from different environmental matrices (such as faeces, manures, soils, stream sediments and waters) is critical to targeting mitigation measures to protect human health (Oliver *et al* 2005; 2010). In addition, the magnitude of FIO transfer across the river-sea continuum is potentially of fundamental importance for the design of the sampling and the delivery of robust classifications for SPAs. However, the word catchment and any reference to catchment-based faecal contamination sources are totally absent from the Guide published in 2017. This is an indication that the expert community on the implementation of the *Regulation (EC) 854/2004* may have not yet caught up with the WFD and the RBMP process.

Q8: Are the semi-quantitative or quantitative data assessments of sanitary survey data recommended in the Guide more robust than qualitative assessments? Experience from undertaking sanitary surveys has shown that the data collected for sanitary surveys include a range of numeric (quantitative) and descriptive (qualitative) data, which must be combined to into an overall assessment to inform the sampling plan (Lee 2009). There is a misconception that numeric (quantitative) data are more relevant to the identification of a robust sampling plan because they allow for statistical comparisons and graphs. However, some numerical data from the SPAs may be irrelevant to the purpose of the sanitary survey and indeed to classification. For example, historical samples for microbiological testing may not have been taken at locations or at times that would detect the impact of the main sources of pollution (Lee 2009; Magil et al 2008) or for commercially harvested species. The Guide does not provide clear recommendations on the framework for the data (numeric or descriptive) that must be used in relation to where or when they were collected.

## III.5 Sanitary surveys and monitoring in SWPAs in France

Sanitary surveys. The SOPs for sanitary surveys on new Professional SWPAs-SPAs are specified in the DGAL / SDSSA Technical instruction protocol (DGAL 2015). Sanitary surveys are undertaken prior to designating new Professional SWPAs-SPAs and include a desk study, a field visit and a sampling to assess the microbiological and chemical quality of the professional SWPA-SPA. Microbiological samples may be collected from one or more points for a minimum of 24 samplings per point for a minimum period of one year to take into account seasonal variability; only shellfish having stayed in the area for at least 15 days (e.g. in bags) can be established for monitoring under sanitary surveys. The points sampled during the sanitary surveys are the most sensitive points for detecting episodes of contamination potential in the area to ensure public health protection. One or several ad hoc chemical analyses may be sufficient during the sanitary survey, given the stability over time of chemical contamination; for chemical monitoring shellfish must have been kept in the area for six months.

Monitoring. The sampling strategy is based on three main elements: locating RMPs for microbiological monitoring, the species sampled and the sampling frequency (IFREMER-REMI 2017):

- Locating RMP. Professional SWPAs-SPAs are considered to be homogeneous in terms of water quality; therefore, as a general rule, only one RMP is defined as representative of an area. The RMP is selected to be a worst-location point as in the Guide. In the case of large areas, a minimum number of sampling points, taking into account the main sources of contamination, is determined. These points are placed so that they are representative of these areas and allow contamination to be detected promptly. Simulation models of FIO transport and dispersion can be used as a tool to assist in the positioning of sampling points, provided that they are available locally and that the results of the simulations are in agreement with the data observed by monitoring results. Simulation models couple a hydrodynamic model and a decay model of bacterial activity. RMP locations are reviewed annually.

- Species sampled. Although classification refers to a group of shellfish, monitoring for classification targets single or multiple species studied during the sanitary survey. In the case where a species is becoming scarce (e.g. in the case of natural beds), monitoring for classification can be applied on another species of the same group; this requires informing the Quadrige data base of monitoring data and the registry of designated areas.
- Frequency. This depends on classification (A, B or C) of the area under *Regulation (EC) 854/2004* and the risk of episodic faecal contamination[7]. The basic frequency is fortnightly or monthly, but it may also be bimonthly (every two months) (if more than three years' worth of data show stability of classification) or adapted to the period of commercial harvesting. The frequency is applied for at least three years (including initial classification data, which are collected during the sanitary survey).

## III.6 Shellfish OCs applying to the USA and New Zealand

*Procedures for sanitary surveys applied in both the USA and N.Zealand (NSSP 2015; NZFSA 2006; 2017; EURL-CEFAS 2017a)*

- The competent authority must perform a sanitary survey that collects and evaluates information concerning actual and potential pollution sources that may adversely affect the water quality in each growing area. The tasks of sanitary surveys are very similar to those described in the Guide for sanitary surveys under the *Regulation (EC) 854/2004*, with the exception of applying a catchment-based rather than shoreline-based approach.
- The sanitary survey report should include:
  - A compilation of relevant data.
  - A data analysis utilizing recognized statistical techniques.
  - Conclusions regarding the appropriate classification of the area.
  - Recommendations on follow-up actions. In areas where historical data are available, three years' worth of data is required to grant classification as the main output of the sanitary survey report.
- The sanitary survey must identify a management plan in case of conditional classification and prohibited zones under certain environmental circumstances. This links the undertaking of sanitary surveys to the designation of exclusion zones around marinas and WWTWs, or closures under high rainfall, to protect shellfish waters from viral contamination.
- Viral data may be collected as part of a sanitary survey to identify the influence of human sources.
- Sanitary surveys must be reviewed annually (because they provide a review of classification grading). Annual review of established classification requires the undertaking of a sanitary survey on the basis of shoreline observations to review pollution sources and adverse pollution conditions identified in the initial (full) sanitary survey.
- The sanitary survey process applies a catchment approach by identifying the boundaries of source-catchments and all types of pollution sources therein.
- There are clear instructions on reporting and creating a GIS-linked database of monitoring data collected during the sanitary surveys.
- Specified frequencies for updating the various sanitary survey components are necessary. Lack of written documentation precludes accurate assessment on a routine basis, and requires that, to protect the public health, the growing area be placed in the prohibited classification or closed status of its classification.

*Viral pathogen control applied in New Zealand (NZFSA2006)*

- Minimum 28-day closure event after a viral illness outbreak
- Minimum 28-day closures after a human sewage event influencing a harvest area.
- Risk Management requirements e.g. samples from at least five sampling sites in a growing area must be found negative for viruses before the area can be reopened after a virus illness event in which the growing area was implicated.

[7] The method involves the estimation of geometric mean of the samples using three years' worth of data.

# Appendix IV - Review of evidence on factors influencing shellfish faecal contamination

## IV.1 Pollution sources

This is a brief review on catchment-based and marine-based sources of faecal contamination in shellfish and shellfish waters.

### IV.1.1 Point sources of faecal indicator organisms (FIOs)

The major point sources of faecal contaminants include discharges from: combined sewage outflows (CSOs); Wastewater Treatment Works (WwTWs); storm tank overflows (STOs); public septic tanks; and poultry or pig units (Rees et al 2010; Campos et al 2013a; Meals et al 2013). Risk of faecal contamination from these sources depends on their proximity to the shoreline, or to waterways discharging at the shoreline, in shellfish waters (Kay et al 2008c). The effects of point-sources on shellfish are summarised below.

- Discharges from CSOs and STOs are associated with both treated and untreated sewage, potentially high volumes of discharge during stormflows, and are characterised by spatial and temporal variability in the patterns of delivery of contaminants (Kay et al 2008a; Campos et al 2013a). FIOs from CSOs and STOs may represent a considerable proportion of the total FIO flux, usually at the first stages of a storm event (Kay et al 2010).
- The impact of discharges from WwTWs on shellfish depends on the level of treatment, population equivalent, volume of discharge, plant performance and age and maintenance of sewage transport infrastructure (Touron et al 2007; Kay et al 2008a; Campos et al 2013a). The treated sewage effluents are often the dominant source of FIOs during dry weather, their FIO content depending on level of treatment and type of system (Kay et al 2010). However, treated sewage effluent may exhibit very different FIO concentrations following rainfall events, both reductions (due to dilution) and increases (due to increased WwTW loadings) (Kay et al 2010).
- Seasonal changes in human population due to tourism exacerbate the effects of CSO, STOs and WwTWs because sewage loadings are at their highest during these periods; however, comprehensive studies on this issue are difficult to design (Meals et al 2013).

### IV.1.2 Diffuse sources of faecal indicator organisms (FIOs)

Diffuse pollution FIO sources include: stormwater runoff from urban and rural built-up areas, agricultural land runoff, on-board ship generated sewage, septic tanks, pets and wildlife.

- Stormwater runoff can derive from roof and road surfaces contaminated with avian, domestic and wild animal faeces as well as from septic tanks and cross-connections to urban sewerage systems (Kelsey et al 2004; O'Keefe et al 2005; Parker et al 2010; Pandey et al 2014). FIO concentrations in stormwater increase with population density, percentage of impermeable cover, density of housing, domestic pet activity, temperature, septic tank usage and tidal stage (Kelsey et al 2004 and literature cited therein; Parker et al 2010). Stormwater impact may be detected some distance offshore and for a few days, potentially more than a week after a storm event (e.g. Parker et al 2010). In addition, there is evidence that concentrations and loads of microorganisms in stormwater vary during a storm event: *E. coli* may decrease but pathogens such as *Bacteroides fragilis* increase (Ahn et al 2005; Converse et al 2009).

- Land runoff. Agricultural land can act as a FIO source under specific types of land use, such as application of animal manures and sewage sludge to arable land (Touron et al 2007); and farm steadings and grazing livestock (Kay et al 2005). FIO transport from source areas to shellfish waters can be controlled by the implementation of manure treatment technologies and specific agricultural mitigation measures (e.g. timing of manure application, slurry storage, and installation of wetlands, buffer strips and retention ponds) to enhance natural FIO die-off or reduce FIO losses to waterways (Chadwick et al 2008; Kay et al 2012; Porter et al 2017). Risk of faecal pollution increases with grazing livestock density, slope, faster flowpaths[8] and limited slurry storage capacity in source-catchments (e.g. Oliver et al. 2009; Kay et al 2012; Murphy et al 2015). FIO export per type of land use is discussed in Appendix IV.6.

- Overboard waste disposal from ships and recreational/ fishing boats can be an episodic-intermittent, marine source of raw sewage to shellfish waters (Kelsey et al 2004; Campos et al 2013a). Untreated sewage can be discharged only at a distance of more than 12 nautical miles from the nearest land according to the International Convention on the prevention of Pollution by Ships (MARPOL). However, MARPOL does not apply to small craft carrying fewer than 15 passengers. In the UK, local byelaws can impose restrictions on disposal of wastewaters; accepted convention is that untreated sewage should not be emptied less than three miles offshore (Green Blue 2010). Generally, the amount of sewage effluent generated depends on the number of people on-board and the type of on-board treatment used (European Maritime Safety Agency-EMSA 2017).

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[8] e.g. hydrological connectivity between sources and waterways or between upstream sources and downstream receiving waterbodies and shellfish waters.

Illegal overboard sewage discharges into shellfish waters have been linked to major outbreaks in the USA (Shieh et al. 2003).

- Domestic septic tanks may discharge their effluent via a discrete point source to surface water (Campos et al 2013a), or they may overspill to a soakaway and therefore contribute to groundwater and, via sub-surface runoff, to surface water as a potential diffuse pollution source (e.g. for Scotland see DPMAG 2011). Septic tanks are usually associated with small volumes of discharge, but malfunctioning or poorly sited septic systems may be locally significant as sources of *E. coli* and Norovirus to shellfish waters (Campos et al 2013a; Campos and Lees 2014). A source-apportionment study by Cahoon et al (2006) showed that improperly performing septic systems located at a high density (20/ha) in soils facilitating discharge (leaching) of septic tank sludge to adjacent estuarine waters can be more important contributors to faecal contamination of shellfish waters than stormwater.

- Pet wastes can be an important source of faecal contamination from a number of pathogens (e.g. *Giardia* sp., *Cryptosporidium parvum,* and *Salmonella* sp), especially in built-up areas (Meals et al 2013).

- Wildlife, both mammals (e.g. seals, otters) and birds, can act as pathogen reservoirs especially when they reach high density close to or in shellfish waters, whereby there is little opportunity for faecal microorganism die-off (Meals et al 2013).

### IV.1.3 Microbial source tracking

The emerging science of Microbial Source Tracking (MST) has the potential to introduce more reliable indicators for routine monitoring of viral faecal pollution. Selected bacteriophage groups, such as e.g. F-specific RNA (FRNA+) bacteriophages and phages infecting *Bacteroides fragilis* have been proposed as indicators for viral contamination (e.g. Jofre et al 2014; McMenemy et al 2018). However, a study on cultivated shellfish at Loch Ettive (Scotland) found that *E. coli* and FRNA+ bacteriophage were poor indicators of norovirus, presumably because of the small influence from human sources of faecal contamination (Magil et al 2007). The study by Magil et al (2007) concluded that *E. coli* may act as a better norovirus indicator in shellfish from more urban/human sewage-impacted catchments and suggested that neither *E. coli* nor FRNA+ bacteriophage should be considered as reliable quantitative risk indicators of norovirus contamination in shellfish on the west coast of Scotland.

## IV.2 FIO transport processes

### Rainfall

- Effect of rain –antecedent rain. Vidon et al (2008) showed that seven-day antecedent rainfall is the best indicator of bacterial loading when no prior knowledge of what constitutes base flow and high flow conditions is available. Campos et al (2017) also provided supporting evidence for the significant effect of the cumulative 7-day antecedent rainfall on shellfish *E. coli.* Others found that FIO concentrations in shellfish remain elevated from one to six days after a rainfall event (Kelsey et al 2004; Campos et al 2011). The duration of elevated FIO concentrations after rain depends on the hydrogeology of the catchment, FIO residence times in the receiving water and the complexity of the riverine network and stream-water residence times (Campos et al. 2011; Kay et al 2008b; 2012; Oliver et al 2009; Murphy et al 2015).

- Rain as indicator of faecal contamination. Vidon et al (2008) showed that river discharges and rainfall are useful indicators of *E. coli* transport to coastal waters through the hydrological network. Campos et al (2017) found that rain is a useful indicator of *E. coli* contamination in oysters. Gourmelon et al (2010) reported the use of rainfall as surrogate for faecal coliform contamination in shellfish production areas in areas where rain is known to be associated with high microbiological loads. In the UK, rain is a strong indicator of FIO fluxes: the delivery of FIOs after rainfall events may exceed 95 % of the total FIO input to coastal waters (Stapleton et al 2007). Rainfall is the most commonly referred factor influencing faecal coliforms, *E. coli* and enterococci levels in coasts and estuaries affected by stormwater runoff (e.g. Kelsey et al 2004). It has been found to significantly increase bacterial levels in shellfish waters (Crowther et al 2001) and shellfish flesh (Campos et al 2011). However, Aslan-Yilmaz et al (2004) showed that sewerage infrastructure improvement schemes and coastal engineering rehabilitation works can potentially reduce the effect of rain on shellfish waters.

### River flow

There is paucity of river FIO data in the UK, therefore it is difficult to predict FIO discharges or fluxes to shellfish waters. However, generic modelling of FIO concentrations collected in relation to land use or type of source suggested that urban (sewerage related) sources dominate the delivery of FIOs during baseflow conditions when there is little or no runoff from rural (agricultural) land (Crowther et al 2011; Kay et al 2008a; b). It has been also predicted that FIO fluxes from both urban and rural catchments are typically around two orders of magnitude greater at high flow than baseflow, with the potential to massively impact water quality in shellfish waters (Kay et al 2008a); see also TableIV.5.1.

### Sediment - Sediment resuspension

Estuarine sediments, when tidally re-suspended or perturbed, release particle-attached pathogens such as coxsackie viruses, *E. coli* and *Clostridium perfigens* to the water column (Smith et al 1978; Desmarais et al 2002). Likewise, coastal sediments act as reservoirs of *E. coli* and coliforms (e.g. Gerba and McLeod 1976) and enteroviruses (Rao et al 1984). Proximity to the shoreline is positively correlated with FIOs in water, especially during the time subsequent to high tide when wash-off from contaminated beach areas occurs (Shibata et al 2004).

Bacterial release into the water column via sediment resuspension may be an important factor affecting shellfish quality in shallow, low-energy and depositional inshore areas impacted by high magnitude storm events or in high-energy areas where waves are large when compared with the overall bathymetric profile (Roslev et al 2008; Wyness et al 2018). A study of over 3500 samples demonstrated a significant relationship between tidally-driven sediment resuspension and *E. coli* in water (Jovanovic et al 2017). However, a number of studies found no or a weak relationship between FIO in sediments and in shellfish (Skanavis and Yanko 2001; Ouattara et al 2011; Clements et al 2015).

### *Tides*

Tides influence levels of FIOs in shellfisheries via dilution during the flood, through drainage of microorganisms from reservoirs during the ebb and through tidal currents. The back-and-forth flow of tidal currents controls the flushing time and water mixing processes in estuaries (Ji 2008). It has been shown that ebb tides increase the faecal contamination levels in estuarine waters (Churchland et al 1982) and in shellfish (Lart and Hudson 1993) in areas where the significant pollution sources are upstream of a given shellfishery. The ebb phase is also considered as an adverse pollution condition by the competent authorities for the classification of harvesting areas in the US (NSSP 2015) and New Zealand (NZFSA 2006). In general, higher FIO levels have been observed in shallow estuaries with long water residence times and receiving high river flow discharges than in in areas impacted by low river flows and long flushing times (Fiandrino et al 2003). Mallin et al (1999) observed that decreases in salinity of over 20% occurring between high and low waters are concurrent with sharp increases in FC concentrations in surface waters (0.1 m depth) in tidal creeks in North Carolina (USA). This was explained by the effect of the falling tide importing contaminated headwater stream waters and, possibly by the stream sediment resuspension.

A hydrodynamic model simulating the dispersion and dilution of FIOs in the Bay of Seine (France) developed by Riou et al (2007) indicated that, during spring ebb tides, the freshwater plume impacts significantly on shellfish beds due to shorter time (<1 h) required for poorly diluted waters to reach them. The simulation showed that the time required for faecal contaminants in the freshwater plume to impact upon the nearest shellfish beds is as short as 3–6h. More recently, tidally-driven hydrodynamics were found to determine the dispersal and concentrations of bacterial indicator/pathogen concentrations near a WwTW plume (Winterbourn et al 2016).

Tidal impacts are more important for intertidal than subtidal shellfisheries because for intertidal beds the tidal state will determine the length of either immersion or exposure of the shellfish to contaminated water. For example, , shellfish harvest sites closest to the Mean Low Water will be submerged at high water for longer periods than during neap tides, but they will be exposed for a longer period during low water during spring tides than neap tides. Clements et al (2015) showed that tidal state will dictate the amount of feeding time available for intertidal shellfish and hence the amount of potential time available for shellfish to accumulate or eliminate micro-organisms.

Variations due to sewage discharges from urban catchments and the die-off rates of each group of microorganisms may confound the effect of tides (Mill et al 2006; Izbicki et al 2009; de Brauwere et al 2014). Further, the association between faecal contamination and tidal cycles may vary by FIO and stage of tidal cycle. For instance, enterococci exhibit extreme variability over timescales less than 24 hours in both turbulent surf zones and wave-protected bays (Boehm 2007). Significant differences in bacterial concentrations in water between high and low tidal cycles have also been reported (Solo-Gabriele et al 2000; Lee and Morgan 2003; Jovanovic 2017). No difference in bacterial concentrations over tidal cycles was observed by Clements et al (2015) in a mussel bed in Wales; however, each season showed a tidal state that appeared to show elevated ***E. coli*** concentrations relative to the other observed tidal states.

## IV.3 Physiochemical parameters in shellfish waters

### *Salinity*

Exposure of catchment-derived faecal microorganisms to saline waters causes osmotic shock and thus enhances die-off rates (Anderson et al 2005). Bacterial concentrations are usually highest in inlets impacted by low salinity agricultural and urban runoff, intermediate in semi-enclosed bays exposed to both freshwater runoff and seawater and lowest in coastal bays (Reeves et al 2004). In estuaries, the large gradients in salinity and over small spatial scales can result in flocculation and deposition of fine particulates with bound bacteria and viruses (Malham et al 2014). Positive associations have also been found between salinity and FIO die-off in shellfish waters on inter-annual scales (Chigbu et al 2004).

Salinity is a good tracer of short-term variations of FIO contamination in coastal waters including shellfish waters

(Brock et al 1985; Moresco et al 2012). McLaughlin et al (2007) developed simple, two end-member water mass mixing models using salinity as a tracer to evaluate FIO impairment of Newport Bay (USA). These models explained up to 20 % of the variance in the data and helped to track changes of *E. coli* concentrations in the upper reaches of the bay.

### *Temperature*

Many studies have assessed temperature effects in relation to the proximity to sewage discharges.

- High temperatures have been linked to bacterial inactivation by increased predation pressure, and the deleterious effect of solar radiation (Sinton et al 2002).
- Concentrations of FIOs naturally increase over the warmer months of spring and summer, and decline over the cooler months of autumn and winter (Chigbu et al 2005; Clements et al 2015).
- Temperature and *E. coli* abundance are negatively correlated in winter season, suggesting a better *E. coli* survival in winter or a greater magnitude of faecal sources in winter (Kay et al 2005; Hassard et al 2017).
- Temperature and salinity are key factors influencing the rates of FIO accumulation and clearance by shellfish (Campos et al 2013a and literature cited therein).
- Pumping rates in bivalve shellfish increase with temperature (Jørgensen et al 1990); see below Annex IV.4.

Faecal coliform levels were positively correlated with temperature away from the influence of sewage outfalls in the Ria Formosa (Portugal) (Catalao Dionisio et al 2000). This indicated that water temperature is a good proxy for FIOs in coastal waters not significantly impacted by freshwater inputs and sewage discharges.

