Technical Report

Review of approaches for establishing exclusion zones for shellfish harvesting around sewage discharge points

Desk study to inform consideration of the possible introduction of exclusion zones as a control for Norovirus in oysters

Project Code: FS513404

30 April 2015

FINAL REPORT, Version 5

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WATER SERVICES LIMITED

Working with:

Aquafish Solutions Limited

European Centre for Environment & Human Health

Intertek

DOCUMENT QUALITY CONTROL AND RELEASE SHEET

CLIENT:	FOOD STANDARDS AGENCY
DOCUMENT TITLE:	TECHNICAL REPORT REVIEW OF APPROACHES FOR ESTABLISHING EXCLUSION ZONES FOR SHELLFISH HARVESTING AROUND SEWAGE DISCHARGE POINTS DESK STUDY TO INFORM CONSIDERATION OF THE POSSIBLE INTRODUCTION OF EXCLUSION ZONES AS A CONTROL FOR NOROVIRUS IN OYSTERS
REPORT NO/REF:	AWS/160215
LEVEL OF ISSUE:	FINAL VERSION 5
DATE:	30 APRIL 2015
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COMMENTS:	STRUCTURAL AMENDMENTS FOLLOWING V2 DRAFT APRIL 2014 EDITORIAL AMENDMENTS FOLLOWING V3 DRAFT NOVEMBER 2014 REFERENCE AMENDMENTS FOLLOWING V4 FINAL FEBUARY 2015

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Glossary

Regulations, Documents and Studies

CODEX	The Codex Alimentarius Commission, develops harmonised international food standards, guidelines and codes of practice to protect the health of the consumers
GMPG	Good Manufacturing Practice Guidance (series of guidance documents produced by Seafish Industry Authority. Includes workbook on Live Bivalves (Ref: Pyke, 2007) and CSO Text Alerts (Ref: Bowes and Pyke, 2013)).
GPG	Good Practice Guide - Technical Application concerned with Microbiological Monitoring of Bivalve Mollusc Harvesting Areas (produced by Cefas, European Union Reference Laboratory for monitoring bacteriological and viral contamination of live bivalve molluscs and agreed by expert EU Working Group) (Ref: EURL 2014b).
IID1	First study of Infectious Intestinal Disease in England (a study of the incidence of infectious intestinal disease based on GP consultations in which microbiological confirmation of the clinical diagnosis was carried out. Also source of 1500:1 ratio of NoV cases in the community: cases reaching national surveillance often cited) (Ref: FSA, 2000).
IID2	The second study of Infectious Intestinal Disease in the community (project aims to estimate the burden and causes of infectious intestinal disease (IID) in the UK population). (Ref: Tam *et al.,* 2012).
SARF	Scottish Aquaculture Research Fund
SHD	Shellfish Hygiene Directive ((91/492/EEC) Formerly the principal regulatory tool to protect Shellfish flesh quality which provided the framework for Classification Scheme and requirements for Sanitary Surveys – now superseded by 853/2004 and 854/2004)
SWD	Shellfish Waters Directive ((2006/113/EC) Formerly principal regulatory instrument to protect shellfish water quality – repealed 2013 and replaced by WFD)
WFD	Water Framework Directive (EU Directive which requires that all surface waters and groundwaters within defined river basin districts must reach at least 'good' status by 2015)

Analytical Related Terms

BAF	Bio Accumulation Factor
CEN	European Committee for Standarisation (with relevance to the standard method developed for RT-PCR detection of NoV in shellfish)
Dt	Digestive Tissue (part of shellfish gut known to concentrate NoV which is extracted for RT-PCR analysis)

FRNA	F+RNA specific coliphage (A variety of bacteriophage which infects *E. coli* bacteria via the F+ pilius site). = see MSC
MSC	Male Specific Coliphage. (A variety of bacteriophage which infects *E.coli* bacteria via a specific site on the pili appendage) = see FRNA
PFU	Plaque Forming Units (a measure of viral concentration where the ‘plaques’ are areas of host cells impacted by a infective virus – units only apply to viruses analysed using tissue culture systems)
PV	Poliovirus (a principal enterovirus studied in environmental behaviour research)
RT-PCR	Real Time Polymerase Chain Reaction (Molecular analytical technique to copy and detect RNA and DNA fragments)

Water Sector Terminology

AMP	Asset Management Plan (5 year investment periods when Water Utilities submit investment plans to the OFWAT regulator)
CSO	Combined Sewer Overflow (sewerage network, pump station or WWTP release points for excessive flow from mixed systems receiving stormwater flows)
Dilution Factor	Dilution volume / wastewater volume (e.g. 1000:1 Dilution Factor would be obtained for 1L of wastewater mixed in 1m^3 of seawater. N.B. 1000:1 dilution will provide 3 ‘$\log_{10}$ reductions’ through mixing)
DWF	Dry Weather Flow (wastewater flow without infiltration of ground or surface water). Many UK WWTP schemes are designed to accommodate 3DWF.
EDM	Event Duration Monitors (CSO system used to monitor qualitative spill status)
EO	Emergency Overflow. (Wastewater discharge arising from a system failure or blockage. e.g. pump station failure)
Log reduction	$\log_{10}$ reduction (Common term used when describing microbial concentration changes. e.g. if faecal coliform levels were to drop from 10^7/100ml to 10^6/100ml this 90% reduction equates to 1 $\log_{10}$ reduction, whilst a 10^7/100ml to 10^5/100ml 99% reduction equates to 2 $\log_{10}$ reductions.)
MBR	Membrane Bio-Reactor (a post-secondary treatment dewatering process within advanced WWTPs)
PE	Population Equivalent (per capita loading rates are used within wastewater engineering assessments – may be on quality basis (e.g. BOD) or quantity basis (i.e. flow)).

PRPs — Pollution Reduction Plans (reports produced by Environmental Agencies to assess Shellfish Water status and improvement plans as part of requirements for the Shellfish Waters Directive (now repealed)).

SUDS — Sustainable Urban Drainage Systems (a key approach to remove surface water from combined sewerage systems which will reduce CSO impact)

Tidal excursion — Distance water particle may travel in tidal period (Lagrangian term). (½ tidal excursion maybe considered to represent the distance covered on a 'flood' or 'ebb' tide. A full tidal excursion maybe considered to represent the 'residual drift' from the original release position.)

WWTP — Waste Water Treatment Plant (or Sewage Treatment Works)

Public Health Terminology

EPT — End Product Testing

HAB — Harmful Algal Bloom (proliferation of phytoplankton capable of producing biotoxins)

HPP — High Pressure Processing

FIOs — Faecal Indicator Organisms (those bacteria which naturally occur in the gut of humans and other warm-blooded animals which are used to indicate the occurrence of faecal contamination).

NOAEL — No Observable Adverse Effects Level. The level of exposure of a population at which there is no statistically significant alteration in health effects in the exposed population when compared to a healthy population. (Often considered <1% illness)

LOAEL — Lowest Observable Adverse Effects Level. The lowest concentration or amount of a substance which causes an adverse alteration of health for a population under defined conditions. (For a population this may be set at a 'significant' threshold e.g 5% illness)

QMRA — Quantitative Microbial Risk Assessment (is a framework and approach that brings information and data together with mathematical models to address the spread of microbial agents through environmental exposures and to characterize the nature of the adverse outcomes)

Organisations and Groupings

ACMSF — Advisory Committee on the Microbiological Safety of Food (a non-statutory independent advisory committee that provides expert advice to Government on questions relating to microbiological issues and food.)

CEFAS	Centre for Environment, Fisheries Aquaculture Science (an executive agency of Defra fully accountable to Parliament through ministers)
DEFRA	Department for Environment, Food and Regional Affairs (UK Government Department responsible for policy and regulations on environmental, food and rural issues)
DG SANCO	Health and Consumer Protection Directorate General, of the European Commission
DOENI	Department of the Environment (Northern Ireland) (Environmental agency with responsibility in Northern Ireland)
EA	Environment Agency (executive non-departmental public body, sponsored by Defra with responsibilities in England)
EFSA	European Food Safety Agency (independent European agency comprising of Scientific Committee and Panels who provide scientific risk assessment advice to risk managers within the European Commission and EU Member States).
EHOs.	Environmental Health Officers (Local Authority staff responsible for administration and enforcement of environmental health legislation)
EPA	US Environmental Protection Agency
EURL	European Reference Laboratory (Cefas is EURL for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs)
FBOs	Food Business Operators (private shellfish industry businesses in the context of this report)
ISSC	Interstate Shellfish Sanitation Conference (Biennial conference to review the NSSP Model Ordinance – see Section 4.2.1)
NRW	National Resources Wales (Environmental agency with responsibility in Wales)
NSSP	National Shellfish Sanitation Program (US scheme with equivalents in US affiliated countries. Periodically revised as ISSC - see Section 4.2.1)
PHE / HPA	Public Health England (formerly Health Protection Agency)
SEPA	Scottish Environment Protection Agency (Environmental agency with responsibility in Scotland)
US FDA	US Food and Drug Administration

EXECUTIVE SUMMARY

There is a recognition from regulatory authorities at a National and European level that shellfish related outbreaks attributed to Norovirus (NoV) in oysters presents a foodborne infection risk. NoV is the most common cause of infectious intestinal disease in the UK. The Second Study of Infectious Intestinal Disease in the Community (IID2 study, Ref: Tam *et al*., 2012)) published in September 2011, suggested that there were approximately 3 million UK cases of NoV annually. Although most cases are caused by contact with an infected person, a proportion of cases are due to contaminated food and drink. In 2011 there were an estimated 314,000 UK cases of foodborne NoV infection.