### *Turbidity/Light*

Touron et al (2007) suggested that the location of the turbidity maximum across an estuary provides information as to where high concentrations of FIOs are more likely to be found. This may be explained by the role of suspended matter in attenuating light penetration in the water column, and therefore reducing bacteria die-off due to solar UV radiation. The existence of a strong turbidity maximum close to the tidal limit, characteristic of many UK estuaries during the summer–early autumn period (Uncles et al 2006), could explain periodic deteriorations in the microbial quality of shellfish waters. In coastal waters, the concentration of total particulate matter (e.g. suspended sediments, organic matter including phytoplankton and detritus) also plays a major role on UV penetration (Tedetti and Sempéré 2006). Generally, *E. coli* can survive 65–80 days in marine sediments as opposed to 3–5 days in seawater (Labelle et al 1980; Young-Joo et al 2002).

The inactivation of enteric bacteria exposed to UV radiation depends upon mixing of the water column and specific vulnerability to UV radiation (Huot et al 2000). Noble et al (2004) found that the combined effect of (high) temperature and solar irradiation has the most significant effect on FIO inactivation in sewage and urban storm drain runoff. In contrast, *E. coli* abundance increased with elevated turbidity (17%) and the lower temperatures (31%) in autumn and winter respectively (Hassard et al 2017). This could be due to the stabilizing effect of suspended particulate matter on bacteria (Gin and Goh 2013; Perkins et al 2014). Adsorption (binding) of microorganisms on sediment particles may mitigate the harmful effects of UV, with turbidity levels >200 NTU in shallow coastal and estuarine waters completely inhibiting the UV-induced decay of intestinal enterococcus (Kay et al 2005).

A recent study by de Souza et al (2018a) in Santa Catharina (Brazil) showed that high rainfall and low solar radiation (winter season) combined were related to increased coliform concentrations in the water column of coastal waters. The Brazilian study concluded that in the summer, coliform die-off due to high solar radiation has the potential to offset higher contamination due to an influx of 1.5m tourists (see de Souza et al 2018a). However, it remains uncertain whether this evidence applies to northern temperate latitudes such as in Scotland.

## IV.4 Accumulation rates in shellfish

It is generally accepted that the accumulation of FIOs by shellfish at any point in time usually reflects bacterial concentrations in the overlying water during the preceding hours or days. Evidence from the literature suggests that temperature and salinity are key factors influencing the rates of FIO accumulation and clearance by shellfish (Perkins et al 1980; Rowse and Fleet 1984; Bernard 1989). Water pumping and filtration efficiency in shellfish reflect the concentration of food in the water (Jørgensen et al 1990). Therefore, the absolute concentrations of FIOs in shellfish may increase at higher temperatures due to higher FIO concentrations in the water during the warmer period or due to higher accumulation rates (Sonier et al 2008).

Bernard (1989) compared the kinetics of accumulation and clearance in the Pacific oyster (*Crassostrea gigas*), blue mussel, the littleneck clam (*Protothaca staminea*) and in the soft shell clam (*Mya arenaria*) immersed in water temperatures of 7, 12 and 17 °C and found different shapes in temperature-specific curves and maximum accumulation levels between species. Interestingly, the lowest accumulation level in mussels was at 12 °C, whereas in the remaining species, it was maximal at 12 °C and significantly lower at 7 °C. This indicates that mussels are particularly resistant to low temperatures and are able to ingest food particles and show some growth during typical winter conditions.

Retention efficiency by filter feeding bivalves depends on the size range of particles. Burrowing and attached filter feeding bivalves (such as those commercially harvested in Scotland) can retain completely particles larger than 1-3μm, which matches the size range of marine phytoplankton and coliform bacteria; however, scallops display a decreasing retention efficiency for particles smaller than 7 μm (Mohlenberg and Risgard 1978).

Evidence from microcosm studies indicates that thermal shock (transfer from lower to higher temperature) increases the volume filtered by mussels (*Mytilus trossulus; Mytilus edulis*) in a given time (Cusson et al 2005). When water temperature decreases towards the minimum for survival, mussel clearance rates decrease significantly, and more time is required for mussels to filter a standard volume of water (Cusson et al 2005). In contrast, native and Pacific oysters are less tolerant to low temperatures. For instance, the feeding activity in native oysters (*Ostrea edulis*) along the Swedish west coast, as measured by the production of faecal material, is significantly lower when oysters are exposed to temperatures of 5 °C compared to 10 °C (Rödström and Jonsson 2000).

Salinity affects both pumping rates and filter-feeding processes in shellfish. For example, Pacific oysters prefer salinity levels nearer to 25psu (Laing and Spencer 2006). Oysters of the genus *Crassostrea* tend to be euryhaline and are often found in shallow waters, whereas those belonging to the genus *Ostrea* are found below low water in the sublittoral zone. Experiments with the Sydney cupped oyster (*C. commercialis*) showed that clearance of *E. coli* from oyster tissues is slow and inconsistent at 16–20psu when compared to that at 32–36psu or 43–47psu (Rowse and Fleet 1984). Feeding rates in *O. edulis* along the Swedish coast begin to decline at 28psu and cease at 16psu (Rödström and Jonsson 2000). On the other hand, mussels grow well above 20psu but usually only feed at higher salinities (20–35 psu). As part of a laboratory investigation into the effect of reduced salinity on the uptake and elimination of *E. coli* by *M. edulis* from the Menai Strait (Wales), Fanshawe (1995 cited in Campos et al 2013a) noted that mussels exposed to salinities of 32psu took less time to accumulate maximum levels of *E. coli* than those exposed to salinities of 20 and 16psu and were able to initiate the clearance period before them.

Campos et al (2013a) reviewed evidence showing that scallops and razor clams are very intolerant of salinities lower than 30psu, so sites with a high inflow of freshwater are not suitable for these species. Razor clams (*Ensis* spp.) commonly inhabit 'fully' saline waters. Younger et al (1999 cited in Campos et al 2013a) also observed that razor clams show very little filtration activity at 20psu.

There is very little evidence linking filtration activity, internal factors (e.g. age, size, maturity, nutritional condition) of individual shellfish and the dynamics of FIO uptake by different shellfish species. A literature review on these factors suggests that the higher FIO accumulation factors in common cockles and blue mussels than in Pacific oysters and native oysters are consistent with higher filtration rates in the former group of species (Campos et al 2013b). Microcosm studies in the common mussel (Plusquellec et al 1990) and native clams (*Tapes decussatus*) (Martins et al 2006) have shown that these species are able to accumulate maximum concentrations of FIOs within 30 min exposure to contaminating source. Teplitski et al (2009) found that mussels are capable of filtering up to 10l/h of water, which makes them capable of effectively removing bacteria from the water column before they can become incorporated into the sediment.

Experiments by Kershaw et al (2013) showed that (i) FIO accumulation in shellfish may exceed that in the overlying waters within 30min exposure to the pollution source (ii) peak FIO concentrations occurred between 12 and 18 h in mussels, Pacific oysters and cockles. Depending on the water temperature, mussels and Pacific oysters could take 40 min to 3h to accumulate 300 FC/100g of FIL. The experiments demonstrated a rapid accumulation phase and that shellfish are able to accumulate *E. coli* to plateau concentrations within 17h of exposure to a contaminating source, then an equllibrium phase appears under chronic pollution beyond 48h exposure to a contaminating source.

Significant differences in baseline levels of FIOs between different species of shellfish have been reported in the literature. In general, mussels and cockles tend to show higher accumulation factors than oysters due to higher filtration activities in the former species (Gerdes 1983; Kershaw et al 2012; Riisgård 2001). The average *E. coli* accumulation in mussels (*Mytilus* spp.) commercially harvested in England and Wales is one to two times greater than that in Pacific oysters (*C. gigas*) (Younger and Reese 2011). Similarly, levels of this indicator in common cockles (*Cerastoderma edule*) commercially harvested in France have been shown to be approximately three times higher than those in Pacific oysters (Amouroux and Soudant 2011). Beucher (1993 cited in Campos et al 2013a) found the following decreasing pattern of contamination levels between species commercially harvested in France: Cockles > blue mussels >(P. oysters, clams, carpet clams) > native oysters.

CEFAS (2014), based on a critical review of the literature, recommended that *Mytilus* spp may be used as an indicator to represent species such as P. oyster, native oyster, *Tapes* clams and hard clams (*Mercenaria mercenaria*), on the assumption that species are co-located both geographically and with respect to depth in the water column. However, Kershaw et al 2013 found significant inter-species variations in shellfish FIO accumulation rates and concentrations and concluded that these differences do not support the application of a single water quality standard for shellfish protected areas where more than one species is commercially harvested.

## IV.5 Catchment FIO modelling

This is a brief review of the potential of catchment FIO modelling to support management of catchment-based sources of FIOs and to assess whether SWPA monitoring or the sanitary survey process can inform source-apportionment modelling.

Very little research and monitoring effort has been directed to define the complex and highly episodic mix of inputs from both point and diffuse catchment-based sources (Kay et al 2010 and literature cited there in). The bulk of available catchment FIO studies aim to explain continued compliance problems (impairment) despite significant financial investment on improving sewage treatment, such as installing ultraviolet (UV) disinfection or increasing CSO or STO storage capacity. A review on source-apportionment studies by Kay et al (2010) concluded that extensive spatial and temporal information on all sources of catchment-derived FIO is required to target FIO control measures. The key management information required are the proportions of the flux derived from all potential inputs from human (sewage) and livestock sources during both low and high flow conditions presented in simple pie charts (Kay et al 2008a, b). To capture hourly or daily FIO fluxes involves: intensive, high frequency in-stream FIO sampling in relation to diffuse sources; and FIO sampling from the sewerage infrastructure during rainstorm conditions (Stapleton et al 2008; Kay et al 2010; Kay et al 2008a; b).

Thereafter, source-apportionment can inform allocation of expenditure between (Kay et al 2010):

(i) Increasing storage to limit CSO discharges.
(ii) Disinfection of treated sewage effluents.
(iii) Implementation of “farm-scale” diffuse pollution control measures.

Available FIO export models are based on multiple regression equations used to predict the low and high flow faecal indicator geometric mean concentrations. When combined with hourly discharge volumes, the predicted FIO concentrations can provide FIO flux estimates, as export coefficients (Kay et al. 2005; Kay et al 2008a).

For example, Kay et al (2008a) estimated FIO export coefficients per type of land use under base-flow and high-flow conditions from in-stream FIO concentrations and discharge estimates collected from 205 catchments in the UK; of these, 52 were in Scotland. The FIO data reported by Kay et al (2008a) reflect the combined point- and diffuse-source inputs to the watercourses monitored. Available land cover and land use data[9] were used in order to calculate the coefficients for improved grassland (IP), rough grazing (RG) and woodland (WL) as well as rural, semi-urban and urban land uses (the various improved grassland categories corresponded closely with land used as improved pasture and the various types of natural grasslands corresponded to land used for rough grazing). Kay et al (2008a) estimated higher FIO export coefficients from semi-urban and urban areas compared to rural areas and from improved grassland compared to rough grassland and woodland. It was also shown that FIO export increases during high flow conditions by one to two orders of magnitude even from rough grassland and woodland. The estimated export coefficients per type of land use are presented in Table IV.1[10].

Finally, a better understanding of catchment FIO dynamics and their effect on shellfish water compliance is required (Kay et al 2010; Cowther et al 2016). Notably, there is a call for more and quantitative information on:

- FIO decay in-stream under different flow and turbidity conditions.
- Seasonal variation in FIO catchment export coefficients.
- The effectiveness of diffuse pollution control measures to reduce FIO flux from livestock and the sewerage system to shellfish waters.
- The relationship between reductions in FIO fluxes from livestock and the sewerage system and compliance with microbiological quality criteria in shellfish.
- The effectiveness of disinfection of treated sewage effluent and increased storage capacity.

[9] For Scotland these sources included the 1988 Macaulay Land Cover of Scotland Map, Ordnance Survey 1:50,000 maps, and digital map information, and Scottish Executive data.

[10] These values were used to predict FIO export from source-catchments to SWPAs in this report.

**Table IV.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 | $2.9 \times 10^{9}$ | $2.7 \times 10^{11}$ |
| | Faecal coliforms | $8.3 \times 10^{8}$ | $1.2 \times 10^{11}$ |
| | Enterococci | $9.6 \times 10^{7}$ | $2.2 \times 10^{10}$ |
| ≥ 75% RG | Total coliforms | $7.1 \times 10^{8}$ | $5.3 \times 10^{10}$ |
| | Faecal coliforms | $2.5 \times 10^{8}$ | $2.5 \times 10^{10}$ |
| | Enterococci | $3.3 \times 10^{7}$ | $3.6 \times 10^{9}$ |
| ≥ 75% WL | Total coliforms | $3.1 \times 10^{8}$ | $1.4 \times 10^{10}$ |
| | Faecal coliforms | $2.0 \times 10^{7}$ | $3.3 \times 10^{9}$ |
| | Enterococci | $8.5 \times 10^{6}$ | $3.8 \times 10^{8}$ |
| < 2.5% BU (Rural) | Total coliforms | $9.3 \times 10^{8}$ | $6.1 \times 10^{10}$ |
| | Faecal coliforms | $4.2 \times 10^{8}$ | $2.6 \times 10^{10}$ |
| | Enterococci | $4.9 \times 10^{7}$ | $4.7 \times 10^{9}$ |
| 2.5< BU <9.9% (Semi urban) | Total coliforms | $4.2 \times 10^{9}$ | $1.5 \times 10^{11}$ |
| | Faecal coliforms | $1.2 \times 10^{9}$ | $4.6 \times 10^{10}$ |
| | Enterococci | $1.5 \times 10^{8}$ | $1.1 \times 10^{9}$ |
| ≥10% BU (Urban) | Total coliforms | $8.5 \times 10^{9}$ | $4.1 \times 10^{11}$ |
| | Faecal coliforms | $2.8 \times 10^{9}$ | $1.3 \times 10^{11}$ |
| | Enterococci | $4.0 \times 10^{8}$ | $2.7 \times 10^{10}$ |

## IV.6 Coastal water FIO modelling

The purpose of coastal FIO modelling is to evaluate and/or to predict the impact of processes related to dilution, die-off and ecology of FIO species on the spatial and temporal variability of FIO concentrations in a coastal area (Gourmelon et al 2010). Available coastal FIO models project FIO fate of faecal coliforms (FC) (Roberts and Williams 1992; Ribeiro and Araujo 2002); *E. coli* and enterococci (Gourmelon et al 2010 and literature cited therein); or F-RNA-specific bacteriophages and astrovirus (Riou et al.2007). These models can be categorised to statistical and process-based dynamic models.

### IV.6.1 Statistical coastal water FIO models

These models are based on linear or logistic[11] regression analyses that link environmental parameters such as rainfall, wind or sunlight to FIO data at specific monitoring stations in the coastal water area (e.g. Crowther et al. 2001; Lee and Morgan 2003; Martinez-Urtaza et al. 2004).

Model output predicts water FIO contamination in relation to input data. Examples of their application include:

- Predictive tools to support conditional management, as in the USA and New Zealand, by using the quantity of rain during the past 24 hours or other relevant meteorological data to build alert curves for shellfish harvesting (Grange 1999; Gourmelon et al 2010; Olyphant 2005).
- Investigation of relationships between FIOs and environmental conditions.
  - o Multiple regression, using the stepwise selection procedure, was used to investigate relationships between FIOs and rain, salinity, temperature and sewage discharges on FIO sampling day ($day_0$) and over the two previous days on the Fylde coast (UK) by Crowther et al (2001). The study evaluated the causes of non-compliances with water quality criteria in selected sampling points.
  - o General linear modelling was performed by Lee and Morgan (2003) to explore the relationship between shellfish *E. coli* contamination and environmental factors in shellfish-farming areas in the UK. The model predicted that season, high/low tidal cycle and rainfall have a significant effect on shellfish *E. coli* levels.
  - o Linear regression modelling and scenario simulations were developed by de Souza et al (2018a; b) to explore FIO variation in coastal waters impacted by sewage in Santa Catarina, Brazil in relation to: catchment variables (spatial model); meteorological variables such as rain and solar radiation (temporal model); and both catchment and temporal variables under different environmental scenarios (integrated model). Scenarios were developed to help understand the effect of human population increase or reduction, WwTW upgrading, normal and extreme rain, and

[11] This would only be suitable for presence/absence data (Jackie Potts, pers. com. 2018).

normal and extreme solar radiation on seawater FIO. The simulation of different scenarios showed that a combination of extreme rainfall and low solar radiation substantially increases the concentrations of FIOs in coastal waters (de Souza et al 2018a). This approach to regression-based models, which is very similar to the approach developed in this report to inform FSS and SEPA, is proposed as a management tool to help stakeholders to predict FIO levels in coastal waters based on geographical and meteorological parameters (de Souza et al 2018b).

  - o Multiple regression techniques were used by Campos et al (2017) to examine the relationship between NoV and *E. coli* concentrations in oysters in SPAs in England and Wales and demographic, hydrometric, climatic and catchment-based-pollution sources. The study showed that variation in *E. coli* concentrations was explained by the cumulative 7-day antecedent rainfall, whereas variation in NoV was explained by seawater temperature, catchment area and the combined volume of continuous discharges in the catchment.

- A mathematical framework was developed by McMenemy et al (2018) to determine the minimum depuration times required to reduce *E. coli* and pathogen (e.g. NoV) levels to below a specified threshold. For example, the framework used parameters derived from data obtained from UK harvester sites and assumed lognormal distribution of pathogens across a shellfish population and exponential decay of pathogens during depuration. The study predicted that minimum depuration times for *E. coli* (range: 25.2 – 30.9 hours) all fall within the minimum 42 hours required for Class B SPAs[12], whereas minimum depuration times for FRNA+ (range: 166.9 – 216 hours) and Norovirus (186.2-326.9) were substantially longer (McMenemy et al 2018). This finding showed that *E. coli* is a poor indicator of viral and pathogen contamination.

A potential limitation of statistical models is their requirement for extensive monitoring data on rainfall (and potentially other relevant environmental parameters) in the source-catchments and on FIO occurrences at the RMP of a shellfish production area (SPA). Further, statistical models are unsuitable for distinguishing between inputs from different sources, or consider processes such as transport, vertical mixing and FIO die-off rate (Crowther et al 2001; Lee and Morgan 2003).

### iv.6.2 Process-based coastal water FIO models

Gourmelon et al (2010) suggested that the development of a shellfish water quality model requires different sub models:

(i) A hydrodynamic model estimating current (i.e. advection) and vertical mixing/turbulence coefficients. This requires knowledge on the FIO input location and estimation of FIO fluxes, which can be derived by estimations of FIO fluxes from specific sewage or diffuse sources based on generic catchment or source-apportionment modelling (e.g. Kay et al 2008a; b).
(ii) A dispersion model predicting FIO transport.
(iii) A FIO decay model predicting the decay of bacteria/viruses in relation to light, salinity, temperature and turbidity (to address adsorption of microorganisms on suspended particles).

The choice of a model depends on local hydrological features and on available resources (Kashefipour et al 2002; Servais et al 2007). Hydrological features are a function of local hydrodynamic processes; resource availability depends on whether the model is open source or on commercial packages. Most applications of process-based coastal water FIO models focus on FC or *E. coli* in coastal water (e.g. Kashefipou et al.2002; Servais et al.2007).

Very few process-based models have addressed shellfish water quality (Pommepuy et al 2005; Fiandrino et al. 2003; Tattersall et al. 2003; Riou et al. 2007; de Brauwere et al 2014 and literature cited therein). Some of these "shellfish" models indicated that when the flushing time is very long, factors influencing FIO die-off and shellfish FIO accumulation rates are more important than physical dilution in determining FIO concentrations in shellfish (Pommepuy and Salomon 1991; Fiandrino et al. 2003; Tattersall et al 2003; Riou et al. 2007). By contrast, in areas influenced by strong tidal currents, processes such as transport, dilution and dispersion are predicted to determine shellfish FIO contamination levels (see also review by de Brauwere et al. 2014 and literature cited therein).

Hydrodynamic modelling indicated that rain was an important determinant too. For example, Riou et al (2007) built a two-dimensional hydrodynamic model to simulate the currents and dispersion of river plumes in coastal environment with shellfish beds in France and included decay rates to simulate microorganism behaviour in seawater. Their model predicted that FIO and pathogen fluxes through land runoff to coastal waters were over 50 times higher than under normal weather conditions (i.e. daily rainfall≤10mm). These increased fluxes were associated with increased rates of shellfish contamination in the shellfish beds influenced by the river plume. Thus,

[12] As of 2018, in the UK, a minimum depuration period of 42 hours exists for shellfish harvested from a Class B area, which has been shown to be sufficient to reduce *E. coli* counts to less than 230 MPN *E. coli*/ 100g FIL. (McMenemy et al 2018 and literature cited therein).

the modelling approach developed by Riou et al (2007) indicated the "risk period" for shellfish harvesting in a particular environment.

Using a similar approach, Dunn et al (2014) developed a three-dimensional hydrodynamic model to simulate the dispersion and fate of a treated wastewater plume in Geographe Bay, Western Australia. The model was validated using field data such as bottom and surface water current speed and direction and in situ FC concentrations. Outputs of the model were used to determine the 'footprint' of the wastewater plume and predict the dilution of contaminants under different discharge scenarios and plume dispersion regimes with a view to identify inter alia where local standards for shellfish and bathing waters are met. Thus, the modelling approach developed by Dunn et al (2014) helped to determine the extent of exclusion zones around sewage outfalls, where the standards for shellfish harvesting may not always be met.

It is also important to note that very few "shellfish" models (e.g. Bougeard et al 2011) have used daily river flows simulated with a watershed model (e.g. SWAT) as an input into another hydrodynamic model to assess daily bacterial concentrations in estuaries and in shellfish. The coupling of these two types of models is of major importance in advancing our understanding and prediction of the microbiological contamination in shellfish waters. Linking catchment and hydrodynamic models is further discussed in Appendix IV.7.