While contaminated food is frequently associated with outbreaks of NoV, or suspected NoV infection, the importance of the food chain as a transmission route for NoV is not well understood. The FSA has therefore commissioned research which aims to accurately define the proportion of UK-acquired NoV infections which is attributable to consumption of contaminated foods (FSA 2015).

With the development of RT-PCR molecular techniques for assessing NoV contamination in shellfish and wastewaters over the last decade, a number of EU countries have undertaken surveillance studies to determine prevalence of NoV contamination in oysters and assess an apparent environmental transmission route via wastewater discharges. In 2013 the standard CEN method for NoV in a range of matrices was accepted at a European level presenting a potential regulatory tool for assessing contamination levels in shellfish (Ref: ISO 2013). Alternative management options are also under consideration such as the development of 'exclusion', 'buffer' or 'prohibition' zones to provide safe separation between wastewater discharges and oyster harvest areas.

No examples of exclusion zoning can be found currently based upon NoV, although examples do exist based on other criteria. There are no easy options for establishing future evidence based exclusion zones to manage NoV risk. Possible options for exclusion zones (Section 6.5) could include geographical proximity, time, dilution or be based on NoV levels in shellfish.

- *Proximity - zoning based on geographical proximity*. Examples include Italy and Netherlands (Section 2.1) where regional and national regulations have set zones ranging from 50m-1500m for wastewater discharges, marinas, ports and freshwater inputs. From a NoV perspective this type of zone scaling might be somewhat arbitrary with limited grounding on a scientific evidence base. This means zones may be poorly targeted and present a risk of legal challenge by negatively impacted industry.

- *Dilution based zones* - Examples include buffer zones for waters adjacent to marinas in US affiliated NSSP (National Shellfish Sanitation Program) countries such as US, Canada, Australia and New Zealand (Sections 2.2 and 2.3). These zones are designed to protect bacteriological water quality standards for shellfish with criteria provided for dilution calculations to determine potential prohibition zone size. Continuous WWTP discharges also have prescribed dilution prohibition zone criteria with 100,000:1 dilution required for a Prohibited : Approved boundary and 1000:1 dilution required for a Prohibited : Conditional boundary (Section 2.3.1 and 4.3.2). From a NoV perspective dilution based zoning would require a target water quality standard which is problematic. At present there is no consensus on an appropriate shellfish flesh NoV standard and limited bioaccumulation data to relate this to a corresponding water quality (Section 3.7.3).
- *Dilution/Time* – A time component is also required for NSSP Prohibited : Conditional zone boundaries. This is on the basis that in the event of a WWTP malfunction, or a spill, there is sufficient response time for reactive management. A number of prescribed system requirements with post-event monitoring and actions are also required. From a UK perspective this time reactive element to zoning would face implementation problems as all of the potential tools and data to deliver this approach are not readily available since they are owned by the Water Utilities. Preliminary CSO Event Duration Monitoring (EDM) text alert trials have recently been conducted in two Water Utility regions (Section 7.2.2). Although technically possible the number of potentially contributing assets which might require inclusion would be prohibitive if conducted for all areas (Section 6.5.3).
- *Viral Shellfish Sampling* – No examples can be found of countries which base wastewater zoning criteria purely on viral sampling and testing. In the US (and within the NSSP Model Ordinance) there is now allowance for using bacteriophage sampling to provide an early re-opening criterion following an 'event' (Section 2.3.2). Similarly in France the 'Winter Norovirus Protocol' allows for early re-opening when 'negative' NoV test results in shellfish samples are obtained (Section 7.1.5). In New Zealand an outbreak closure requires a 'negative' all clear viral sample after a minimum 28 day closure period (Section 4.3.2). The US and

Canada are currently in the process of undertaking a comprehensive risk assessment of NoV impact upon shellfish (Section 2.3.3). As part of this program the US Food and Drug Administration (US FDA) has been assessing NoV and bacteriophage shellfish quality in relation to wastewater discharge proximity in parallel with dilution dye studies (Section 4.1.3). This work has been undertaken on a number of trial sites to establish whether the current Conditional 1000:1 dilution criteria are acceptable (Section 2.3.2).

Shellfish testing (Section 7.1), although a useful management tool, has limitations as NoV analysis using RT-PCR provides no indication of viability. A UV disinfected WWTP will discharge NoV with a probable ~1% viability (Section 3.3.3). Any shellfish impacted in the receiving water could exhibit flesh testing genomic results which over-estimate risk as disinfection efficacy cannot be demonstrated directly for NoV. Similarly, *in-situ* environmental degradation will yield differential genomic and actual viability decay rates. A preliminary computer modelling tool has been used to illustrate how a dual T_{90} decay approach could be used to help risk managers with aggregated sources (Section 6.3.3).

A number of plume impact studies have been undertaken to assess the potential influence of wastewater discharge proximity upon shellfish quality. These could be used to assess the possible extent of zone scaling if the objective were to provide shellfish at an acceptable 'harvesting' standard (Section 3.1.2). Plume impact examples are provided for Australia, New Zealand and the UK (Sections 4.2.3, 4.3.3 and 4.4 respectively). These examples range from long sea outfalls serving large WWTPs to small septic tanks for small population numbers (Section 4.5). In all cases NoV is detected over a number of kilometres, although the gradient of reduction with distance is very site specific with lowest rate of reduction expected within poorly flushing estuarine systems. Current work by Cefas on behalf of FSA and Defra will continue to explore the relationship between shellfish quality against proximity (Ref: FSA/Defra, 2015). This project may possibly employ the US FDA dilution studies approach.

All zoning options face a fundamental difficulty within many UK shellfish waters. In essence, the NoV risk profile does not fit the *E. coli* indicator around which all food and environmental regulatory systems have been designed (Section 3.7). Under winter worst case conditions when NoV is widespread wastewater can be highly contaminated (Section 3.1 and 3.2). The relationship between the NoV level of infection in the sewerage connected population and the NoV concentration in the wastewater is termed '*Catchment Health'* and is an indicator of load to the environment, which varies on a seasonal basis for NoV which has a much higher loading in winter compared to summer. When catchment health is poor even small volumes of inadequately treated discharge can have an adverse impact upon shellfish. This means that potentially multiple wastewater discharges are implicated as possible contributory sources.

Intermittent CSO (Combined Sewer Overfow) discharges are particularly problematic for most regions (other than Scotland) with shellfish receiving impacts from a high number of inputs (Section 6.2). The relative magnitude of NoV loading from these untreated sources can far outstrip that from treated continuous WWTPs (Section 4.4.1 and 4.4.4). Whilst CSO spill impact is considered to last only a day from an *E. coli* perspective, it may last many weeks for NoV due to selective binding and retention within oyster tissues (Section 3.6). As wastewater systems are designed to allow 10 'significant' spills a year this could mean extensive shellfish closure periods with potential to impact industry over the peak commercial winter season. As most CSO EDMs are qualitative it will be difficult to assess the potential impact of actual spills on shellfish NoV quality (Section 7.2.2). The extensive geographical coverage of CSOs in many shellfish waters would result in major commercial impact if precautionary zoning were continually implemented for all potential wastewater discharges (Section 6.5.3).

Freshwater diffuse wastewater sources (e.g. septic tanks, small private WWTPs and potentially biosolids disposal) may all contribute NoV to rivers (Sections 4.2.3, 4.4.2 and 6.3). By virtue of the extended persistence in the environment (Section 3.5) these could also be considered NoV sources and require zoning (Section 6.5.4). Similarly *vessel based discharges* direct into coastal waters can contribute a significant load (Section 6.5.5) and theoretically require zoning. Geographical proximity-based zones are applied around river and marinas in some Italian regions and in the Netherlands.

Alternative management measures may provide scope for more flexible and reactive zoning to account for seasonal and 'event' based conditions where the NoV risk profile varies. These are outlined in Section 7 and developed with catchment Scenarios in Section 6. The type of management measures most appropriate or available varies on a regional basis (Section 6.7.1) and is likely to involve a combination of shellfish and wastewater management options. For example, many English and Welsh areas are currently supported by computer models which could be adapted for NoV management purposes (Section 5.3.3). In SW England, where there is a strong emphasis on UV treatment and good stormwater storage, FBOs in these areas may consider reactive EDM spill management supported by NoV testing as management options. In Scotland NoV testing alone may be appropriate.

A *generic model* approach for risk management has been developed with two risk scoring systems used:

- The 'whole system' approach is focussed on the NoV environmental transmission pathway where scoring at each stage is factored on its relative significance. This system allows a responsive approach to events (e.g CSO spills) which could implement a layering of composite zones (and corresponding shellfish management actions) according to risk score. This approach has been termed 'enhanced management' zones.
- The *E. coli* NoV proxy approach was developed by Cefas with the FSA data and has the advantage of using the historical classification and sea temperature data which would allow a rapid and easy impact assessment of UK stocks. The disadvantages of this approach are that the *E. coli* NoV relationship varies on a catchment basis, the NoV data takes no account of NoV viability and there is no capacity for responsive scoring.