### *IV.6.3 Real-time data (i.e. information that is delivered immediately after collection) for early warning*

Presently, the process for evaluating public health risk from consumption of shellfish is a time-consuming process: it requires monitoring, microbiological analyses and classification. In addition, faecal contamination cannot always be predicted by classification (see Section 3.3 and Appendix III.4). Early warning systems are based on parameters which are proven, through statistical modelling, to be correlated with FIO contamination in shellfish (Gourmelon et al 2010). Parameters commonly used for the development of early warning FIO contamination systems include salinity or turbidity because real-time sensors are available for these parameters (Grange 1999; Olyphant 2005; Le Saux et al 2006; Pommepuy et al 2008). Further, real-time data for early warning systems can be collected from sewage network key-points and from disease outbreak monitoring networks (e.g. Le Saux et al. 2006; Gourmelon et al. 2010 and literature cited therein).

## IV.7 Linking catchment and hydrodynamic modelling

It has been argued that catchment source-apportionment and FIO export modelling alone does not address the processes influencing the fate and transport of faecal contaminants in the coastal/estuarine environment and the highly dynamic nature of FIO fluxes (e.g. Falconer et al 2014; Kay et al 2010). Predicting FIO concentrations in coastal waters requires linking catchment and nearshore hydrodynamic models to facilitate accurate prediction of FIO concentrations at certain locations of public health or policy importance and to target diffuse pollution measures at the waterbody and farm-scale (e.g. Kashefipour et al 2006; Kay et al 2007).

Studies linking catchment and nearshore hydrodynamic models in the UK demonstrated strong interactions between event-driven FIO-contaminated riverine discharges to a coastal area and dispersion of contaminants with the tidal cycle (Bougeard et al 2011; Boye et al 2015;; Falconer et al 2014; Gao et al. 2015; Huang et al 2015; Kashefipour et al 2006; Stapleton et al 2007; Wilkinson et al 2006;). For example,

- Gao et al (2015) The study showed that both FIO inputs from upstream catchments and tidal processes play a significant role in the distribution of FIOs from river discharges but not from CSOs, and WwTWs in the Ribble Estuary, with the tidal cycle significantly enhancing dilution of FIO concentrations in the lower estuary area but not in the upper estuary.
- Huang et al (2017) integrated an integrated hydrodynamic model with a 1D and 2D catchment model to simulate the adsorption-desorption processes of *E. coli* to and from sediment particles in river, estuarine and coastal waters. The integrated five sub-models used were validated using hydrodynamic data and suspended sediment and water column *E. coli* concentrations. The study showed that (i) sediment transport is a key process by which FIOs can be transported from river basins to coastal waters and (ii) excluding adsorption and desorption processes during sediment resuspension from modelling may lead to significant underestimation of FIO levels in coastal waters.

However, it must be noted that there is paucity of evidence on integrated catchment and hydrodynamic modelling for lochs and inlets such as those where shellfish cultivation usually takes place in Scotland.

## IV.8 Monitoring considerations

### *Spatial and temporal variability within a shellfishery*

Clements et al (2015) studied the distribution and abundance of *E. coli* in FIL within a single intertidal commercial mussel bed in Wales classified as B on the basis of routine monitoring from the RMP and concluded that there was significant patchiness in relation to season and tidal state. Clements et al (2015) demonstrated the presence of clear "hot spots" of faecal contamination within the mussel bed, indicating that mussels from these patches were unsafe for consumption; conversely, other patches, yielded shellfish *E. coli* results below the threshold for class B. This means that routine monitoring samples taken from patches outwith the RMP, may skew the classification, potentially downgrading SPAs from Class A to B or from B to C, with important implications for post-harvest treatment and cost (Clements et al 2015). Further, seasonal and diurnal variation in the abundance of FIOs in shellfish waters has been shown to vary with water temperature and light intensity (Kay et al. 2005). Arguably, the sampling point (RMP) for routine FIO monitoring has a direct impact on the final classification awarded to commercial shellfish beds and, by extension, on the economic prosperity of the shellfish industry and the assessment of risk to shellfish consumers (Kay et al 2005; Clements et al 2015).

Levels of FIOs in surface waters show seasonal cycles which closely mirror hydrological events (e.g. rainfall, river flows), inputs of microbial pollutants associated with these, differential survival of FIOs, climatic factors and abundance of phytoplankton and zooplankton (Campos et al 2013a and literature cited therein; Mote et al 2012). Kay et al (2008b) argued that FIO monitoring in shellfish or shellfish waters should target high flow events in areas where the intention is to design sewerage infrastructure to effectively reduce the flux of FIOs. This will help to avoid confounding of FIO monitoring results due to seasonality and any potential monitoring bias towards avoiding storm events. Likewise, a study of the risk factors associated with cultured shellfish at Loch Ettive concluded that historical routine stream monitoring data (if available) will be biased to 'low flow' conditions and use of such data to estimate catchment FIO flux "will produce erroneous and dangerously optimistic conclusions concerning the total pollutant flux to adjacent harvesting waters" (Magil et al 2007). As a solution, Magil et al (2007) recommended that high frequency sampling during storm events should be included in sanitary surveys in order to (i) aid selection of routine monitoring points and b) assess the impact of high flow events on shellfish production areas. This recommendation particularly refers to new designations and SPAs at risk from microbiological non-compliance.

### *Factors that should be taken into accounted when handling monitoring data*

Transformation. Because shellfish microbiological data are often highly right-skewed, i.e. few high values, logarithmic transformation can help to make a data distribution more symmetric; this distribution is referred to as lognormal (ISSC/FDA 2007 cited in NSSP 2015); see also calculation of percentiles.

Calculation of percentiles There are two different approaches to estimating percentiles from a set of sample values. The first is the parametric approach which assumes knowledge of the statistical distribution from which the samples have been drawn (typically lognormal), and the second is the nonparametric approach which requires no such assumption[13]. One method is not universally better than the other, but provided that there is reasonable justification for assuming a lognormal distribution, the parametric method will be more efficient in the sense that it will be possible to achieve a given degree of precision with fewer samples.

Percentile evaluation of the log normal probability density function of microbiological data acquired from a particular dataset is as follows:

- Take the log (10 or e) value of all bacterial enumerations in the data sequence to be evaluated. (If a zero value is obtained, take the log(10 or e) value of the minimum detection limit of the analytical method used instead.)
- Calculate the arithmetic mean of the log(10or e) values (μ).
- Calculate the standard deviation of the log(10or e) values (σ).
- Multiply the standard deviation by 1.28 for 90th percentile and by 0.84 for the 80th percentile. Add the product to the arithmetic mean.
- Estimate the percentile according to the equations:

$$80\text{th-percentile}=(10 \text{ or } e)^{\mu+s*0.84}$$
$$90^{th}\text{-percentile}=(10 \text{ or } e)^{\mu+s*1.28}$$

The parametric method for calculating the probability that the percentile is below the threshold is described in Appendix 5C of WRc (1989). However, it is important to remember that the method does not take into consideration the effect of the uncertainty in the estimation of the mean and standard deviation, particularly when there is a low number of samples[14].

Non-parametric method. Under *Regulation (EC) 854/2004*

[13] SEPA uses the parametric approach, whereas FSS uses the non-parametric approach.

[14] SEPA therefore consider the confidence of the classification to be very low when there are less than 10 samples regardless of the estimated probability of being in each class.

the 80th-percentile must be £230 MPN of *E. coli* /100g of FIL for class A. Using the non-parametric approach this percentile is obtained simply by sorting the samples into increasing order and counting the percentage of the way along. For class A, *Regulation (EC) 854/2004* has an additional criterion that all samples must be < 700 MPN *E. coli*/100g FIL. However, it is possible that a single exceedance may be a rare event. Assuming that the *E. coli* values follow a lognormal distribution probability of exceeding the threshold of 700, this can be calculated by using the standard normal distribution function, e.g. by using the NORM.S.DIST function in Excel. If $\bar{x}$ and *s* are the mean and standard deviation of the log-transformed values then the probability is given by 1-NORM.S.DIST((LN(700)-m)/s).

Database. Building a database and establishing procedures for data input and storage are important steps in ensuring the accuracy and integrity of a dataset (Meals et al 2013). Spreadsheets and interactive databases are useful tools for this task. The data in the files created can be sorted, merged, and aggregated in different ways to provide the information required. Because working with the data involves updates, checks, and validation to ensure that data are free from entry errors, typos, and other mistakes before carrying on any further, the database has the potential to facilitate inspection and use of data (Meals et al 2013). It is advised that an outlier value should never be rejected (Meals et al 2013).

### IV.9 Criteria for exclusion zones and buffer zones

- There are no specific requirements for the application of buffer zones in the UK; however, modelling of sewage' zone of influence predicted a requirement for an exclusion zone of >2km (EURL-CEFAS 2017a).

- In the EU, the most common practice referring to buffer zones is to establish fixed zones at a pre-specified or modelled distance from the contamination sources such as active harbours and marinas, continuous or intermittent sewage or animal slurry discharges (EURL-CEFAS2017a).

- Possible options for criteria to exclusion zones could include (Fitzgerald 2015):

  - o *Define a distance from a known pollution source*, which is easy to apply but poorly targeted.

  - o *Perform dilution* analysis to delineate areas within and adjacent to marinas, which may be difficult/ expensive to apply.

  - o *Identify the interaction of Time with dilution* to delineate areas around a WwTP in relation to storm events, which may be difficult/expensive to apply.

  - o *Monitor NoV in shellfish*, which is a potentially an effective approach but technically challenging.

# Appendix V - Review of current practices and available data in Scotland (trial results)

### V.1 Responsibilities of Local Authorities in SPA management in Scotland

Local Authorities (LAs), and particularly the local Environmental Health Officers, have multiple responsibilities at the local scale. These include (FSS protocol 2017):

- Submitting applications for classification of "shellfish harvesting areas", i.e. SPAs, in conjunction with the applicant harvester. LAs and harvesters must identify any specific sources of faecal contamination to the area that they are aware of or concerned about, such as: livestock, stable waste or slurry stores, sewage treatment works, storm sewer outfalls, septic tank outfalls, wildlife, boating activity or marinas, or other.
- Ensuring the safety of live bivalve shellfish "from farm to fork".
- Informing the harvesters about the results of monitoring and classification in their jurisdiction.
- Ensuring that harvesters continue to supply live bivalve shellfish in compliance with the health standards when results out-with classification are found by establishing Local Action Groups, which will develop a Local Action Plan to address the issue.
- Investigating the causes of any unusual or rare high shellfish *E. coli* results and, if appropriate in the interests of public health, issuing a "temporary closure notice" (TCN);
- Making a decision on the status of the TCN based on supplementary sampling results.

### V.2 FSS protocol

FSS has specified the programmes of official controls (OCs) implemented in line with the *Regulation (EC) 854/2004* in the FSS protocol (2017 and FSS links cited therein). The FSS protocol also specifies the following:

- Monitoring regimes for awarding established classification: these require three years' data (minimum of 24 samples) collected according to the sampling plan justified by the sanitary survey
- Monitoring for review established classification: this requires at least 8 samples per year to grant "B" or "C" classification and at least 10 samples per year

to grant "A" classification. Each classified area can have two separate seasons or parts per year, if data show significant differences. However, the method for detecting significant differences is not reported.

- Preliminary classification can only be graded as "B" and applies to areas that have been previously classified following a full sanitary survey (within two years of application) for the same or another species. No pre-classification monitoring is required, thereby potentially failing to account for species-specific responses to changes in faecal contamination risk.

- Provisional classification can be graded as "A", "B", "C", or "prohibited" and applies to "new areas" with no previous monitoring record. At least 10 pre-classification samples, collected at least one week apart, are required.

- Annual classification can be graded as "A", "B", "C", or "prohibited" and applies to provisionally classified areas or "new areas", where harvesters have requested so. Monitoring requirements for classification grading are as for established classification.

- Areas are categorised by status as classified, provisionally classified, dormant or de-classified.

- SPAs that become inactive for 6 months to two years or are graded as "C" for an extended period of time, may be award a dormant status. Quarterly monitoring during the period of dormant status allows for the shellfishery's grade to be re-instated according to the results after removing dormant status.

- SPAs that are unable to comply with the number of samples required to review the established classification are given de-classified status for two years.

- Enforcement mainly refers to (i) awarding a dormant status; (ii) de-classification; (iii) awarding classification only after FSS collects the specified number of samples and issues a letter of classification; and (iv) initiating an incident state and potentially immediate closure of the area.

- The FSS protocol prescribes a risk-management approach for local action groups (including harvesters and SEPA) to deal with high *E. coli* results from classified SPAs in accordance with local management plans.

## V.3 Description of sanitary survey process undertaken by CEFAS on behalf of FSS

The key components of the current approach applied by FSS to comply with the requirements of the *Regulation (EC) 854/2004* are presented in the recently updated FSS protocol (FSS 2017) and FSS web site (FSS n.d.). These are summarised below:

- CEFAS have previously undertaken sanitary surveys in Scotland on behalf of FSA[15] in Scotland (FSAS) since 2007 and until 2014. The sanitary survey reports are available on line (CEFAS (2018).

- The following information is reviewed and assessed in sanitary surveys.

    - o Location and extent of the SPA
    - o Type of shellfishery (species, method of harvest, seasonality of harvest)
    - o Location, type and volume of sewage discharges
    - o Location of river inputs and other potentially contaminated water courses (from OS maps/nautical charts)
    - o Location of harbours and marinas (from OS maps/nautical charts)
    - o Hydrographic and hydrometric data
    - o Existing microbiological data from water quality or shellfish monitoring undertaken in the same area or adjacent areas.
    - o Available information is supplemented by a practical shoreline survey.

Available information is supplemented by a practical shoreline survey. No further description on how this information should be collected or stored to underpin identification of representative monitoring point and frequency with each SPA or used in classification updates is given. CEFAS' sanitary survey reports described in length (i.e. typically exceeding 80 pages) the type of information collected and its use in determining the sampling plan. It is worth noting that sanitary surveys started to be undertaken after 2006/2007 in already classified SPAs, i.e. areas where commercial harvesting had been authorized. Therefore, many of these sanitary surveys confirmed or slightly revised already designated SPA boundaries and the monitoring point and frequency for classification.

The existing sanitary survey reports refer to (i) data from desk-top surveys; (ii) information sampled or observed during shoreline surveys; and (iii) analyses of desk-top survey data. The types of data and analyses are detailed below.

**Desk-top surveys.** These include the collection and evaluation of data extracted from existing databases of various organisations or maps and refer to data such as:

- Type of shellfishery, e.g. species commercially harvested, subtidal or intertidal

[15] On 1 April 2015, SG through FSS assumed responsibility for functions carried out by the FSA in Scotland. However, in line with UK policy, the direct UK contact point in relations with the EU on food and feed matters will be FSA, as mentioned in the Memorandum of Understanding (MOU) between FSS and FSA (MOU 2015).

- Human Population
- Nearshore sewage discharges. This further specifies the locations per type of source (i.e. septic tanks, combined sewage outflows-CSOs, Waste Water Treatment Works-WWTW, Emergency Outflows-EO); type of discharge (continuous or intermittent); level of treatment (primary, secondary, tertiary, septic tank or CSO, trade/other effluent and SUDS) and faecal coliform (FC) loadings in final effluent.
- Land cover maps (LCM07) and discussion about predominant land use based on visual map observations of the nearshore areas but without explicit reporting of percentage land cover on a catchment basis. Where data are available from literature, land management practices in the nearshore area bordering the SPA is reported.
- Geology and soils. Some reports include this information, which was derived from maps developed by the Macaulay Institute, in order to identify areas with the highest potential for runoff.
- Numbers of farm animals in the nearshore areas bordering a production area. The reports used Agricultural census data to parish level requested from the SG Rural Environment, Research and Analysis Directorate (RERAD). However, data on a catchment level were not available. As many sanitary survey reports mention: "Therefore, the figures are of little use in assessing the potential impact of livestock contamination to the fishery; however, they do give an idea of the total numbers of livestock over the broader area." (e.g. CEFAS-Loch Ryan 2014 uploaded to CEFAS 2018).

Wildlife numbers in the vicinity of a production area. This information was derived from a range of sources and organisations depending on site-specific availability of data or observations, not always scientifically or reliably quantified. For example, numbers of seals (*Phoca vitulina* and/or *Halichoerus grypus*) were based on anecdotal accounts of Tourist Information offices and reports by Special Committee on Seals (SCOS) and the Scottish Natural Heritage (SNH). More specific information was available for birds due to the Seabird 2000 census data (Mitchell et al 2004) but also from tourist information offices and local councils. Information on designations of Special Areas of Conservation and Natura sites was also reported to underpin discussion over potential risks from wildlife such as waterfowl and migratory birds. However, the estimation of impacts from other wildlife species, including dolphins, porpoises, otters and deer, was problematic due to the lack of information. No specific locations were reported.

- Meteorological data i.e. rainfall and wind. These data were derived from weather stations in the vicinity of a production area and were, usually briefly, discussed in the context of their seasonality and intensity; direction of prevailing winds was also flagged. Daily rainfall data was used for analysing the effects of rain on historical shellfish *E. coli* data. Wind and rainfall data were fed into hydrodynamic modelling, where this was undertaken.
- Bathymetry and bottom topography data were collected to assessing gradients from the shore to inner reaches of lochs and the potential for turbulent flow. Depth data was derived from Admiralty charts[16] or the Scottish Association of Marine Science (SAMS) and was briefly discussed in the context of movement of contaminants and observations or sampling during shoreline surveys but not explicitly due to the lack of current or dispersion measurements. Bathymetry supported hydrodynamic modelling, where this was undertaken.
- Current measurements. Such measurements were available for areas where Scottish Water or fish farms had commissioned a hydrographic study of the area. In Shetland, NAFC Marine Institute provided current meter data. Current measurements were discussed in the context of movements of contaminants and hydrographic assessments.
- Tidal data. This information was obtained from charted information by the UK Hydrographic Office (i.e. the tools EasyTide and TotalTide. Software such as POLTIPS-3[17] was used to compute the tidal cycle in the SPAs. The output summarises mean tidal (spring/neap) range and mean high and low water at spring and neap tides. This information was used to compare shellfish *E. coli* data between different stages of the tidal cycle. The extent of tidal transport of contaminants was also assessed but not explicitly due to lack of current and dispersion measurements. Tidal data was used for hydrodynamic assessment and was briefly discussed in the context of movement of contaminants and observations or sampling during shoreline surveys. Data from sites closest to the production area were used assuming that tidal flow will be very similar between sites.
- River flow. This information is available only where a gauging station is available in the shoreline bordering a production area.
- Historical shellfish *E. coli* data from FSS' post-classification monitoring data and, where the boundaries of SPAs were delineated withinSWPAs, from SEPA's monitoring for the Shellfish Water Directive. Collection of this data aimed to assess the potential for conducting statistical analyses and to summarise data per production area as follows: site, species, sampling locations, total number of samples per year, minima and maxima concentrations over the available record, as well as

[16] Electronic bathymetry data was obtained from SeaZone initially, but this source was abandoned due to the cost of licensing. That is why bathymetry data were redacted from a number of the earlier reports (pers com. M. Price-Hayward, CEFAS).

[17] An easy-to-use software package for Microsoft Windows.

geometric mean and concentrations at 90th and 95th percentiles. This summary informed the decision on sampling point. However, the majority of reports mention that "A spatial assessment of the sampling data could not be undertaken due to the majority of samples being identified at the nominal RMP." Depending on available historical data per SPA, some reports contain data on more than one shellfish species.

- Historical classification results in each production area derived from FSS. This information was presented and discussed, where relevant, in the context of sampling frequency. For example, "when a production area has held the same (non-seasonal) classification for three years, and the geometric mean of the results falls within a certain range it is recommended that the sampling frequency be decreased from monthly to bimonthly." (CEFAS-Dornoch Firth 2010).

**Shoreline surveys.** The observations and samples collected during the shoreline surveys comprise the most detailed part of the available sanitary survey reports. Generally, this is due to the lack of historical data on the potential factors influencing shellfish *E. coli* concentrations such as salinity temperature, water *E. coli* (aka as bacteriological surveys), tides, livestock, FIOs in sewage discharges and FIOs in the vicinity of wildlife colonies. As a result, the types of information derived from desk-top surveys were supplemented by shoreline observations and sampling. However, these data could not be used in the statistical analyses as there collected at different dates compared with the historical classification data. In addition, shoreline surveys typically took place within one or two consecutive days in each SPA. Therefore, the data derived from shoreline survey sampling were not representative of the environmental variability in any area.

**Analyses of data**. Two types of analyses are described in the sanitary survey reports.
The first type involves statistical analyses to assess the variability in historical shellfish *E. coli* concentration data in relation to seasons and other variables such as temperature, salinity, tides and rainfall 2- and 7- days before sampling. Analyses mainly included:

- Scatterplots of shellfish *E. coli* data by date, month and season fitted with a lowess trendline, which allows for locally weighted regression scatterplot smoothing.

- One way Analysis of Variance (ANOVA) to compare shellfish *E. coli* data by season.

- Scatterplots between shellfish *E. coli* and rainfall, salinity and temperature data

- Polar plots of shellfish *E. coli* on tidal data (spring/neap and high/low cycles).

**Hydrographic assessments.** These estimate surface flow and exchange properties on the basis of measurements of model output data. Modelling was rarely feasible. The value of hydrodynamic modelling can be clearly illustrated in predictions of the direction and dispersion of contaminated flows. For example, the sanitary survey report for Loch Ryan used a layered box model approach to quantify surface flow and exchange mechanisms such as tidal volume versus estuarine circulation volume flux, entrainment between upper and lower water layer (wind versus tidal density driven), and flushing time (median and 95%-ile) (CEFAS Loch Ryan 2014 uploaded to CEFAS 2018).

**Conclusion.** Each sanitary survey report concludes with a sampling plan on the basis of the data collected and analysed, explicitly reports the:

- **Boundaries of SPAs**. A number of reports recommended reorganisation of SPAs, by splitting or agglomerating them to reflect the findings of the sanitary surveys. (e.g. CEFAS-Vaila Sound 2010 uploaded to CEFAS 2018). Generally, the major reason for recommending changes in boundaries was to exclude areas lying nearer to contaminating sources (e.g. CSOs or river/stream mouths) while still including commercial shellfish harvesting sites.

- **Location of representative monitoring point(s) (RMPs).** As explicitly mentioned in the sanitary survey reports the selection of RMPs was intended to take account of the full extent of the shellfishery, the position of local point and diffuse faecal pollution sources, tidal flows and other relevant factors. Practically, RMPs reflect the location at highest risk of faecal pollution within the classified area. The majority of reports recommended only one RMP per SPA; however, there have been examples of recommending parallel monitoring two RMPs e.g. at Riskaness and Lera Voe sites for 1 year to determine whether these sites should be monitored separately (CEFAS-Vaila Sound 2010 uploaded to CEFAS 2018).

- **Sampling tolerance**. This ranged between 10 to 500ml. A greater tolerance was recommended for dredged shellfisheries and where there was potential for the line to shift in the wind and tides.

- **Depth of sampling.** This parameter is not consistently reported. One reason for this maybe that depth is not relevant to subtidal shellfisheries.

- **Sampling frequency.** All reports recommended monthly sampling to reflect the significant effect of rainfall and season on shellfish *E. coli* data.

## V.5 Results of GIS and statistical analyses

The data on catchment indicators can be divided into two categories: data providing both spatial and temporal information for the period 1999-2017[18], such as rain and livestock; and data referring only to a specific location within the source-catchment or a specific sources catchment, such as septic tanks and catchment area.

A broad range of values was observed for both categories of catchment indicators (Table V.1). The greatest range of values was observed for total annual rain (range: 119 to 4114mm), showing that some source-catchments had no rain at all a whole year during the study period (1999-2017); number of septic tanks (range: 0-1046); and catchment area, which ranged from 0.1 km$^2$ (Sound of Barra, SWPA=72, SPA=473) to 688km$^2$ (Loch Fyne, SWPA=39, SPAs=151, 634, 635, 569, 571, 714). Seal colonies were also considered as a catchment indicator, but it is recognised that information on catchment-based wildlife population is scarce and incomplete, and rather qualitative than quantitative, i.e. there may be information on presence of a type of wildlife but not on their density.

Table V. 1. Overview of desk data for all SWPA source-catchments (aka catchment indicator data)

| Spatial and temporal data | Minimum value at source-catchment | Maximum values at source-catchment |
|---|---|---|
| Total annual rain (mm) | 119 | 4114 |
| Cattle density | 0 | 669 |
| Sheep density | 0 | 422 |
| Pig density | 0 | 5 |
| Poultry density | 0 | 211 |
| Goat density | 0 | 1 |
| Horse density | 0 | 8 |
| Deer density | 0 | 1 |
| | | |
| **Only spatial data** | **Minimum value at source-catchment** | **Maximum values at source-catchment** |
| Septic Tanks numbers | 0 | 1046 |
| Number of Effluent point sources (CSOs, Sewage trade effluent, WwTP) | 0 | 7 |
| Catchment Area (km$^2$) | 0.1 | 669 |

### *V.5.1. Shellfish E. coli variation in SPAs sitting within SWPAs (Figure V.5.1)*

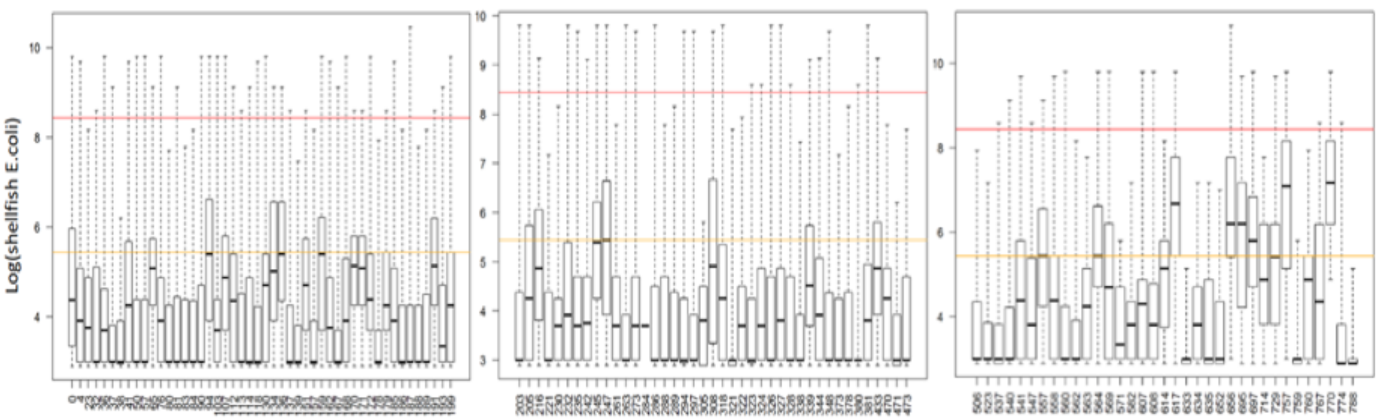



**Figure V.5.1.** Boxplots showing differences between SPAs. The mid-line shows the median and the box shows the interquartile range. The orange line indicates the 230 E-coli per 100g of flesh threshold and the red line the 4600 E-coli per 100g of flesh threshold.

[18] Data for livestock were available until 2015.

### V.5.2 Shellfish E. coli variation in SWPAs (Figure V.5.2)

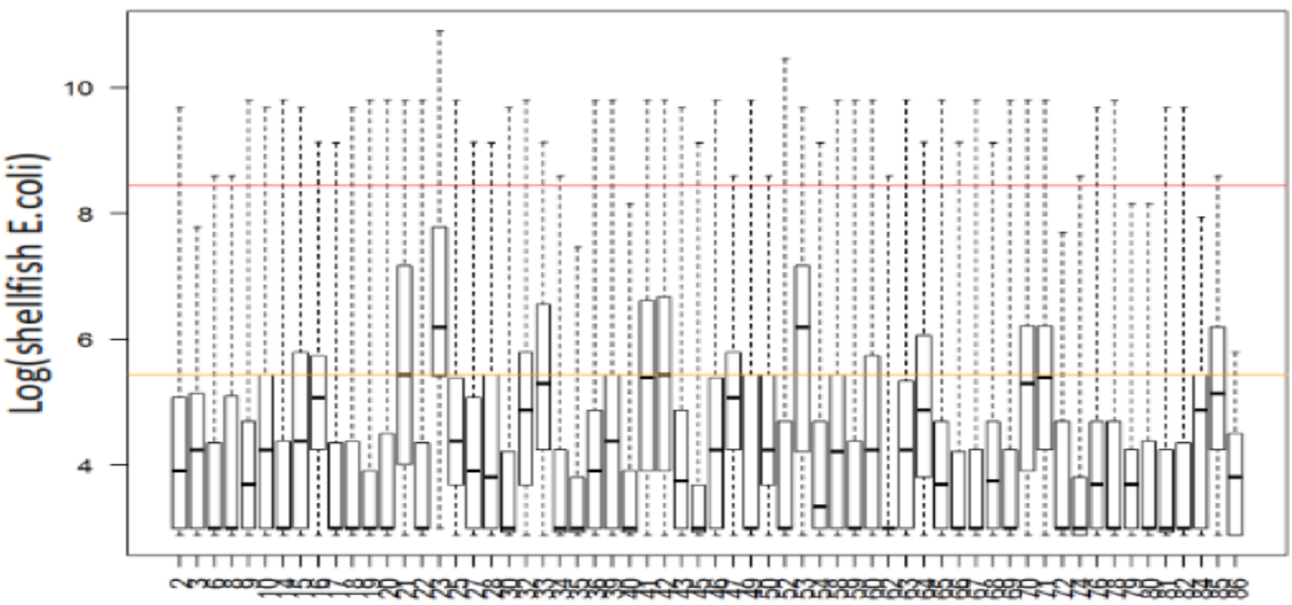



**Figure V.5.2**. Boxplots showing differences between SWPAs. The mid-line shows the median and the box shows the interquartile range. The orange line indicates the 230 E-coli per 100g of flesh threshold and the red line the 4600 E-coli per 100g of flesh threshold. Data from SPAs within SWPAs have been pooled.

### V.5.3 FIO export coefficients in relation to land use (Figure V.5.3)

Examination of land use in the source-catchments and waterbodies showed that:

- Land use was predominantly rural in 82 SWPA source-catchments.
- In three SWPA source-catchments (i.e. 15, 16 and 85) land use was semi-urban, i.e. built up areas comprise between 2.5% and 9.9% of the catchment area. According to Kay et al (2008a) it can be assumed that the export of FCs from the semi-urban source-catchments ranges from 1.2 X $10^9$ cfu/km$^2$/h at baseflow to 4.6 X $10^{10}$ cfu/km$^2$/h at high flow.
- Improved grassland or rough grassland did not exceed the 75% of the total area of any of the SWPA source-catchments. However, two SWPA source-catchments were covered by more than 70% improved grassland which may mean increased FIO export during baseflow and high flow.
- Woodland covered more than 95% only in one source-catchment (i.e. 24). Interestingly, this catchment could be characterised as remote, but the study by Kay et al (2008a) indicates that during high flow an amount of 3.3 X $10^9$ cfu/km$^2$/h of faecal coliform can be exported from this catchment, which is higher than the amount of FCs exported from semi-urban areas during baseflow.
- Small waterbodies covered by more than 75% improved or rough grassland or woodland were found to occur in areas upstream of SWPAs, thus indicating that "hot spots" of livestock and wildlife FIO sources exist within the SWPA source-catchments (Figure V.5.3).

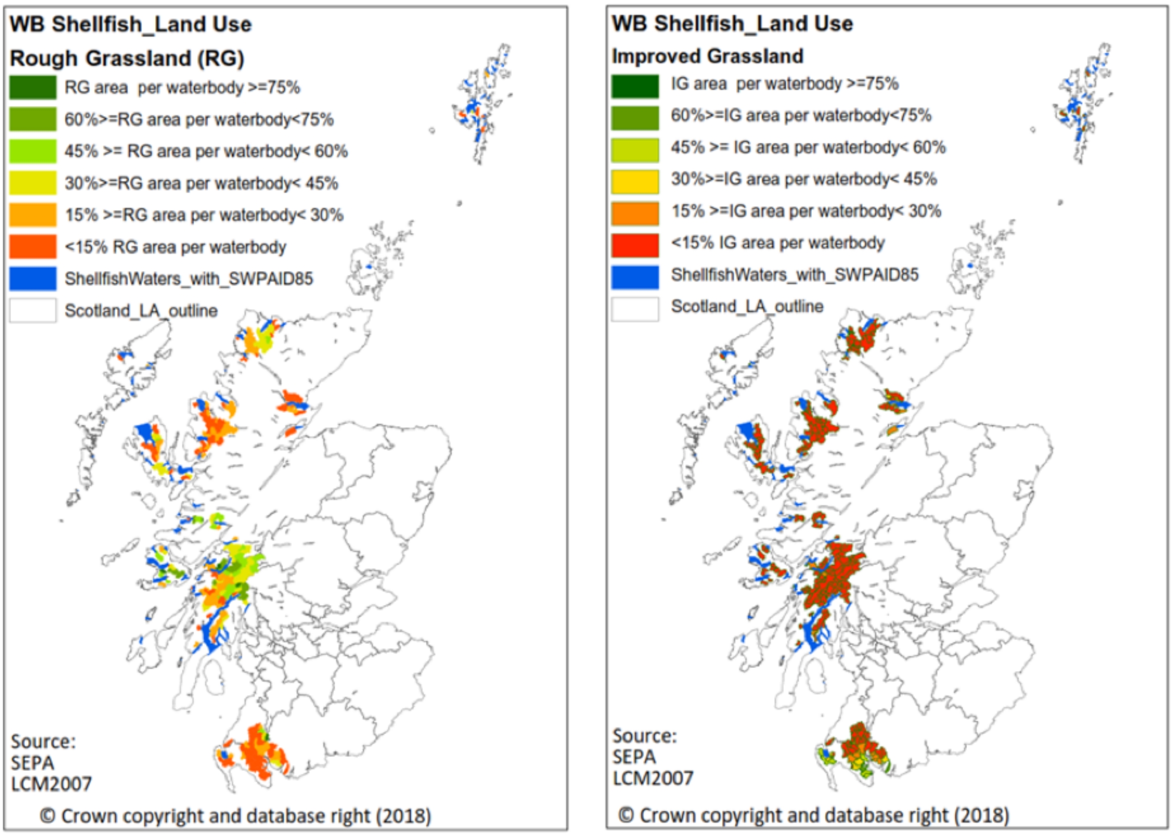



**Figure V.5.3.** Land use as a proxy for FIO export coefficient from waterbodies draining to SWPAs. Export estimates have been given by Kay et al (2008a) for catchments where rough grassland or improved grassland exceed 75% of land cover (see text).

## V.5.4 Regression analyses

Shellfish E-coli significantly varied with shellfish species, month of sampling (season), and antecedent rainfall (Table V.2). Sheep grazing had a marginally significant effect on shellfish *E. coli* but there were no significant effects due to the density of other livestock species. No significant effects on shellfish *E. coli* could be detected due to the numbers of septic tanks or population size per source-catchment. Overall, some variation could be explained by differences between SWPAs but there is a large component of unexplained variation between samples from the same location collected at different times even after allowing for the effects of month and rainfall.

**Table V.2. Significance levels for the explanatory variables considered. * Effect is significant at p<0.05.**

| Variable | p-value |
|---|---|
| Species | <0.001* |
| Month | <0.001* |
| Two-day antecedent rain | <0.001* |
| Three to seven-day antecedent rain | <0.001* |
| Septic tanks | >0.05 |
| Population | >0.05 |
| Sheep | <0.05* |
| Cattle , Poultry, Pigs (separately) | >0.05 |
| Trend with year | <0.001* |

### V.5.4.1 1999-2017 Shellfish E. coli variation by month

Shellfish *E. coli* concentrations varied by the month of sampling (Figure V.5.4.1). Maxima were observed for samples collected in August and September and minima in April. It should be noted that this does not reflect the seasonal pattern of rainfall; rainfall is highest during the winter months. *E. coli* concentrations above the value of 4600 MPN counts / 100g of FIL were recorded in all months except February (Figure 3). However, only in July, August and September the upper quartile of the shellfish *E. coli* concentration boxplots were above the value of 230 MPN counts of *E. coli* /100g of FIL.

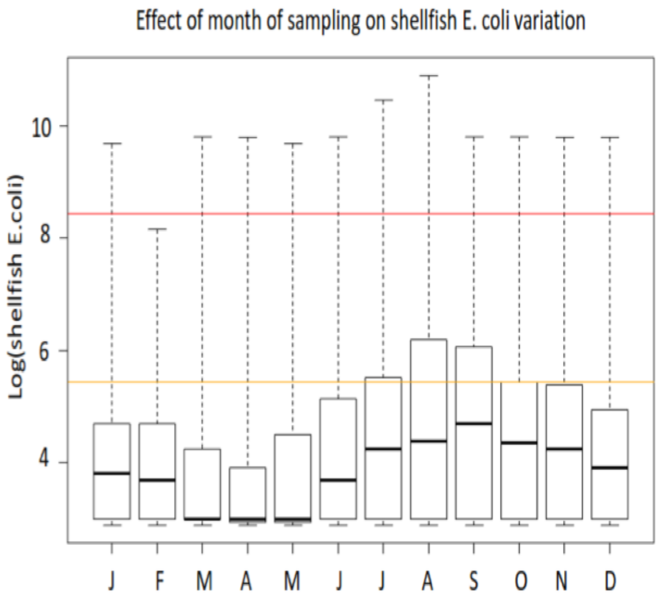
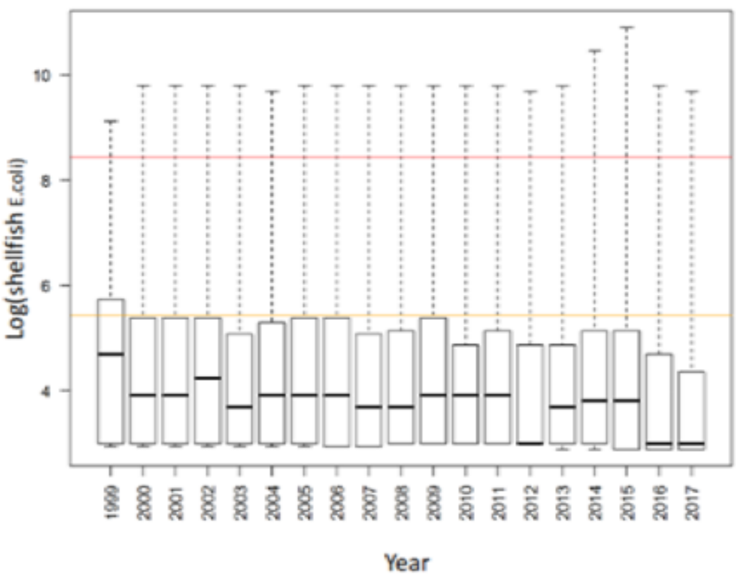



**Figure V.5.4.1**. Boxplots showing shellfish *E. coli* concentrations by month. The mid-line shows the median and the box shows the interquartile range. The orange line indicates the 230 E-coli per 100g of FIL threshold for granting A classification for SPAs, and Good classification for SWPAs. The red line shows the 4600 E-coli per 100g of FIL threshold for granting B classification for SPAs and Fair classification for SWPAs.

### *V.5.4.2 1999-2017 Shellfish E. coli variation by year*

Shellfish *E. coli* concentrations slightly declined from 1999 to 2017 (Figure V.5.4.2). Despite occurrences of shellfish *E. coli* data above the value of 4600 MPN counts /100g of FIL, the upper quartile of *E. coli* concentrations is consistently below the value of 230 MPN counts /100g of FIL 230.



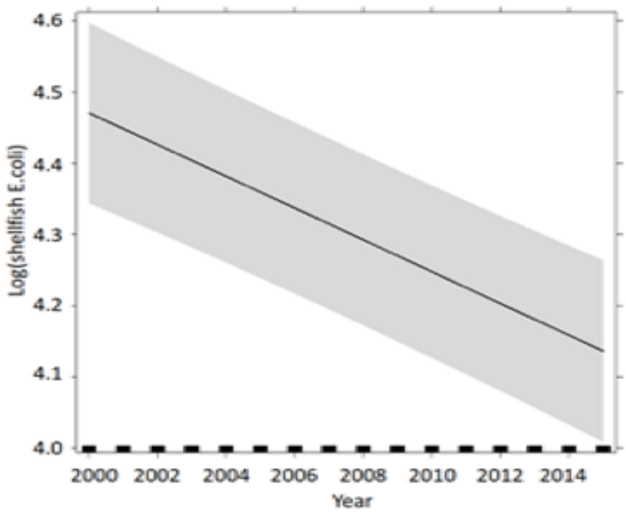




**Figure V.5.4.2.** Left: Boxplots showing shellfish *E. coli* concentrations by year. The mid-line shows the median and the box shows the interquartile range. The orange line indicates the 230 E-coli per 100g of FIL threshold for granting A classification for SPAs, and Good classification for SWPAs. The red line shows the 4600 E-coli per 100g of FIL threshold for granting B classification for SPAs and Fair classification for SWPAs. Right: Effect of year on shellfish *E. coli* concentrations.

### V.5.4.3 Effect of the interaction "shellfish species by month of sampling" on shellfish E. coli

Shellfish *E. coli* concentrations varied by species, with cockles displaying the highest contamination levels and (Figure V.5.4.3A). The most commonly commercially harvested species in Scotland, i.e. native (n.) oysters, Pacific (P.) oysters, cockles and common mussels, displayed a strong seasonal cycle, with 1999-2015 maxima in August or September and 1999-2015 minima in March and April for P. oysters, n. oysters, cockles and mussels (Figure V.5.4.3B).

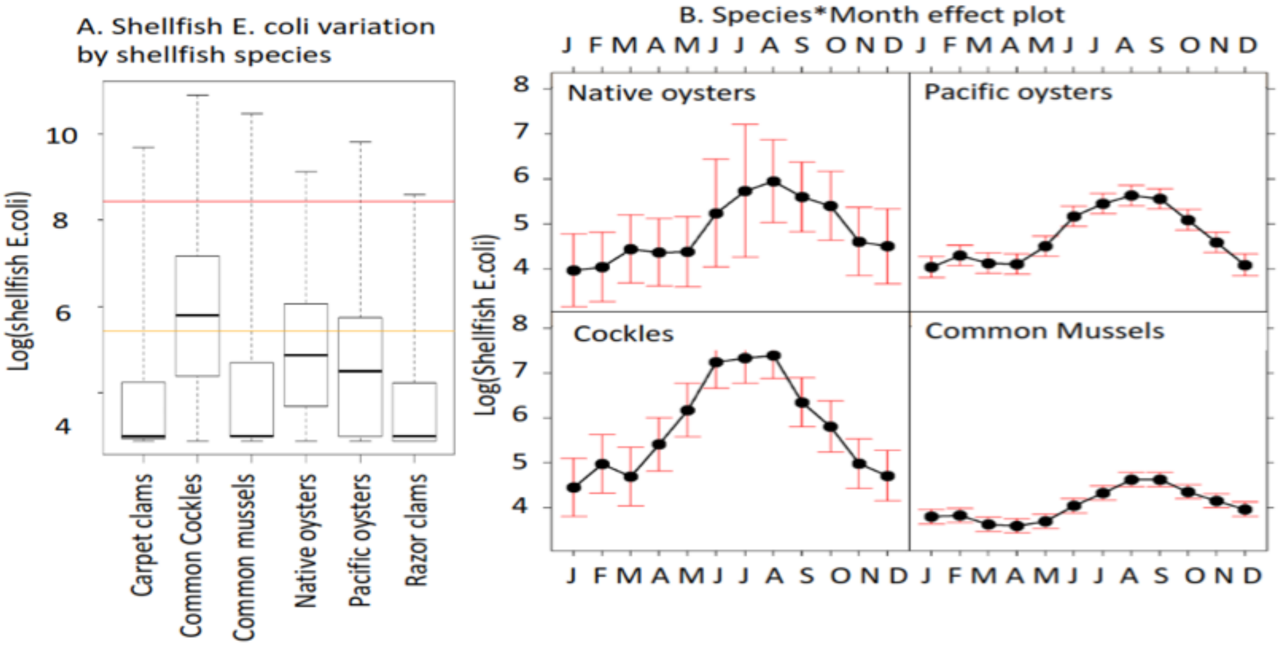



**Figure V.5.4.3.** A. Boxplots showing differences between species. The mid-line shows the median and the box shows the interquartile range. The orange line indicates the 230 E-coli per 100g of flesh threshold and the red line the 4600 E-coli per 100g of flesh threshold. B. Estimated effects of species and month after fitting a linear mixed model with species, month, the interaction between species and month, antecedent rainfall, sheep and year as fixed effects, and SWPA and SPA as random effects. This covered the period 1999-2015 for which rainfall data were available.