In an ideal world the 'whole system' approach would be informed by a comprehensive NoV based sanitary survey and trials/research undertaken to optimise the scoring factors and any zone scaling. In view of timescales and resources a hybrid approach has been put forward for a staged approach using a combination of both systems. Following a UK impact assessment the *E. coli* NoV proxy score system could be linked to target default zone thresholds (e.g. to deliver harvest NoV standards) – these would be open to consultation and adjustment. Alternative evidence based options could be available for those areas wishing (and capable) of establishing 'enhanced management' zones.

Resource and regulatory implications are considered (Section 6.7.2) and reflect the difficulty of a cross-sector issue which is regulated by a number of different Governmental bodies. Recommendations are provided for research (Section 8.1), implementation (Section 8.2, 8.3 and 8.4) and at a strategic level (Section 8.5).

1 PROJECT OVERVIEW

1.1 Project Aim

The Food Standards Agency (FSA) has commissioned a literature review of the available information on approaches for establishing exclusion zones for bivalve shellfish harvesting around sewage discharge points in the UK. More specifically this project is a desk study to inform consideration of potential for establishing and use of exclusion zones as a control for Norovirus (NoV) in oysters.

The FSA appointed Aquatic Water Services Ltd with the University of Exeter Medical School (UEMS) to carry out a desk study to review relevant literature relating to approaches that have been or may be used for establishing exclusion zones for bivalve shellfish harvesting around sewage discharge points. This review incorporates reports and literature available up to June 2014. The study also uses shellfish and water industry expertise from Intertek Ltd and Aquafish Solutions Ltd to assess technical and practical applicability of the various approaches identified to the UK's shellfish harvesting waters.

The project will help the FSA deliver its aim of ensuring that food produced or sold in the UK is safe to eat. The study will provide an evidence base to inform development of UK policy and contribute to risk management discussions within the EU in respect of possible introduction of exclusion zones, as a potential control for NoV.

The drivers behind this research requirement are considered further in Section 1.2 and 1.3 whilst an overview of the tasks and scope of the project is outlined in Section 1.4.

This Technical report has been produced in parallel with an Executive Summary report.

1.2 Health Implications of Norovirus (NoV) and Shellfish as a Foodborne Vector

NoV is a gastroentric viral infection primarily acquired through community based infection via a faecal-oral route. Contamination is persistent and can be spread via person to person contact although airborne vectors have been recorded. It is widely accepted that human NoV (Genogroups GI, GII and GIV) is largely species specific with limited indications of potential zoonotic connections (Ref: Caddy, 2013).

In the US the NoroCORE project (Section 4.1.5) is currently looking at all aspects of NoV contamination and infection including some work on foodborne risks. There is also mounting evidence of NoV infection through waterborne contamination via drinking water, recreational exposure or contact with contaminated waters. The recent EU Viroclime study (Ref: Kay, 2013) examined waterborne contamination and highlighted the importance of NoV as a waterborne contaminant particularly following storm and flood events.

NoV infection via a foodborne vector is most commonly related to contamination during post-production food handling through poor hygiene particularly by infected food handlers (Ref: FSA, 2013) who may be shedding NoV particles.

This study only considers potential control measures for NoV with respect to shellfish as a potential foodborne vector. As highlighted within risk profiles (Section 1.3) raw bivalve shellfish are widely recognised to be a high risk commodity with regard to viral contamination and NoV in particular (Refs: CODEX 2008 and 2012, EFSA, 2012).

NoV is thought to be one of the most significant causes of shellfish related foodborne illness with oysters often implicated. Updated public health outbreak data for England and Wales over 22 years (1992-2013) indicates 176 outbreaks attributable to crustacean/shellfish (Ref: Harris 2013). Whilst a significant proportion of this group had 'unknown' (30 outbreaks), or suspected viral causes (56 outbreaks), the greatest identified illness type was attributable to NoV (58 outbreaks). Of 280 outbreaks reported attributed to seafood 120 outbreaks were associated with oysters with 2064 people affected. High profile shellfish related gastroenteric outbreaks have long been reported (e.g. Ref: Kohn *et al.*, 1993). However, the relative contribution of different sources and transmission routes (including foodborne transmission) to the overall burden of NoV in the community is not yet established (Ref: FSA, 2013). Seafish have sought to balance the relative contribution of shellfish NoV outbreaks to wider community IID infections (Ref: Pyke, 2010). This information leaflet using 2000-2009 public health outbreak data for England and Wales highlighted that just 25 outbreaks were attributable to live bivalve shellfish out of 679 IID outbreaks.