### V.5.4.4 Effect of antecedent rain on shellfish E. coli

Shellfish *E. coli* increased linearly in response to both 2-day and 3 to 7-days antecedent rain (Figure V.5.4.4a). The analyses showed that rain events in the source-catchment in the period of 2 days and 3 to 7 days before a sample is collected in SWPAs tend on average to increase *E. coli* levels above the baseline level for the particular species and month. Rain increased towards the winter months and reduced in the summer, with greatest levels observed in November, December and January and lowest in June (Figure V.5.4.4b).

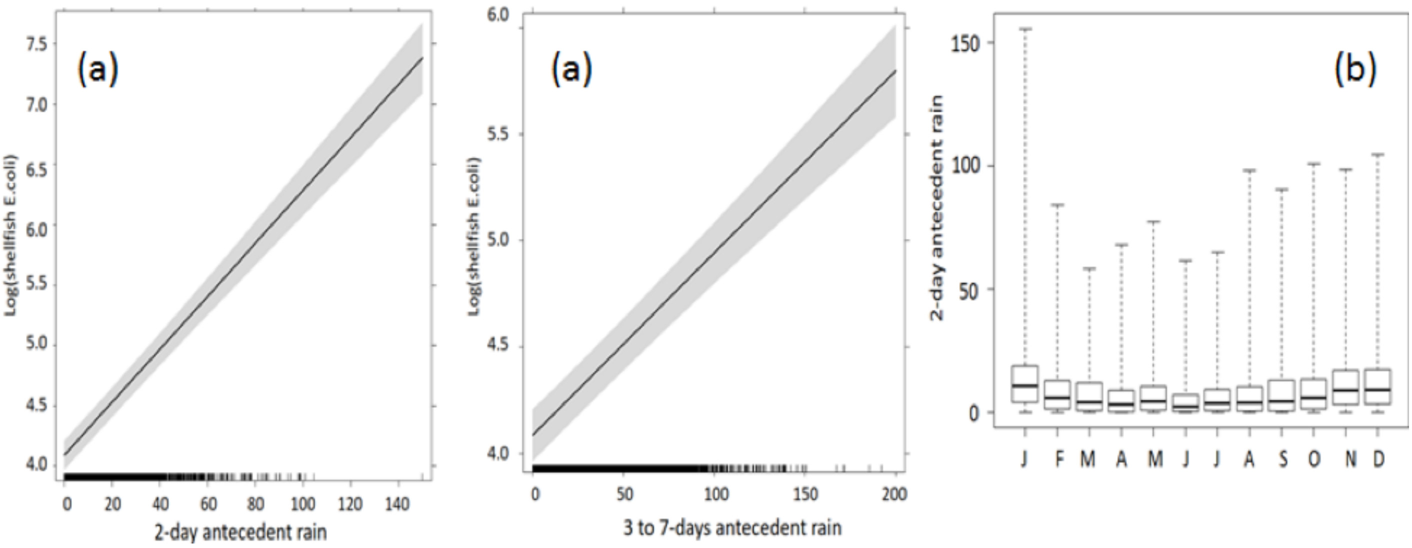



**Figure V.5.4.4a.** Effect of 2-day and 2-7 days antecedent rain on shellfish *E. coli* levels. b. Seasonality in 2-day antecedent rain.

### V.5.4.5 Effect of sheep density on shellfish E. coli

The analyses showed that higher sheep densities of source-catchments were associated with a slight increase in shellfish *E. coli* concentrations (Figure V.5.4.5).

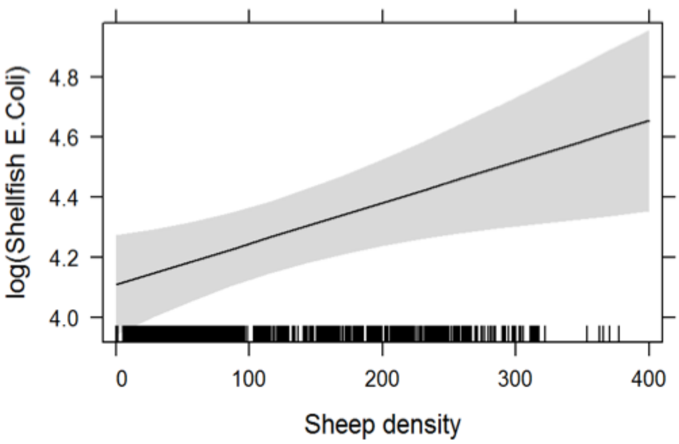



**Figure V.5.4.5.** Effect of sheep density in source-catchments on shellfish *E. coli* concentrations.

### V.5.5 Multivariate analysis

A principal component analysis of catchment indicators was carried out using the 1999-2015 average of rain and livestock and the available septic tank data (2016) and population (2011 census). The aim of this analysis was to explore any spatial patterns indicating which catchments pose a high risk from faecal contamination to SWPAs based on the assumption that groups of source-catchments with higher livestock density, rain, septic tank or population will signify a higher risk from diffuse pollution to their SWPAs. Catchment area was used a proxy to the size of the river network in each catchment and was used to explore whether smaller source-catchment, whereby FIO transfer may be faster, are grouped apart from larger catchments on the basis of livestock density and rain.

The first principal component (PC1) explained 57% of the variation and the second principal component (PC 2) 30%. The loadings are given In Table V.3.

**Table V.3.** Relative contribution of variables to the Principal Components.

| (1999-2015 Average) | PC 1 (57%) | PC2 (30%) |
|---|---|---|
| rainfall | 0.33966 | -0.55963 |
| livestock | -0.23361 | 0.67863 |
| log(area) | 0.56362 | 0.04706 |
| log(population) | 0.48045 | 0.41094 |
| log(septic tanks numbers) | 0.53062 | 0.23493 |

The biplot (Figure V.5.5) showed a continuum of source-catchment indicator data across PC1 on the basis of numbers of septic tanks, population and catchment size, i.e. mainly human sources. The biplot was a straightforward way to visualise which SWPA source-catchments have a higher population and number of septic tanks, i.e. those on the right hand side of the arrangement in the biplot) and which source-catchments have higher rain (i.e. those towards the top of the biplot) or livestock density (i.e. those at the bottom of the biplot) (Figure V.5.5). The arrangement in the biplot also shows that SWPA source-catchments at the range of higher rain levels have lower livestock density (Figure V.5.5).

Univariate analyses on each of the variables used for the PCA showed no significantly different groups of SWPA source-catchments. The results of the univariate and multivariate analyses on these catchment indicators suggested that the SWPA source-catchments cannot be categorised in different groups on the basis of the quantitative data on catchment indicators such as rain, septic tanks and livestock density.

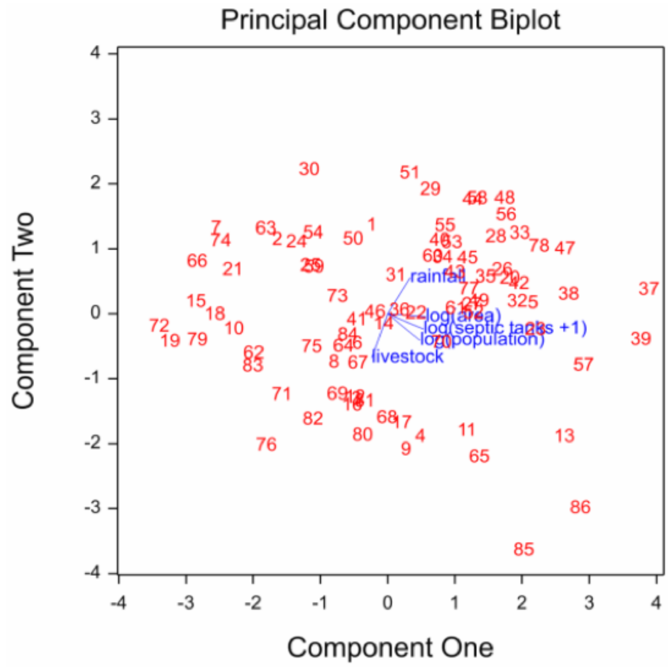



**Figure V.5.5.** Biplot of principal component analysis on catchment characteristics. The red symbols indicate the SWPAs; those which are more similar to each other are closer together. The direction of the blue lines indicates how each of the variables contributes to the principal components. SWPA source-catchment 86 is the catchment draining to Wigtown Bay, which is not designated as a SWPA but has currently classified areas. Number denote the code number of SWPA.

### V.5.6 Exceedances and heavy and extreme catchment rain events

By reference to Kendon et al. (2016), "heavy rainfall" and "extreme" rainfall events were estimated as the rainfall levels above the 95th- and 99th-percentiles of the whole rain data series from all source-catchments during 1999-2015, respectively. The analyses showed that:

- The number of heavy rainfall events in the catchments draining to SWPAs on days when *E. coli* samples were taken for the period from 1999 to 2015 ranged between 1 event (in three SWPAs) and 99 events in one SWPA (i.e. 65).
- The number of extreme rainfall events on days when *E. coli* samples were taken in the catchments draining to SWPAs for the period from 1999 to 2015 ranged between 1 event (in 14 SWPAs) and 29 events in one SWPA (i.e. 65).

The number of *E. coli* samples taken on days when there was heavy or extreme rainfall was compared with the number that would be expected by chance given the sample size. The same was done for the number of days on which rainfall exceeded the 25$^{th}$ and 50$^{th}$ percentiles. There was no evidence to suggest that rainfall events were avoided when sampling, or that sampling was not random with respect to rainfall.

In addition, the rainfall levels above the 95th- and 99th-percentiles of the whole rain data series from all source-catchments during 1999-2015 varied considerable between SWPAs. The level of heavy rain for a two-day period varied by catchment-SWPA and ranged between 17.20 and 63.39mm. The level of extreme 2-day antecedent rain, defined as the level above the 99$^{th}$ percentiles of the whole rain data series in a source-catchment during 1999-2015 also varied by catchment-SWPA and ranged between 29.24 to 94.98mm.

Only 1507 out of 17940 shellfish *E. coli* results were found to exceed the level of 700MPN/100g of FIL. It was observed that the greatest shellfish *E. coli* levels did not coincide with all heavy and extreme rain events; conversely, shellfish *E. coli* levels above the threshold of 4600 MPN/100g FIL were observed at rain levels below the 30mm (Figure V.5.6).

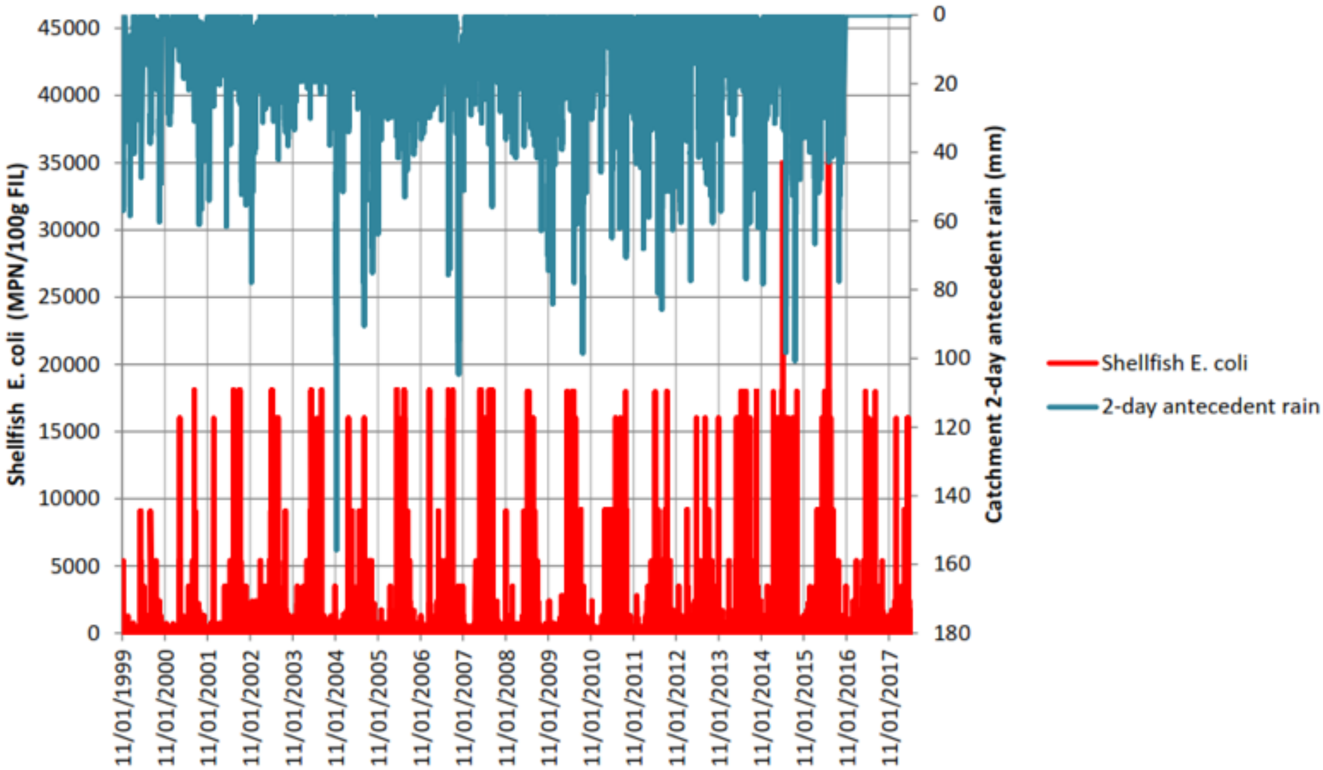



**Figure V.5.6.** Shellfish *E. coli* results in relation to two-day antecedent rain intensity (mm).

## V.6 Trial desk studies

### *V.6.1 Cat Firth (SWPA€8, SPA€32 classified for commercial harvesting of mussels)*

Blue mussels are collected from the RMP of SPA=32 at the centre of Cat Firth (Figure V.6.1), which completely sits within SWPA=8. The results of the desk study are summarised below.

- Land Use. Source-catchment is dominated by bogland (60% of total area), with smaller areas of improved and rough grassland at the northern shoreline (Figure V.6.1a).
- Seasonality of shellfish *E. coli* data Mussel *E. coli* levels varied by month of sampling with highest values in

August and September and lowest values in January and April (Figure V.6.1b). Using shellfish *E. coli* data collected post-2014 resulted in high confidence of a class A for the SPA=32 or Good classification for the SWPA=8 with no exceedances of the 230MPM/100g of FIL.

- Effect of rain. Rain was seasonally variable with maxima in October and minima in January (Figure V.6.1c). Two-day antecedent rain had no significant effect on shellfish *E. coli* concentrations This may be partly explained by the different patterns in *E. coli* and rain seasonal variability. However, it may also be due to sampling bias regarding heavy and extreme rain. Very few heavy and extreme rain events were observed. Minor freshwater inputs to the area were observed in an earlier observation by SEPA (2011).
- Livestock. Overall, sheep density declined from approximately 250 sheep /$km^2$ in 1999 to Post-160-166 sheep /$km^2$ post-2014 (Figure V.6.1d). This density is above the national scale threshold for observing a significant effect of livestock on shellfish *E. coli* levels. This is an indication that sheep in Cat Firth may affect the microbiological quality of the area.
- Septic Tanks. An estimated 17 septic tanks were observed at the northern shoreline of Cat Firth (Figure V.6.1a).

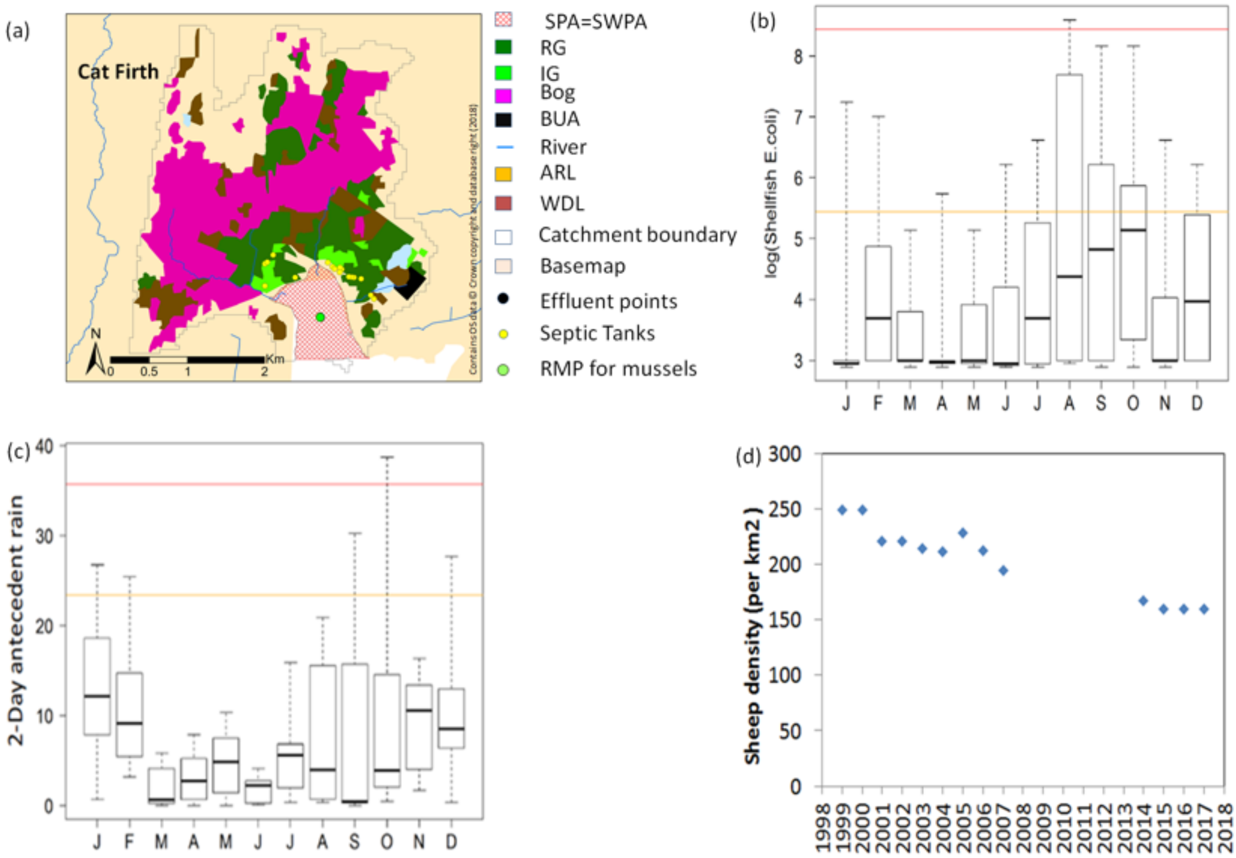



**Figure V.6.1.** Desk study in Cat Firth. (a) Land use map, boundary of SWPA (here, it is the same as the SPA) and location of RMP Shellfish *E. coli* results. RG: Rough Grassland; IG: Improved Grassland; BUA: Built-up areas; ARL: Arable land; WDL: Woodland. (b) Shellfish *E. coli* results. (c) Two-day antecedent rain intensity (mm). (d) Sheep density per km2. Data sources: FSS, SEPA, CEH, OS Open Data.

- Population. The 2011 Population Census result for the parishes comprising the source-catchment showed a resident population of 318 people.
- Sanitary surveys. No sanitary survey report is available for this area.
- SEPA 2011 report under Shellfish Water Directive. A descriptive report is available. No significant point source or diffuse pollution pressures were identified in the report. Additional information refers to low freshwater inputs and estimates for flushing time (4 days).
- Overall conclusion on the risk of faecal contamination from catchment based sources. On the basis of the desk-based evidence the overall conclusion is that there is low risk of faecal contamination from livestock and septic tanks.
- Conclusion on the sampling plan. As data came from one RMP, it is not possible to assess whether it is representative of the greatest impact of pollution sources to the shellfishery. The monthly variability indicates that monthly sampling, as currently applied, is necessary.
- Conclusion on the use of this information as part of the sanitary survey process. The desk study presented here helped to quantify the potential effects of rain on shellfish *E. coli* results and map the rural land uses generating faecal contaminants, i.e. improved and rough grassland and septic tanks. Therefore, it meets the requirements of the *Regulation (EC) 854/2004* for undertaking surveys on pollution sources and quantifying these sources. The desk study presented here is also in line with the recommendation of the Guide for assessing historical shellfish *E. coli* data in the context of desk-based surveys on pollution sources. The results of this desk study can be supplemented by shoreline and catchment observations, which can be recorded during the ongoing sampling by FSS. Given the high confidence of class A or Good classification, and the assessment based on the GIS and statistical analyses, it is suggested that this desk-based assessment of pollution sources and any field observations are used for completing a sanitary survey report for Cat Firth. The sanitary survey for Cat Firth could be used by harvesters and LAs before submitting an application for a different site for mussels within Cat Firth or a different species. In the case of an application for a different species, E coli data for this species would be needed in order to identify the species-specific RMP.
- Supplementary sampling by SEPA or FSS. This sampling can take place as part of the sanitary survey to account for the effect of the relatively long flushing period by targeting worst-case conditions with regard to rain (i.e. rain above 23 and 35mm in the two days prior to sampling[19]) and any event causing turbulence or sediment resuspension in the embayment. This will help to better understand all processes and pollution sources potentially impacting microbiological quality, as required by the *Regulation (EC) 854/2004* and the SG Directions (2015, 2016). However, current evidence is not supportive of prioritising additional monitoring in this area.