A number of other food types such as fresh produce have been implicated potentially as a result of contamination during production or post-harvest handling. A systematic review of NoV Genogroup attack rates and risk factors (Ref: Matthews *et al.*, 2012) indicated that outbreak patterns tend to differ relative to causal vectors and seasonality which presents a complex picture within which to frame foodborne shellfish related outbreaks.

NoV sources and illness dynamics within the population are poorly understood and as such the relative importance of NoV in shellfish as a foodborne vector varies in importance from country to country. Reports from the US (Ref: Hall *et al.*, 2012) indicate on average, 365 foodborne norovirus outbreaks were reported annually, resulting in an estimated 10,324 illnesses. However, it should be noted that of 364 outbreaks attributed to a single commodity, only 13% were attributed to molluscs as opposed to 33% to leafy vegetables and 16% to fruits/nuts. Furthermore, 53% of these outbreaks were attributed to infected food handlers and may have contributed to 82% of outbreaks from a range of commodities. It is understood that in the US fresh produce is the principal food group of interest in relation to NoV outbreaks, whilst *Vibrio spp.* contamination of shellfish is the major pathogenic threat under consideration. In southern European countries the incidence of Hepatitis A (HAV) is the principal viral pathogen of concern with respect to shellfish. At a global level a systematic review of 359 shellfish viral disease outbreaks (1980-2012) showed that NoV was the most commonly involved pathogen in ~84% of shellfish viral outbreaks (Ref: Bellou *et al.* 2013). EU and UK authorities have also recognised the importance of NoV as a potential foodborne vector and as such the regulatory climate is moving towards improved risk based control measures. This report is intended to help inform some components within this process.

Most outbreaks have been associated with shellfish harvested from waters affected by untreated sewage for example from storm overflows or from overboard disposal of faeces from boats (Ref: Campos and Lees, 2014). However, there has been a growing recognition over recent years that the current wastewater treatment provision does not remove viral pathogens as effectively as the Faecal Indictor Organisms (FIOs) upon which food and environmental regulations are based. In consequence, even treated wastewater discharges which may meet the environmental regulatory requirements can adversely impact shellfish hygiene quality from a public health perspective.

The FSA's Advisory Committee on the Microbiological Safety of Food (ACMSF) has recently released a draft report looking at viruses in the foodchain (Ref: ACMSF, 2014) including NoV in shellfish. This report has considered new technology and developments to identify data gaps and research priorities. Part of the role of this report was to review the previous recommendations from the 1998 report and the Governments' responses, whilst making new (or renewed) recommendations for prevention and control measures which may influence future regulatory climate with respect to NoV.

1.3 Regulatory Climate with respect to NoV

1.3.1 Public Health Data on Numbers of NoV Cases

It is widely acknowledged that person-to-person contact and contamination within the community is the principal cause of NoV related outbreaks and as such most information has been generated from outbreaks in communal social settings (e.g. hospitals, care homes, hotels and cruise ships).

Public Health England (PHE), formerly known as the Health Protection Agency (HPA), gathers statistics on the annual, regional and seasonal pattern of illness although it should be noted that the data is somewhat semi-quantitative. A number of reasons why health data requires careful interpretation are as follows:

- Laboratory analytical techniques have advanced considerably in recent years, particularly with the development of RT-PCR techniques. As such it must be accepted that long term datasets probably have better identification of NoV as a causal pathogen in recent years whilst 'unknown virus' may have been suspected at the onset of records.
- NoV is a not a notifiable disease and therefore reporting from health service representatives and assessment by regional PHE Public Health Laboratories is not universally consistent.
- Under-reporting of illness is common. Whilst NoV is an unpleasant illness for most healthy people who become symptomatic the most debilitating effects are overcome within a few days. Such patients are still shedding NoV as are other potential carriers who may be infected but asymptomatic.

It is probable that NoV and other viral gastroenteric illnesses have long been a poorly defined infection. Increased awareness can raise the profile of outbreaks resulting in increased public and political pressure.

Figure 1.1: Total annual NoV cases in UK
(*Source*: FitzGerald 2008a using HPA data)

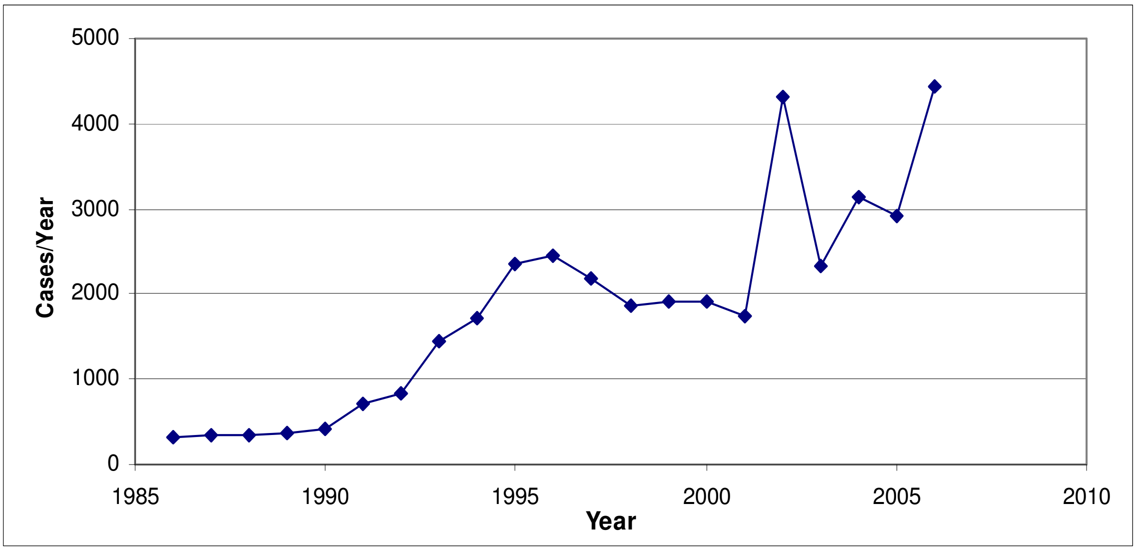
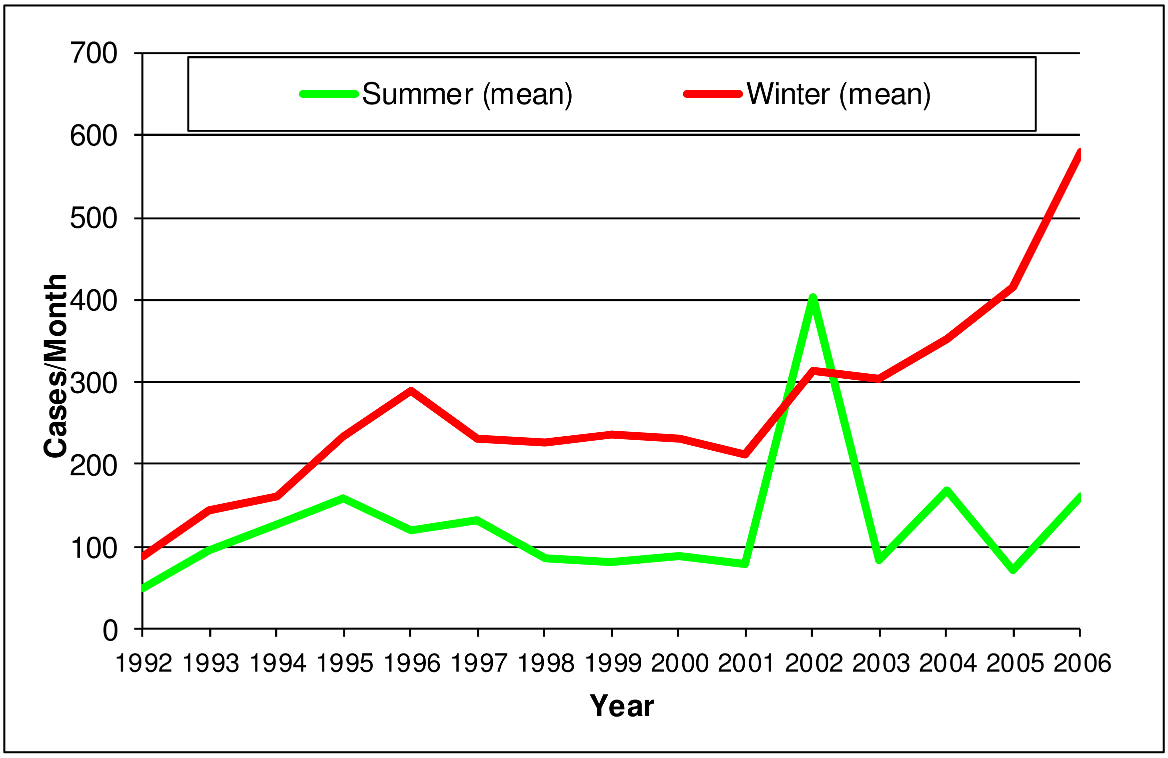


Figure 1.2: Monthly and seasonal variation UK NoV cases
(*Source*: FitzGerald 2008a using HPA data)



As indicated above improvements in analytical techniques and reporting schemes have indicated an apparent increase in the incidence of NoV cases as highlighted in Figure 1.1. The strong seasonal pattern of increased prevalence over the winter months (Figure 1.2), which gives NoV its 'winter vomiting bug' title, has led to the recognition that NoV has significant human health and resource implications with high profile impacts reported within the NHS (Ref: FitzGerald, 2008a).