### V.6.2 Cromarty Bay (SWPAϵ11, Not currently classified SPA and therefore no data for SWPA classification)

The location of shellfish *E. coli* samples from declassified SPAs is presented in Figure V.6.2. Mussels and P. oysters had been sampled in the past. The results of the desk study are summarised below.

- Land Use. Source-catchment is characterised by mixed farmland (over 50% of total area), mainly at the shoreline and woodland (37%) further upstream (Figure V.6.2a). Built up areas are located at the East part of the Bay at Jemimaville.
- Seasonality of shellfish *E. coli* data. No shellfish *E. coli* data.
- Effect of rain. No shellfish *E. coli* data.
- Livestock. Overall, livestock density remained below 100 individuals / km$^2$. However, post-2013 poultry density increased above 200 individuals / km$^2$. (Figure V.6.2b). This is an indication that poultry may affect the microbiological quality of the area; however, it is not known where these operations are located within the source-catchment.
- Effluent. The recent map of Effluent locations (SEPA 2016; see Figure V.6.2a) shows no waste water treatment plans, or discharges from trade effluent or CSOs within the designated area. However, a number of potential sewage sources can be seen around Invergordon (across from Cromarty Bay) and at Cromarty.
- Septic Tanks. An estimated 326 septic tanks were observed at the source-catchment (Figure V.6.2a), many of them located around the Newhall Burn which discharges into the west part of the Bay.

[19] MetOffice forecasts can help to prepare for a sampling targeting such "worst-case" conditions.

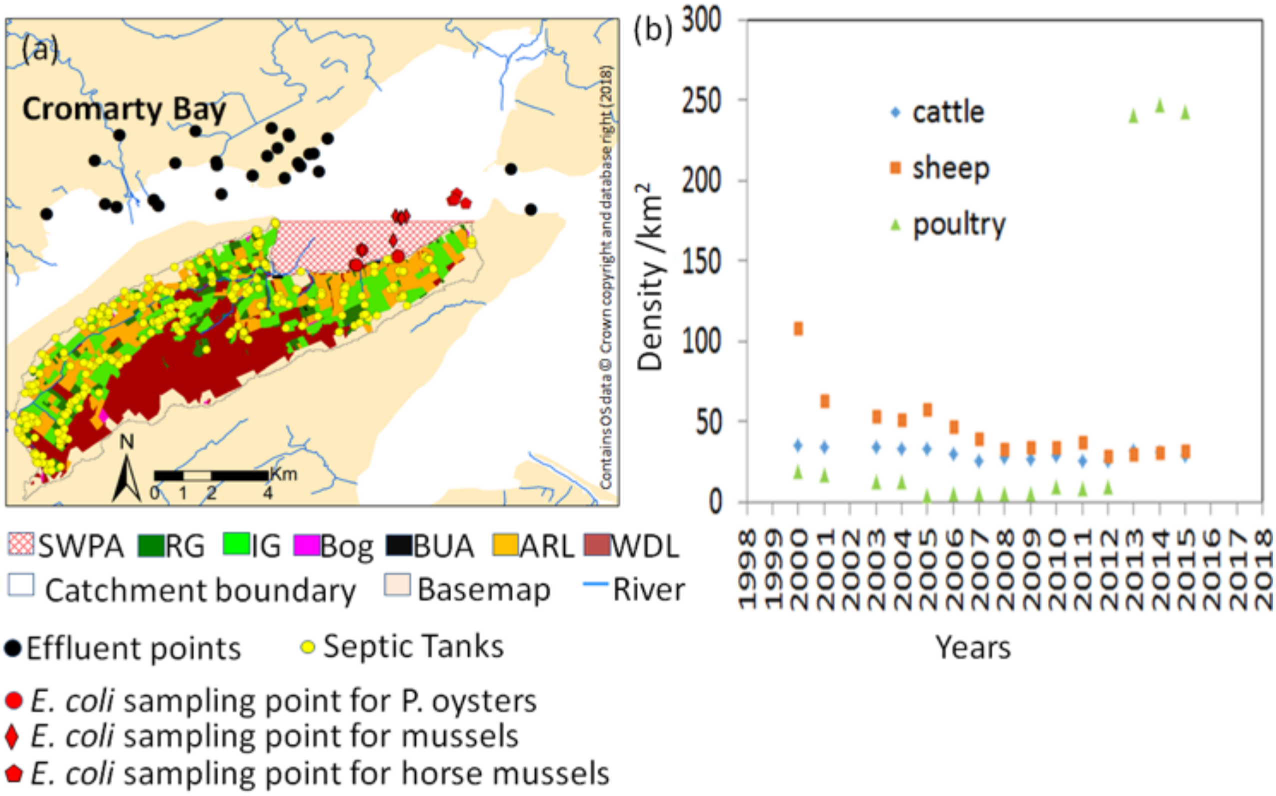



**Figure V.6.2b.** Cromarty Bay: (a) Land use map, boundary of SWPA=11 and locations of shellfish *E. coli* monitoring stations in the past. RG: Rough Grassland; IG: Improved Grassland; BUA: Built-up areas; ARL: Arable land; WDL: Woodland. (b) Livestock density per km2. Data sources: FSS, SEPA, CEH, OS Open Data.

- Population. The 2011 Population Census result for the parishes comprising the source-catchment showed a resident population of 1796 people.
- Sanitary surveys. The available sanitary survey report for this area has assessed pollution sources impacting all the bay and presented *E. coli* results for two shellfish species, mussels and P. oysters, based on samples from bags deployed to study the effect of specific sources (i.e. Cromarty Harbour, eastwards currents and river discharges). The report assesses shellfish *E. coli* data collected during shoreline surveys in the context of a desk-based and shoreline surveys of pollution sources as well as hydrodynamic modelling. The report concluded that the major risks regarding faecal contamination are related to
  - o Sources of human and animal faecal contamination outwith Cromarty Bay due to water circulation patterns in Cromarty Firth (i.e. hydrodynamic modelling showed westward transport of contaminants from Cromarty to mussel operations within the Cromarty Bay and eastward transport of contaminants from sources to the west of the Bay.
  - o Sewage and wildlife around and west of Jemimaville and ShoreMill (western end) due to eastward shoreline currents.
  - o Likely increases in livestock and tourism during summer.
- SEPA 2011 report under Shellfish Water Directive. A descriptive report is available indicating that point sources at Jemimaville and diffuse pollution from livestock and septic tanks may cause failures with respect to the standards for the Shellfsh Water Directive
- Overall conclusion on the risk of faecal contamination from catchment-based sources. On the basis of the desk-based evidence the overall conclusion is that there is medium to high risk of faecal contamination for intertidal species from point sources, river discharges and wildlife at west end because of eastward shoreline transport of contaminants. There is also medium to high risk of faecal contamination for subtidal sites at the north-eastern parts of the Bay due to westward transport of contaminants from Cromarty.
- Conclusion on the sampling plan. As a time-series of historical data is not available the selection of RMP requires sampling data form points selected to address the findings of the available hydrodynamic model output. It is suggested that worst–conditions at Cromarty Bay are tested against the tidal movements as well as rain. The SWPA is spatially heterogeneous, therefore an area-based classification cannot be representative of the greatest impacts from faecal contamination on a local scale.
- Conclusion on the use of this information as part of the sanitary survey process. The evidence presented and

analysed here helped to quantify catchment indicators and to understand that the designated area is spatially heterogeneous. Each part of the designated area is influenced by different sources of faecal pollution, some of which come from outwith the source-catchment. It is suggested that any sampling during a future sanitary survey to identify a sampling plan addresses this evidence. Without shellfish *E. coli* sampling data it is not possible to identify the RMP for shellfish *E. coli* levels and therefore it is not possible to identify a species and site-specific sampling plan. That said, the evidence presented here can be used by harvesters and the LA to inform application for a new harvesting area within Cromarty Bay.

- Supplementary sampling by SEPA or FSS. This sampling may be used to assess the FIO content of shoreline sources such as wildlife, any discharges from Newhall Burn and Shoremill. In addition, SEPA could deploy bags with mussels or oysters or both at least two weeks before sampling to explore levels of shellfish faecal contamination in relation to specific events, (e.g. rain) or sources to assess whether a site has the potential for development of shellfish aquaculture. However, bag samples could be used for the classification of a SWPA only

### V.6.3 Loch Ryan (SWPA€85, SPA€191)

Loch Ryan (SWPA=85) can be divided to south (inner) and north (outer) part. The south part is currently classified (SPA=191) for commercial harvesting of n. oysters, whereas SPAs for the harvesting of cockles and mussels with the south part have been declassified. The north part has also been declassified for the harvesting of razor clams. SEPA has also collected samples from a declassified site for cockles at the beach north of Sandmill Farm and from the n. oyster bed 200m south of the Cairnryan Ferry Terminal and near the RMP for SPA=191. Supplementary water samples have been taken by SEPA at the cockle and oyster sample point and at Sole Burn, which is potentially a source of contamination from livestock (SEPA 2018). It must be noted that these water samples may not be useful in terms of classification but may be useful in modelling transport of contaminants. Loch Ryan produces the greatest number of native oysters in Scotland. Figure V.6.3a shows the sampling points for each sampled from currently classified and declassifed SPAs within the Loch Ryan.

- Land Use. The shoreline is dominated by grassland (46% of total area of source-catchment) (Figure V.6.3a). Built areas cover more than 2.5% of the total catchment area, thus the source-catchment is characterised as semi-urban. The implication of this is that the FIO export from this catchment is one order of magnitude higher that from predominantly rural areas with less than 2.5 % of built up areas. The major built up area lies at the head of the Loch (Stranraer) (Figure V.6.3a).
- Seasonality of shellfish *E. coli* data. N. oysters were more contaminated from September to December and in June for the study period 1999-2017; data from June and July exist only for the 2016 and 2017. (Figure V.6.3b). Exceedances of the value of 230MPN *E. coli*/100g of FIL were recorded year-round but in September and November there were exceedances of the value of 4600MPN *E. coli*/100g of FIL. High confidence of a class B or Fair classification was estimated for the SPA=191 for the south part of Loch Ryan using the entire data set and data from three most recent years. This shows the persistent presence of faecal pollution sources at least in the south part of Loch Ryan.
- Effect of rain. The effect of two-day antecedent rain on n. oyster *E. coli* levels is statistically significant (Figure V.6.3c).
- Livestock. Overall, sheep density varied between the range 100-200 individuals / $km^2$ and had a significant effect on shellfish *E. coli* levels (Figure V.6.3d) during the study period. Other livestock present in the source-catchment were cattle and poultry, the latter doubling its density post-2012 (Figure V.6.3d).
- Effluent. The recent map of Effluent locations (SEPA 2016; see Figure V.6.3a) shows the presence of point source discharges of sewage and trade Effluent from the Caledonian Cheese Company, east of Stranraer[20] and discharges from Auchneel Water Treatment Works. However, a review of the two sanitary survey reports for Loch Ryan and the report by SEPA for the designated area under the Shellfish Water Directive mention many more consented discharges directly to the SWPA. This shows that desk-based data should always be crosschecked against any published literature and field observations.
- Septic Tanks. An estimated 365 septic tanks occur within the source-catchment many of them located at the shoreline immediately bordering the designated area and along the burns discharging to the west side of the Loch (Figure V.6.3a).
- Population. The 2011 Population Census result for the parishes comprising the source-catchment showed a resident population of 13,258 people; of whom 10,851 live in Stranraer.
- Sanitary surveys. There are two sanitary survey reports for Loch Ryan, one for each part. Each of these reports assessed historical data from the routine monitoring of the species harvested in the context of thorough desk-based and shoreline surveys supported by hydrodynamic

[20] This had been found to be a significant source of faecal contamination to the south Loch Ryan in the sanitary survey report by CEFAS (CEFAS 2013).

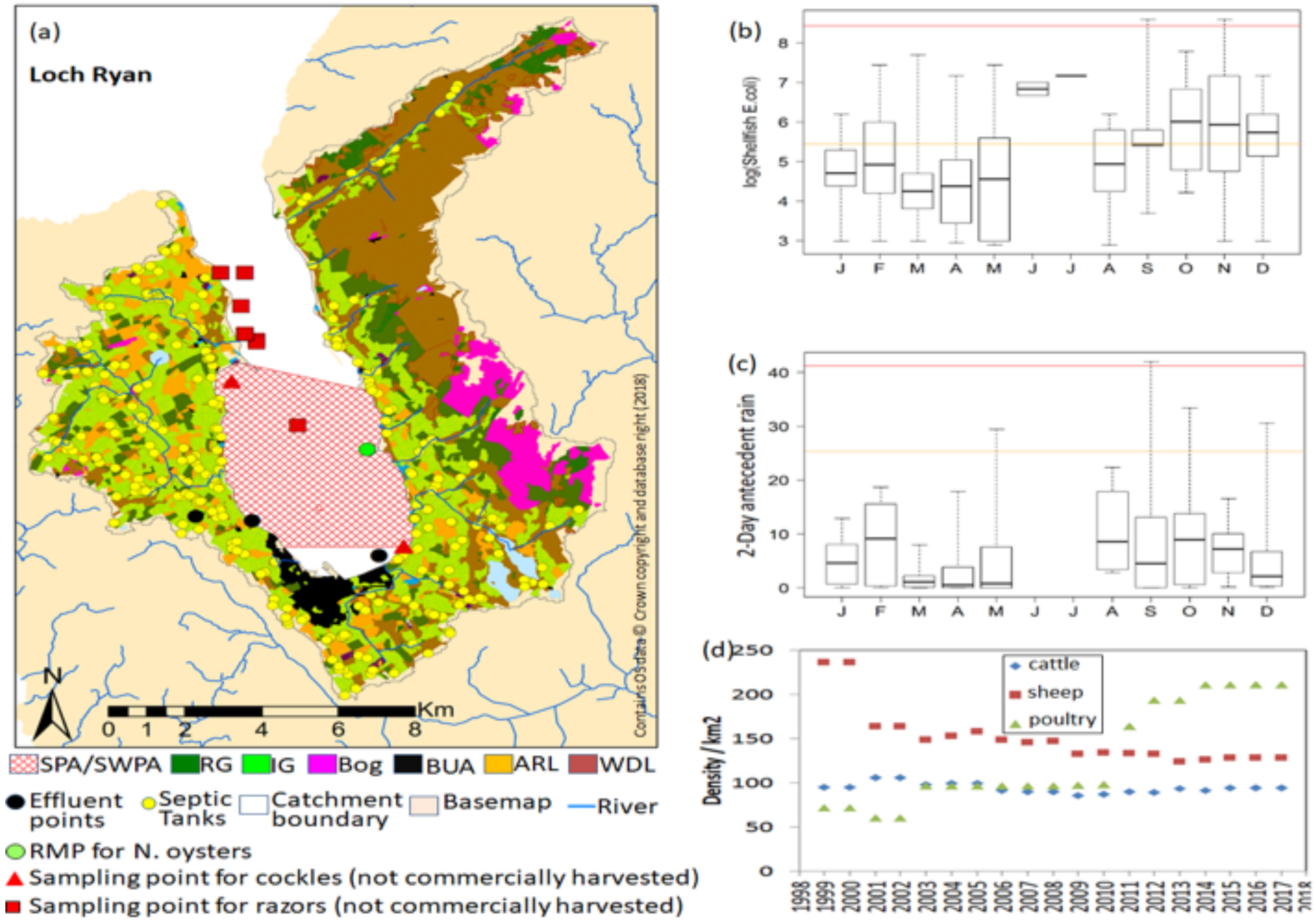



**Figure V.6.3.** Loch Ryan. (a) Land use map, boundary of currently classified SPA and location of RMP in currently classified SPA and montoring points for not currently commercially harvested species. RG: Rough Grassland; IG: Improved Grassland; BUA: Built-up areas; ARL: Arable land; WDL: Woodland. (b) Shellfish *E. coli* results by month. (c) Two-day antecedent rain by month; rainfall data was missing for June and July. (d) Livestock density per km2 by year. Please note that the current boundary of SWPA=85 (including both the inner and outer part of Loch Ryan) was not available. Data sources: FSS, SEPA, CEH, OS Open Data.

modelling. Earlier hydrographic assessments at Loch Ryan have provided evidence for a fast flushing period (1 day) (Edward and Sharples 1991 cited in the report for north Loch Ryan). The studies during the sanitary surveys showed that contaminants can be transported along the north-south axis at a distance of 5-10km. The strongest currents were estimated to occur along the eastern coast at the vicinity of the Cairnryan ferry port.

- o The report for the northern part of the Loch assessed pollution sources and transport of contaminants to the production area for razors, which was declassified in 2012. The risk of faecal contamination is mainly related to public and private sewage discharges at Kirkholm as well as to diffuse pollution sources related to livestock and the streams discharging north of the razor beds. Contaminated discharges to the south part of the Loch towards the end of spring and ebb tide have the potential to influence the razor beds though water circulation.
- o The report for the south part of the Loch assessed the influence of pollution sources on the extensive n. oyster bed, which covers the majority of this part. N. oysters are harvested by dredging from September to April, which explains why there were not any time-series data for the summer months (see Figure V.6.3b). The RMP is located to represent the impact of the strongest currents along the east coast and the sewage discharges from the WWTP at the ferry terminal and the Cairnryan holiday park. It was concluded that the major risks to the shellfishery are related to septic tanks and combined sewage outflows, the Cheese processing plant (Stranraer), river runoff (from Bishop, Kirlachie, Sole Burns), birds, overboard discharges from boats and sediment resuspension.

- SEPA 2011 report under Shellfish Water Directive. This report identifies in detail all the sources of effluent into the south and north Loch Ryan. Sampling was based on mussels which are not commercially harvested at Loch Ryan.
- Overall conclusion on the risk of faecal contamination from catchment-based sources. On the basis of the desk-based evidence the overall conclusion is that the n. oyster bed of south Loch Ryan is at risk from faecal contaminants of both human and animal origin on a year-round basis.

- Conclusion on the sampling plan. The evidence shows that RMP for n. oysters is indeed located at site representative of the greatest impacts from faecal contaminants. However, due to the presence of intermittent and continuous sewage discharges, sampling must also address worst-conditions for their input and transport to the shellfishery. Worst-conditions can be (i) heavy or extreme rain, which can trigger overflowing of treatment plants and combined sewage outfows and (ii) the tourist season (worst-season) because the Cairnryan holiday park is a known and important source of faecal contamination. It is possible that FSS random sampling has not captured the greatest impact due to rainfall; therefore, sampling must target high rainfall, which in the case of Loch Ryan is rain intensity above 25.32mm in the two days before sampling. Sampling can be predominantly random but also target rain events, in such a way that half data can come from worst-condition sampling. In addition, no samples have been collected during the worst-season. Examination of the data used for classification showed that FSS collects samples only for three to four months per year. As a result, there are only 21 samples from January 2014 to June 2017. This means that less than the minimum number of samples is collected each year from this area despite explicit reference to the risk from both sewage and animal faecal contamination, which would require a higher number of samples to ensure that contamination events are captured.
- Conclusion on the use of this information as part of the sanitary survey process. The evidence presented and analysed here can be considered as an update of the evidence presented in the two sanitary survey reports for north and south Loch Ryan. Therefore, it can be used for informing the review of the sanitary surveys and improvements for the sampling plan such as those referring to targeting worst-conditions. It must be emphasised that although there is a sanitary survey report for the razor beds at the north Loch Ryan the review of the existing sanitary survey must consider the collection of a time series of razor clam *E. coli* samples during worst-conditions before identifying the sampling plan because the north part of the Loch is influenced by the point source discharges at the south part through the currents.
- Supplementary sampling by SEPA or FSS. SEPA is already undertaking this type of sampling. The data cover a limited period of time i.e. 2017 to 2018 and therefore it is uncertain whether they show a spatial or temporal trend in the levels of contamination. Interestingly, SEPA's water samples indicate higher levels of contamination from Sandmill Farm and Sole Burn than from the area 200m away from Cairnryan ferry terminal. This result should be taken into account in the review of the sanitary survey report for south Loch Ryan because there is a risk that the greatest impact on the n. oyster bed is near the west side where Sole Burn is discharged and not on the east coast. This means that the review sanitary survey must consider collection of n. oyster *E. coli* samples in the vicinity of the mouth of Sole Burn.

### V.6.4 Loch Creran (SWPAϵ32)

The designated area for Loch Creran is commercially harvested for three different species: P. oysters, cockles and carpet clams from five different SPAs (130, 433, 547, 564, 729) (Figure V.6.4a). Poor recording of actual sampling locations has made it difficult to depict the locations of RMPs for each of these SPAs on the map.

- Land Use. The source-catchment is dominated by woodland (at the south) and bogland and woodland at the north part (Figure V.6.4a). Rough grassland is found in the upland areas at the north east part of the catchment and along river Creran, which discharges at head of the loch. Built up areas cover less than 0.5% of the total catchment area. The implication of this is that there is low risk from FIO export from this catchment.
- Seasonality of shellfish *E. coli* data. There are considerable differences between the species (Figure V.6.4b). The pattern for species contamination levels is not the same as that observed when national scale data were used. Specifically, at Loch Creran oysters had higher levels of contamination than cockles. However, this depended on the site of production; for example, oysters grown ta SPA=130 had lower levels of contamination than oysters grown at SPA=564. Cockle *E. coli* levels also differed between SPAs. This suggests that location and species are both important in determining levels of faecal contamination in commercially harvested species at Loch Creran. All species at each location have a high confidence of a class B or Fair classification, when the historical data (1999-2017) are considered.
- Effect of rain. The effect of two-day antecedent rain on n. oyster *E. coli* levels was statistically significant (Figure V.6.4c).
- Livestock. Overall, livestock density was very low and post-2014 sheep density was below 40 individuals/km2 (data not shown).
- Effluent. There is no evidence of consented waste water discharges on the basis of SEPA's database on effluent locations (SEPA 2016).
- Septic Tanks. An estimated 227 septic tanks occur within the source-catchment the majority of them located at the south shoreline immediately bordering the designated area and along the rivers discharging to the south coast side of the Loch (Figure V.6.4a).

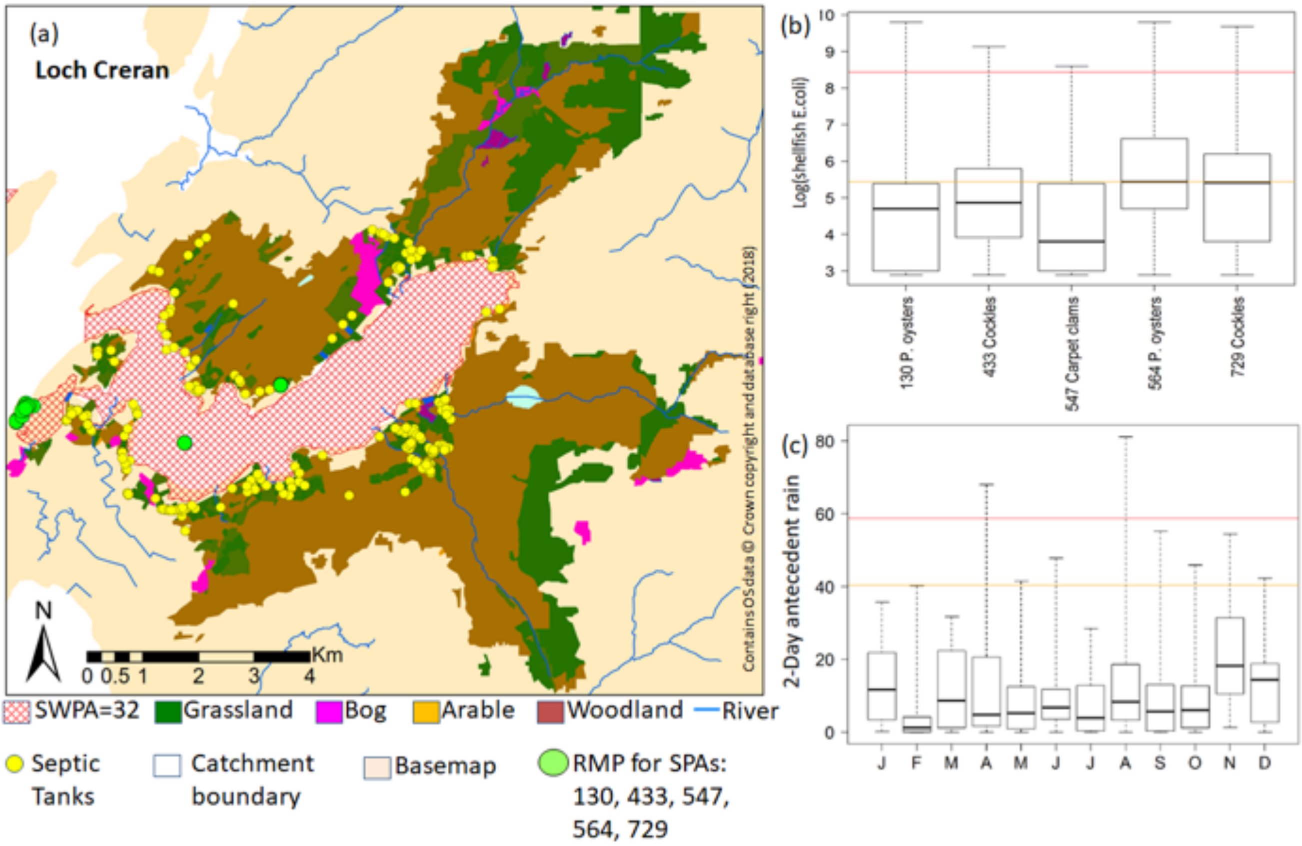



**Figure V.6.4**. Loch Creran. (a) Land use map, boundary of SWPA=32 and locations of RMPs for shellfish *E. coli* monitoring in currently classified SPAs. (b) Shellfish *E. coli* results by month. (c) Two-day antecedent rain by month. Data sources: FSS, SEPA, CEH, OS Open Data.

- Population. The 2011 Population Census result for the parishes comprising the source-catchment showed a resident population of 1313 people.
- Sanitary surveys. There is a sanitary survey report from 2007 and a review from 2013 for mussels and oysters harvested in Loch Creran and for cockles harvested in Eriska Shoal. These reports referred to all the production areas classified at the time of the writing of these reports. The currently classified production area for carpet clams located at Eriska Shoal was classified in 2014, and therefore there is no sanitary survey for carpet clams. The hydrographic data suggested a fast flushing time (three days) and dilution greater at the mouth than at the head of the loch. Freshwater inputs, potentially contaminated from livestock faecal sources, were found to be relatively low but create a persistent seaward movement of water at the surface above a more saline tidally circulated water layer. An earlier hydrographic survey also revealed that vertical mixing of water layers and dilution varies locally (Black 200). The sanitary surveys concluded that tidal movement and circulation pattern plays an important role in keeping faecal contamination at high levels despite the observed low pressures from livestock. The risk of faecal contamination to the shellfisheries of Loch Creran was found to be related to: inputs from River Creran (at the head of the Loch) which drains the grassland in the northwest part of the source-catchment; septic tank discharges in the vicinity of shellfisheries; tourism and recreational boating in the summer months; and potentially to wildlife (e.g. seal sanctuary, overwintering bird populations[21], and mammals living in forested areas). The sanitary survey for Eriska Shoal concluded that there are no major sources of contamination other than wildlife feeding on the cockle bed but local sources in the immediate vicinity of the shellfishery (such as grazing cattle) may increase faecal contamination in cockles.
- SEPA 2011 report under Shellfish Water Directive. This report also mentions the local pressures from livestock and the seasonal effect due to tourism in Loch Creran but it does not refer to Eriska Shoal.
- Overall conclusion on the risk of faecal contamination from catchment-based sources. On the basis of the desk-based evidence the overall conclusion is that the species grown at Loch Creran are at low risk from faecal contamination from catchment-based sources due to the population of humans and livestock. Marine-based sources in summer months and unpredictable (spatially and temporally) inputs from wildlife in combination with circulation pattern and the relatively lower mixing in the inner Loch may be responsible for the exceedances of the

[21] There is no RSPB observatory in Loch Creran.

threshold for class A or Good classification.

- Conclusion on the sampling plan. Due to poor recording is difficult to assess the location of RMPs for oysters and cockles. Monthly sampling, as currently undertaken, is in line with best practice.
- Conclusion on the use of this information as part of the sanitary survey process. The existing sanitary primary and review reports for Loch Creran contain complete and thorough information for pressures in Loch Creran. This shows that SWPA-wide sanitary surveys are critical to understand the interplay between catchment inputs and hydrodynamic processes. The desk study presented also established that differences between species are location specific, therefore the SWPA is spatially heterogeneous. It also showed that pressures from livestock are relatively low, suggesting that other sources must be addressed and controlled.
- Supplementary sampling by SEPA or FSS. SEPA is already undertaking this type of sampling. The data cover a limited period of time i.e. 2017 to 2018 and therefore it is uncertain whether they show a spatial or temporal trend in the levels of contamination. Interestingly, SEPA's water samples indicate higher levels of contamination from Sandmill Farm and Sole Burn than from the area 200m away from Cairnryan ferry terminal. This result should be taken into account in the review of the sanitary survey report for south Loch Ryan.

# Appendix VI - Standard Operating Procedures (SOP) for Sanitary surveys

A sanitary survey may involve four elements:

1. A desk study to identify pollution sources processes contributing to faecal contamination to shellfishery and identify likely worst-locations and worst-conditions at the broader scale (sealoch or SWPA).

2. Shellfish *E. coli* and salinity sampling and field observations to test initial findings of the desk based study.

3. Data assessment to identify the effect of pollution sources, rain, salinity and temperature on shellfish *E. coli* concentrations to confirm or revise findings of the desk based study

4. A report providing:

   (i) Description on data collection, methods (sampling, catchment data analysis, hydrographic data analysis, statistical analysis).

   (ii) Assessment on whether hydrodynamic or catchment modelling are required to undertand risk from faecal contamination in the broader area.

   (iii) Assessment and conclusion on the sources and processes contributing to risk of faecal contamination to the shellfishery.

   (iv) The sampling plan of each species and SPA delineated in line with the conclusion.

   (v) A classification grade, if between 12 to 24 samples have been collected during the sanitary survey, the report may grant an initial (12 samples) or annual (24 samples) classification grade.

5. A database with the all data, numerical and descriptive collected during the sanitary survey

## 1.0 Desk study

The following steps should be undertaken during the desk study:

| ***STEP 1. Characterisation of the shellfishery/ production area*** |
|---|
| Approach: Through consultation with the shellfish industry, FSS must identify and record the characteristics of the shellfishery(ies) in the area described in the application |
| 1. Location and extent (description and map) |
| 2. Bivalve species |
| 3. Aquaculture or wild stocks |
| 4. Capacity of area |
| 5. Whether it is a production area or relaying area |
| 6. Seasonality of harvest |
| 7. Growth and harvesting techniques |
| 8. Any conservation controls (e.g. closed season) |
| 9. Existing classification data or Classification history |
| 10. Designation (yes/no): SWPAs, Bathing Waters, designations under the Habitats Directive and the Birds Directive, Marine Protected Areas |

***STEP 2. İdentification of pollution sources as of Regulation (EC) 854/2004 : Annex İİ:A:par. 6)***

Approach: FSS should obtain, where practically possible, this information through available online or open access databases, or affordable published information. Data should be collected at the source-catchment scale and at the broader coastal area (e.g. sea-loch, or SWPA-scale).

| STEP 2: Pollution Sources | | |
|---|---|---|
| **Type of source** | **Potential Data source: Point-sources** | **Information** |
| Continuous sewage discharges | Effluent shapefile from SEPA | Location (Latitude/longitude and/or relevant National Grid Reference (NGR) |
| | | Size (dry weather flow, maximum flow; population equivalent if other information not available) (cubic metres per day). |
| | | Treatment level (e.g. untreated, primary, secondary, tertiary, disinfected, septic tank, soakaway) |
| | | Tidal phasing or other periodicity if relevant |
| | | Microbial content (results of any monitoring undertaken on the discharge together with information on the flow conditions pertaining) |
| | | Sanitary content (as surrogate if microbial content not available) (measured levels of ammonia, biochemical oxygen demand (BOD), suspended solids together with information on the flow conditions pertaining) |
| | | Seasonal variations in any of the above. |
| Rainfall-dependent sewage discharges | Effluent shapefile from SEPA | i.e. combined sewer overflows (CSOs) or storm tank overflow (STOs) and other rainfall-dependent discharges (storm water discharges) |
| | | Location (latitude/longitude and/or relevant NGR). |
| | | Measured or predicted spill frequency (per annum). |
| | | Treatment level (if any). |
| | | Tidal phasing or other periodicity if relevant. |
| | | Maximum flow rate (litres per second) together with information on the flow conditions pertaining). |
| | | Sanitary content (as surrogate if microbial content not available) |
| | | Seasonal variations in any of the above. |
| Emergency discharges | Effluent shapefile from SEPA | Location (Latitude/longitude and/or relevant NGR). |
| | | Circumstances under which the discharge may operate. |
| | | Maximum predicted flow rate (litres per second). |
| | | Microbial content of the associated continuous flow (results of any monitoring undertaken on the discharge together with information on the flow conditions pertaining). |
| | | Sanitary content of the associated continuous flow |
| | | Seasonal variations in any of the above. |
| Industrial discharges | Effluent shapefile from SEPA | If they have significant sewage content, the proportion of sewage and the effects of any antibacterial action of the chemical constituents should be estimated. |

| STEP 2: Pollution Sources | | |
|---|---|---|
| **Type of source or process** | **Potential Data source: Diffuse pollution sources** | **Information** |
| Land use | Edina AgCensus and CEH LC07map (25*25m) | Percentage of Broad Habitat per source-catchment: Improved grassland, Rough grassland (natural, acid, etc.), woodland, bogland, built-up areas, industrial areas, arable, horticulture |
| | | Livestock density /$km^2$ (cattle, sheep, goat, pig, horse, deer, poultry), each type of livestock separately |
| | SEPA shapefile (confidential information not available but recommended as essential) | Farm-scale hotspots of faecal indicator sources to watercourses (If SEPA does not have information on this, then this may be part of the field observations).<br><br>Slurry storage locations<br><br>Information on the implementation of general binding rules (GBR) and diffuse pollution control measures |
| Other pollution sources | LAs or SG, maps | Ships, boats, marinas |
| | LAs or SG | Tourism |
| | SG | Wild animals (coastal and terrestrial) |
| | SG | Spreading of bio solids on land. |
| | SEPA | Septic tanks per source-catchment |
| | Scotland's Census | Population |
| Processes transporting faecal contaminants | MetOffice | Daily rain |
| | SEPA | River flow |
| | · UKHO (Hydrographic Office) –Marine Scotland<br><br>· Nautical charts (admiralty charts) either within a GIS or hard copy.<br><br>· Mariners Handbook,<br><br>· Tidal charts/tidal stream software or simple hydrodynamic modelling. | Hydrographic data[22] |
| | Complex hydrodynamic models | Prediction of transport of faecal contaminants<br><br>(Where available this information may be used to interpret the significance of the data gathered during the sanitary survey) |
| Historical data | FSS | Shellfish *E. coli* concentrations from commercially harvested species |
| | CEFAS or SAMS | Sanitary survey reports or pRMP assessments in the vicinity or within the broader area or in the same area for a different species |
| | SEPA | Shellfish *E. coli* concentrations from naturally occurring shellfish beds |
| | | Bathing Waters |
| | Marine Scotland | Any marine information |

If this is a sanitary survey for a new harvesting area, the desk study must assess available data and identify likely worst-locations and worst-conditions. Each potential pollution source should be assessed for:

- The microbial load of the source
- The distance from the shellfishery
- The seasonality of the faecal inputs
- Currents, mixing and dilution

[22] Potential sources for hydrographic data include: EDINA (Digimap Edina-marine GIS 2018); the Aquaspace project- Argyll case study (n.d.); and the the Marine Scotland Interactive (MSI) data for Shetland (MSI-Shetland 2012). The Marine Environmental Mapping Programme (MAREMAP 2017) may be useful in the future, once it maps areas within SWPAs.

## 2.0 Sampling and field observations

| **STEP 3.1. Sampling and field observations during sanitary surveys** | |
|---|---|
| **If there are historical shellfish *E. coli* data, the sanitary survey process can skip this phase** | |
| **Approach: Samples for Shellfish *E. coli* must be collected in tandem with salinity and temperature at the likely worst-locations and worst-conditions, if the extent of contamination is not clear after completing the desk study.** | |
| **Equipment** | Map of the broader area to be surveyed, including source-catchment |
| | GPS |
| | Notebooks and writing materials |
| | Digital Camera |
| | Personal Protective equipment as required |
| | Map of the area to be surveyed |
| | Sampling equipment (if field observations accompany shellfish *E. coli* sampling) |
| **Sampling/Field observations: For shellfish *E. coli* sampling see FSS protocol (2017). In addition, provision should be made for the measurement of salinity and temperature (and/or turbidity). Sampling during sanitary surveys should target worst-conditions, i.e. August and September, tourist (yachting) season, ebb tide, rainstorm events. Field observations in the vicinity of the production area must account for:**<br>· **Presence of wildlife and pets**<br>· **Presence of sewage effluent outflows**<br>· **Weather at the time of sampling (wind, rain)**<br>· **Tidal stage at the time of sampling**<br>· **Visibility in water or the presence of sediment in streams discharging in the vicinity of the shellfishery**<br>· **Presence of recreational boating (number of boats, number of people)**<br>· **Tourists**<br>· **Anything relevant to the microbiological quality of the shellfishery** | |
| **If it is decided that sampling is not required as part of the sanitary survey, or it is not undertaken for any reason, and that there was not sufficient or up-to-date desk-based data, then a detailed field survey must be undertaken.** | |

| **STEP 3.2. Detailed field survey** | |
|---|---|
| **Approach: Sample officers must acquire some information about the area to be surveyed and consult with SEPA about catchment-based pollution sources identified in the desk study. Sample officers should decide whether the broader area is to be surveyed by boat (coastline observations), or by car (shoreline and catchment). Field observations may also be made on foot (observations on the shoreline immediately bordering with the SPA.** | |
| **Procedures** | Identify and visit the location of each WwTW, industrial source |
| | Identify in consultation with SEPA and visit the agricultural hotspots of faecal contamination. |
| | Identify and visit the location of marinas |
| | Note any populations of wild animals that may influence the shellfishery |
| | Identify and visit sites of freshwater inputs (streams, drainage ditches) near the shellfishery |
| | Describe and evaluate the extent of influence of each source (distance). |
| | Identify sources which require sampling for analysis; this can inform supplementary sampling by SEPA. |
| | Describe weather conditions during the field survey |
| | Record the (descriptive) data in a spreadsheet (fields as above) together with the location of the observation so as the database of field observations can be projected on a map (GIS). |
| | Compile a report:<br>· Describing the field observations and showing maps of confirmed faecal sources to the shellfishery<br>· Evaluating the risk from each source<br>· Confirming worst-locations or recommending further investigations on a source's impact, including sampling (for catchment-based sources, this may be part of SEPA's supplementary sampling by SEPA) |

## 3.0 Data assessments

If a long-term time series of historical shellfish *E. coli* data is available, then through statistical analyses must be performed to explore the effect of data on pollution sources and rain on the shellfish *E. coli* contamination. However, this may still be a small dataset and it will be impossible to establish significant relationships between variables. The Spearman rank correlation coefficient can be used to explore whether shellfish *E. coli* concentrations sampled during the sanitary surveys are related to data collected concurrently with shellfish *E. coli* such as salinity and temperature.

## 4.0 Compilation of sanitary survey report

The report should include the following information:

1. Executive Summary describing conclusions and sampling plan and, if relevant, recommended classification grade

2. Overview of the shellfishery/production area (the survey may examine more than one commercially harvested species)

3. (For SPA located within SWPAs) Description of SWPA and source-catchment - this can be written in consultation with SEPA

4. Hydrography / hydrodynamics

   - Tides (type and amplitude/stage)

   - Currents (velocity and direction)

   - River discharges (volume and seasonality)

5. Human sources of contamination (public and domestic sources sewage, marine-based sources, tourism)

6. Agricultural sources of contamination –For SPAs within SWPAs, this can be written in consultation with SEPA. Include:

   - SEPA's farm scale information, data on slurry storage, "hot spots" locations
   - LC07 map or more recent maps
   - FIO export coefficients reported by Kay et al (2008a) (Discussion and maps) –
   - Livestock numbers per type (Method, Table of data, Discussion)

7. Significant wild animal/bird populations.

8. Meteorological Data (as part of a desk-based survey)
   - Rainfall (Annual, Monthly, Daily) (Seasonality and effects on pollutant transport)
   - Winds (Seasonality and effects on pollution dispersion)

9. Records of field surveys (Validate desk-based findings)

10. Records of sampling results collected during sanitary surveys

11. Historical shellfish *E. coli* data (historical classification, trends for each species)

12. Designations in the broader area – Fish farms

13. Assessment of effect of pollution sources and processes on contamination of shellfish.

14. Conclusion on the risk from each source and the overall risk

15. Sampling plan (see below 4.1)

16. Recommended classification grade

    - Initial classification: if less than a years' worth of data are available (at least 12 samples)

    - Established classification: if 24 samples within a year or for a longer period are available

17. Recommendations for sanitary survey improvement, e.g. changes in monitoring schedules, addition of sampling stations or station relocation)

The report should contain maps indicating the location of inputs into the shellfishery and detail seasonal effects on contamination and the effect of rain in the context of agricultural and point sources of pollution. Evidence gaps should be assessed. For example, the need for further hydrodynamic assessment should be evaluated and included in the conclusion. The report should be available from the FSS website with a link to SEPA's web site for sanitary surveys referring to SWPAs. The report should be made available to all stakeholders.

### 4.1 Sampling plan

The sampling plan should refer to the following:

- Boundaries of production area or relay area

- Site Name

- Site Identifier

- Species

- Geographical location (grid reference and/or latitude/longitude) of representative sampling point (RMP)

- Allowed maximum distance from identified RMP

- Depth of sampling (if relevant)

- Frequency of sampling

- Responsible authority

- Authorised sampler(s): name(s) and reference number(s)
- Other relevant information

There must be at least one sampling point per site. Each sampling point should be at a fixed geographical location, identified by latitude/longitude reference to an accuracy of 10 metres.

## 5.0 Database for the data collected during the sanitary survey

A database for each sanitary survey will contain both quantitative and descriptive data. All data from desk studies, field observation sand sampling must be linked to a location (longitude/latitude). The database for sanitary surveys referring to SWPAs must be shared with SEPA. Authorised staff from Local Authorities should also have access to inform the application process for new harvesting areas within the broader area.

The database should contain the following fields (at least):

- Information on the sampling plan:
    - Boundaries of the SPA
    - (Boundaries of the SWPA)
    - RMP
    - Tolerance
    - Depth
    - Frequency
    - Species
- shellfish *E. coli* sample NGR
- date of each sample
- Shellfish *E. coli* concentrations collected for classification from each sample
- Monitoring strategy: Random, worst-condition, supplementary, investigative (specify who took the sample, why, what for)
- Information on any other supplementary samples collected outwith strict use for classification:
    - Medium: Shellfish, Seawater, River outflow, stormwater or point discharge outflow, sediment
    - Type of microorganism tested
    - NGR and date of sampling
- Temperature at the shellfish *E. coli* sample NGR
- Salinity at the shellfish *E. coli* sample NGR
- Additional shoreline observations on the date of sampling
- SPA Classification per year; Type of test for seasonal classification
- SWPA classification per year (potentially this should be referring to the same data).
- Information on the Sanitary Survey process (i.e. Full primary survey or pRMP assessment, Annual review, Full review, dates)
- Desk-based data collected for the Sanitary Survey process (Catchment data, after their processing to calculate them on a <u>catchment scale:</u>
    - Source-catchment boundaries
    - Type and number of point source discharges, population equivalent, treatment (this may have to be a separate shapefile)
    - Population
    - Boundaries or point locations referring to wildlife colonies or sightings.
    - Livestock numbers
    - Daily Rain 1-2 and 3 to 7 days before sampling (i.e. 2-day and 3-7 days antecedent rain)
    - Number of septic tanks
    - Data on recreational boating
    - (For areas with historical data: tidal stage at the date of sampling)
    - (For areas with historical data: Wind at the date of sampling if possible)
    - If hydrodynamic modelling is available: predictions per area on tidal volume versus estuarine circulation volume flux, entrainment between upper and lower water layer (wind versus tidal density driven), and flushing time
- Information on pollution events
- Information on the implementation of GBR and diffuse pollution control measures (this infomration can be given by SEPA)

The data should be subject to appropriate verification procedures.

## 6.0 Review of the sanitary survey report

Reviews of sanitary surveys should be undertaken by FSS[23] to ensure that the risk from the interplay of pollution sources and processes, which was identified in the sanitary report, has not changed and that the classifications are still valid. There should be two types of reviews:

- <u>Annual Review:</u> This should review the shellfish *E. coli* data collected during the past year in the context of the conclusions of the primary sanitary survey report. It should also include the field observations made during ongoing monitoring. The database should also be updated every year so that the data can be used for the Full Review of the sanitary survey report.

- <u>Full review:</u> The database of sanitary survey data should be updated every year. The Full Review should deliver a report reviewing the conclusion on risk of shellfish faecal contamination, the sampling plan, and the classification grade. FSS should review the conclusion on risk in consultation with SEPA, especially in areas where the greatest risk from faecal contamination was due to catchment-based point and diffuse pollution sources.

[23] In SWPAs where there are not any SPAs, it is recommended that SEPA undertakes sanitary surveys

It is recommended that a complete re-evaluation of sanitary surveys (Full Review) be undertaken by FSS once every up to six to 12 years, depending on availability of up-to-date desk-based data. Results from both the Annual Review and the Full Review should be available online.

# Appendix VII –Full list of technical recommendations

## Recommendations to FSS

*Recommendation #1 for the tasks in revised full sanitary survey process*
The revised (full) sanitary survey process should include the following tasks (see Figure 1a):

- A desk study
- Sampling of shellfish *E. coli* and salinity and field (shoreline and catchment) observations
- Analysis of shellfish microbiological data in the context of the desk-based data
- A report
- A GIS-linked database of the data collected during the sanitary survey accompanying the report.

*Recommendation #2 for the monitoring strategy of routine monitoring*
The monitoring strategy should be tailored to the risks and type of pollution sources identified in the sanitary survey, as follows:

- Apply the random strategy to areas predominantly influenced by farmland runoff with year-round sampling on a monthly basis.
- Apply the worst-condition strategy in areas influenced by point sources of human sewage discharges. For example, depending on the findings of the sanitary survey, samples may be collected: within two to seven days after a heavy or extreme rainstorm event; weekly or fortnightly during the tourist season or in August-September; and during the ebb phase of the tidal cycle.
- Apply a hybrid strategy (i.e. a combination of random and worst-condition sampling), if the number of samples collected under worst-conditions is not sufficient for the review of classification (e.g. when there are fewer than 24 samples under worst-conditions in the last three years).

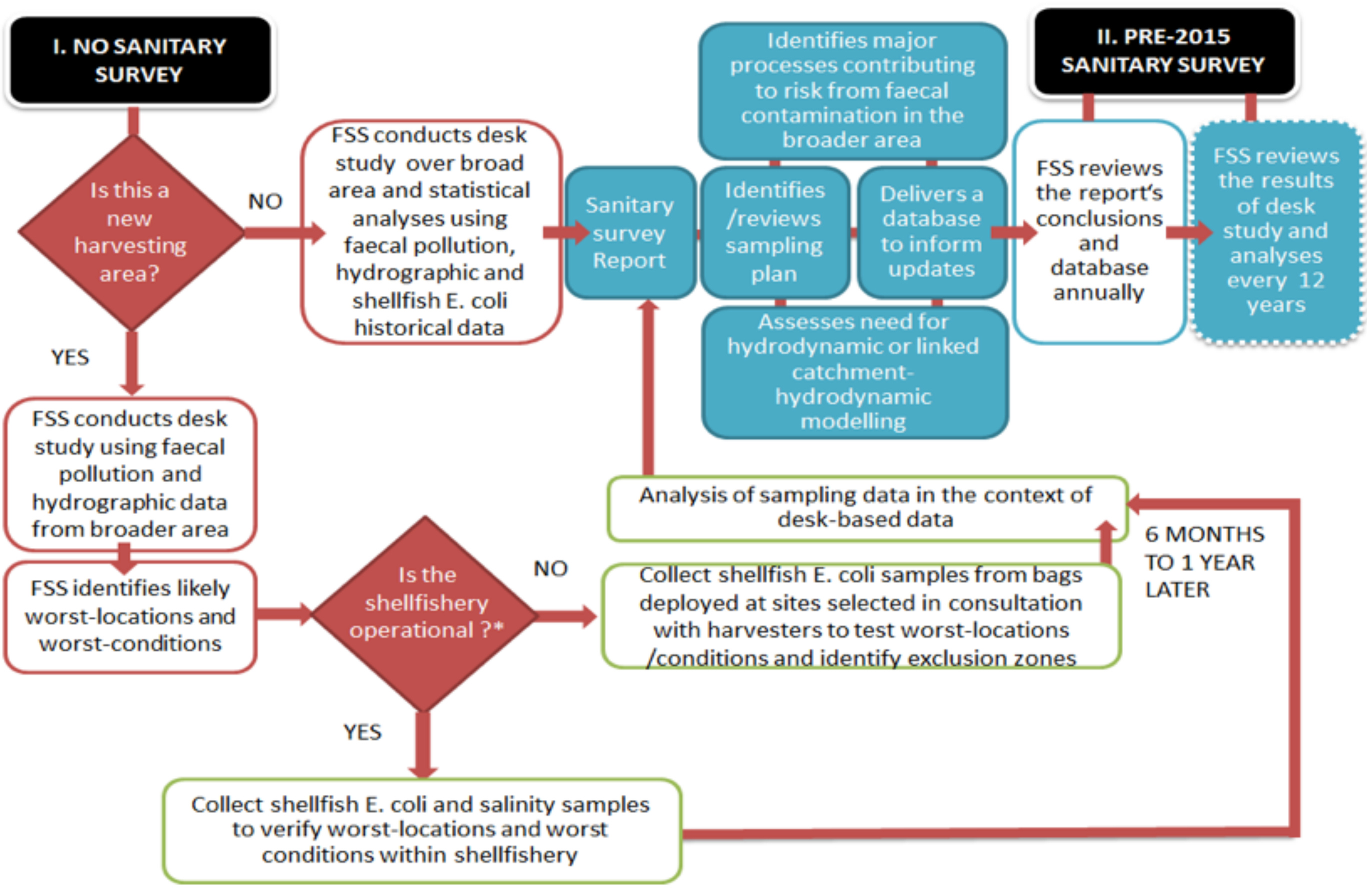



**Figure 1a.** Decision-tree for sanitary surveys in SPAs by FSS. *Operational: ready for harvesting.

*Recommendation #3 for monitoring seasonally or part-year classified areas*

- Apply year-round monitoring for SPAs classified for one season or part-year due to seasonal harvesting.
- Apply a higher frequency than monthly for SPAs where exceedance of class A criteria are likely. Other than that, seasonal or part-year classification based on 24 samples from three years' worth of data should be maintained.
- Start monitoring for classification at least one month prior to the harvesting season for class A areas and two months prior to the season for class B areas.

*Recommendation #4 for sampling seawater parameters and field observations with shellfish E. coli*

- Collect seawater salinity and temperature samples with shellfish *E. coli* concentration at the RMP (or any other sampling point) during sanitary surveys and routine monitoring for classification.
- Gather and record field observations on potential activities or processes affecting the shellfishery at the time of sampling (e.g. tidal phase, wildlife, pets, domestic sewage outflows, grazing livestock, recreational boating, wind direction and rain).

*Recommendation #5 for revising currently applied classification types*

- Remove preliminary classification from the classification programme.
- Remove provisional classification with 10 weeks' worth of data from the classification programme.
- Grant initial classification based on six months' to one year's worth of data collected fortnightly at the worst-location and including the period August-September, or any other worst-condition identified in the sanitary survey.
- Grant annual classification based on fortnightly bias-free monitoring for a year, i.e. 24 samples.
- Make a rule that all classifications are based on 24 samples regardless of whether they are based on six months', one year' or three years' worth of data.

*Recommendation #6 for using a parametric method to estimate percentiles of shellfish E. coli data for classification of SPAs.*

It is recommended that the parametric method of fitting a lognormal distribution by calculating the mean and standard deviation of log-transformed data is used when fewer than 24 samples or fewer than three years' worth of data is available. The following equations can then be used for calculation of 80th- and 90th-percentiles must be based on lognormal data and the equations:

$$80\text{th-percentile} = (10 \text{ or } e)^{\mu + s*0.84}$$

$$90^{th}\text{-percentile} = (10 \text{ or } e)^{\mu + s*1.28}$$

*Recommendation #7 on the resources (e.g. time, expertise) required to build the catchment-shellfish E. coli database*

- It is recommended that provision should be made for two months' worth of work for an expert team to undertake a broad-scale (SWPA-scale) desk study for a SPA and deliver a report and a catchment-shellfish *E. coli* database when historical shellfish *E. coli* data and a sanitary survey report is available. More time will be needed if there are discrepancies in the historical data set.
- The expert team should include a GIS analyst and an environmental scientist with an understanding of both catchment and hydrodynamic processes as well as competence in statistical analysis.
- If sampling is involved, then the timescale of a year should be considered before granting a classification grade.

*Recommendation #8 on cost-benefit implications*

The revised approach to the implementation of official control programmes by FSS is premised on tightening the link between sanitary surveys and classification and applying good practice in the monitoring of SPAs and the recording and storage of data. If the recommendations provided here are taken forward, then:

- The cost-benefit implications in the timescales and application procedure for new SPAs must be evaluated in consultation with the shellfish industry.
- Training should be offered to sampling officers to ensure that sampling data and field observations are appropriately recorded. The benefit is significant for both FSS and SEPA. However, it must be evaluated against other priorities for the programme.
- Both a GIS analyst and a scientist with a background in environmental sciences are required to undertake the assessments of the data collected during the sanitary surveys. Additional cost may refer to purchasing hydrographic data or software for hydrodynamic modelling. Whether the undertaking of sanitary surveys is kept in-house or not is a decision FSS must make in view of a cost-benefit analysis.
- An option for FSS is to classify areas only within SWPAs or prioritise the undertaking of sanitary surveys for SPAs within SWPAs; however, this is a policy issue beyond the scope of this project.

## Recommendations to SEPA

*Recommendation #9 for sanitary surveys undertaken by SEPA*
Undertake sanitary surveys in SWPAs where no sanitary survey has been undertaken and there are not any currently classified SPAs (see Figure 1b).

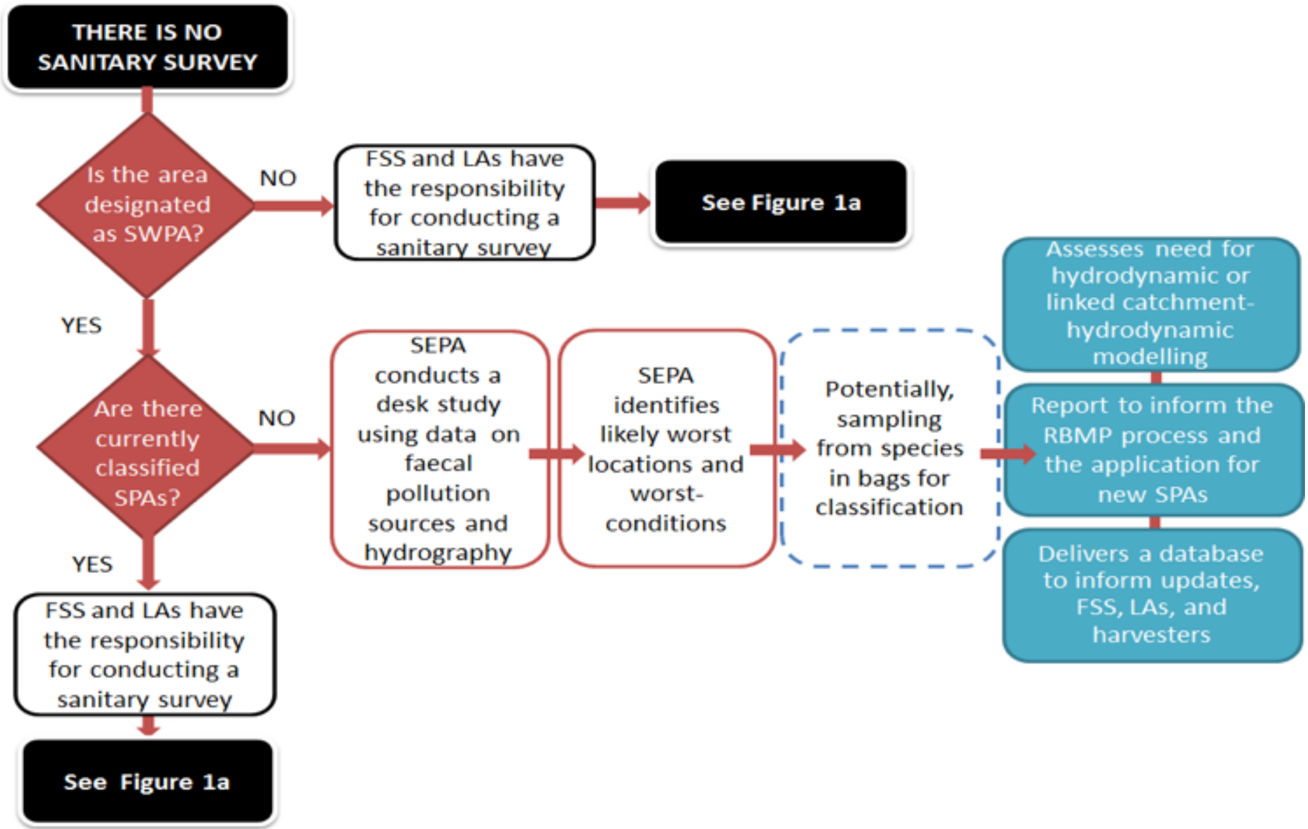



**Figure 1b.** Decision-tree for undertaking sanitary surveys in SWPAs where no sanitary surveys have been undertaken in the past.

*Recommendation #10 for the classification of SWPAs*

- Classify SWPAs based on data from commercial aquaculture bivalve shellfish species, i.e. from SPAs.
- Do not grant a single classification grade for large, spatially variable (heterogeneous) SWPAs with more than one SPAs within their boundaries to avoid misrepresentation of local risks and locally favourable conditions for shellfish harvesting.
- Classify each species from different SPAs within a SWPA separately in SWPAs where more than one species is commercially harvested.
- Where data is available from more than one SPA for the same species, examine whether the SPAs can be grouped into a single, homogeneous area influenced by the same faecal pollution risks and processes on the basis of the results of a sanitary survey at the SWPA-scale. Then, a representative monitoring point (RMP) should be identified for the classification of the group. If this is not possible, each SPA should be classified separately.

*Recommendation #11 for monitoring in SWPAs without classified SPAs within their boundaries*
In SWPAs where no commercial harvesting is practised, SEPA should consider the following options:

- o No monitoring until commercial harvesting begins.
- o Monitoring of shellfish *E. coli* from species deployed in bags in areas at risk from faecal contamination to inform the RBMP process; or at sites prioritised by the shellfish industry.
- o Monitoring of shellfish *E. coli* from naturally occurring (not commercially harvested) species found in the area. The species or the locations may be selected in consultation with the shellfish industry to inform on the potential for development of commercial harvesting.

*Recommendation #12 for supplementary monitoring in SWPAs by SEPA*

Undertake supplementary monitoring within or outwith the boundaries and the RMP of currently classified SPAs in order to provide "supplementary" information on the risk from faecal contamination in relation to specific catchment-based faecal sources of pollution or hydrographic parameters within the SWPA. Supplementary monitoring must be in line with Recommendations #4 and #5.

*Recommendation #13 for investigative monitoring in SWPAs by SEPA*

Apply investigative monitoring for faecal indicators and to account for the presence of pathogens in shellfish and water in areas where commercial harvesting has not yet started; and in areas potentially influenced by human sewage discharges and/or agricultural land runoff. For example, investigative monitoring can include shellfish microbiological monitoring from bags or non-commercially harvested beds to inform application for new areas; and microbial source tracking monitoring and in-stream sampling (aka blitz pressure investigations) to inform source-apportionment modelling for faecal microorganisms. This monitoring can be undertaken regardless of whether commercial harvesting is practised in SWPAs.

*Recommendation #14 on modelling faecal inputs to SWPAs and hydrodynamic processes*

- Use the developed catchment-shellfish *E. coli* database to verify linked catchment-hydrodynamic models on faecal indicator inputs and transport (once the models are developed).

- Add in-stream *E. coli* monitoring[24] data in source-catchments draining to priority SWPAs[25] to the catchment-shellfish *E. coli* database in order to enable linking source-apportionment studies with shellfish *E. coli* data.

- Support development of hydrodynamic (process-based) modelling to SWPAs where:

    - o Shellfish *E. coli* results do not match the desk-based assessment of pollution sources.
    - o There is a large bivalve shellfish production.
    - o Shellfish *E. coli* levels frequently exceed the classification grade granted to production areas.
    - o There is a potential link between bivalve shellfish harvested from a SWPA and a disease outbreak.
- Support and promote the development of linked catchment-hydrodynamic models to inform the RBMP process and the integrated management of shellfish waters in collaboration with Marine Scotland and FSS and other organisations such as research institutes and universities in Scotland and the UK.

## Recommendations for integrating FSS and SEPA programmes

*Recommendation #15 for alternative implementation of the SWPA programme*

- Consider the benefits for Scotland of the French paradigm, whereby the requirements of the *Regulation (EC) 854/2004* and the WFD have been fully integrated enabling complete alignment of sanitary surveys, monitoring and classification procedures and the RBMP process for SPAs and SWPAs.

*Recommendation #16 for a database on sanitary survey data[26] shared by SEPA, FSS and Local Authorities (LAs)*

- Data from each sanitary survey undertaken at the SWPA- and SWPA source- catchment scale should be compiled and stored in a database that is shared between FSS, SEPA and LAs. This database may contain both quantitative, numerical data and qualitative, descriptive data (e.g. description of current circulation, presence of wildlife and tourist influx). Numerical data entries may refer to raw data (i.e. shellfish *E. coli* data, tidal stage, salinity); modelled data (i.e. number of septic tanks, daily rainfall data, livestock density; current speed); or presence-absence data (e.g. 0 for no and 1 for yes). Both sample data on behalf of FSS and supplementary sample data on behalf of SEPA collected during sanitary surveys must be recorded.

- FSS, SEPA and LAs should name dedicated expert staff from each organisation with access rights.

- FSS and SEPA should name staff with write-access to the database to ensure control of the data introduced into the sanitary survey database, as follows:

    - o In SWPAs where there are SPAs and applications for new SPAs, both FSS and SEPA will have write-access (Figure 1a).

    - o In SWPAs where there are not any SPAs, SEPA will have write-access (Figure 1b).

[24] aka Blitz monitoring.

[25] Priority for the shellfish industry in terms of production and economic output

[26] It must be reminded that this project did not use sanitary survey data but combined desk-based data on catchment faecal sources (i.e. catchment indicators) with historical shellfish *E. coli* monitoring data.

*Recommendation #17 for using a shared catchment-shellfish E. coliE. coli database*[27]*.*

- SEPA should provide GIS-linked data on catchment hot spots (farm-scale) of faecal inputs.

- SEPA and FSS should use and regularly update the catchment-shellfish *E. coli* database produced during this project. Updates must be undertaken by authorised staff in consultation with GIS analysts. SEPA can update the catchment data; FSS can update routine shellfish E coli data.

- The catchment-shellfish *E. coli* database could be broken down into different periods i.e. from 1999- 2013 and post-2013, to mark when the designation of SWPAs came into force; or, alternatively, there is a separate database for each SWPA with historical data since 1999.

- FSS should use the catchment-shellfish *E. coli* database for the review of sanitary survey reports in combination with available pre-2015 sanitary survey reports.

SEPA should use the catchment-shellfish *E. coli* database to plan supplementary monitoring. For example, in SWPAs where there are no catchment pressures (e.g. very low livestock and septic numbers, and low rain) but shellfish *E. coli* results from SPAs indicate elevated faecal contamination levels, SEPA may collect supplementary samples outwith the SPAs to identify whether there is scope for delineating exclusion zones within the SWPA in relation to catchment "hot spots" of faecal inputs.

*Recommendation #18 on the protection and improvement of SPAs outwith SWPAs*

It is recommended that SEPA provide catchment data and SPA source-catchment boundaries to FSS to support catchment-based sanitary surveys for SPAs outwith SWPAs.

[27] It is reminded that this is the database developed in this project.

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