1.3.2 Infectious Intestinal Diseases (IID) Studies

Comprehensive studies have been performed in the UK to better understand the patterns and incidence of gastroenteric illness within the wider community through the IID1 and IID2 studies.

UK the 1993-1996 IID study reported 3.7 million NoV cases/year in England (~6% of population) (Lawrence *et al.*, 2004) – see FSA website link in Glossary. Similar incidence levels have been reported for the US with 21 million NoV cases/year (~7% of population) (Hall *et al.,* 2013). Re-analysis of IID study samples to pick up lower NoV loads potentially shed by asymptomatic patients, suggested a wider incidence of 10 million cases/year (reviewed Ref: Lawrence *et al.,* 2004) and revised in Phillips *et al.* (Ref: 2010) to a prevalence of ~12% asymptomatic infection in England. In consequence, asymptomatic NoV cases are believed to be much more broadly spread than symptomatic cases and contribute to endemic re-infection.

The impact of NoV on the general population health and the degree to which foodborne illness contributes to 'seed' outbreaks is considered in depth in Lawrence *et al.* (Ref: 2004). The study was not able to differentiate between foodhandlers, fresh fruit and vegetables and shellfish.

A number of knowledge gaps were identified by Lawrence *et al.* (Ref: 2004), some of which have been addressed by the second study of infectious intestinal disease in the community (IID2 study). However, shellfish requirements have still not been attained:

- Dietary surveys on raw and cooked oyster consumption
- Point-of-sale survey of oysters for NoV contamination, although the recently commissioned 'attribution study' should address this need (Ref: FSA 2015).

1.3.3 NoV Risk Profiles

Although a number of foodborne NoV sources exist (Section 1.2) the relative contribution of shellfish derived outbreaks relative to wider food handling outbreaks is likely to be a critical feature in influencing any future integrated FSA /public health policy in the UK.

The FSAs 2010-2015 Food Disease Strategy (Ref: FSA 2011) highlighted a range of programmes and components with a focus on priority organisms most likely to have the greatest public health benefit. Although NoV incidents arising from consumption of raw shellfish is highlighted the foodborne disease contribution from food handlers is also acknowledged. It should also be noted that whilst many risk profiles focus upon specific food:pathogen pathways, the FSA Food Disease Strategy concludes: "*that a pathogen-specific rather than commodities focussed approach to tackling foodborne disease will be taken*." Risk profiles have been used in a number of countries and are instrumental in linking public health evidence to actions on specific food groups.

A NoV risk profile was prepared by the Codex Committee on Food Hygiene (Ref: CODEX, 2008) with a specific focus upon shellfish. This specific viral agent/product combination was deemed necessary due to the complexity of issues which would have been hard to assess in a generic study. The CODEX risk profile set out the knowledge gaps including the need for a better understanding of the level and incidence of NoV contamination, which in the UK was addressed by the FSA's survey to determine NoV prevalence in UK oyster harvesting areas (Section 1.3.4).

It is understood that since the publication of the CODEX 2008 a number of countries have conducted, or are in the process of undertaking, their own national NoV risk profile such as New Zealand (Ref: Greening *et al.,* 2009), Australia and the US/Canada. These studies are critical to assess the potential impact upon their native population as they consider shellfish consumption patterns for different age groups and cooking ratios for shellfish meals within their own countries. Computer modelling of health outcomes may also be incorporated (see Section 5.1). Health impact issues relating to infection, illness and viability are considered further in Section 3.1.

Following the identification of the risk profile for NoV in shellfish there was an acknowledgement that remedial efforts should focus upon pre-harvest prevention of contamination rather than post-harvest decontamination with a draft CODEX produced in 2010 (now formally adopted CODEX 2012). This guidance document has significant implications for wastewater treatment and management and is considered further in Sections 5.2 and 7.2. Recognition of zoning as a potential control measure is also provided in this guidance: *"The use of a prohibition zone for the harvest of bivalve molluscs near a wastewater treatment plant is another option the competent authority may use."* (Ref: CODEX, 2012).

It is recommended that the UK generate a national risk profile in conjunction with the proposed impact study (Section 8.4). It should also be acknowledged that risk profiles for various devolved countries may differ. It may be wise to progress with any UK risk profile once the current US/Canadian study has been published.

1.3.4 NoV in Oysters 'Prevalence' Study

The FSA commissioned a study for the "*Investigation into the prevalence, distribution and levels of norovirus titre in oyster harvesting areas in the UK*" Project reference FS23500) which was carried out by Cefas (Refs: Lowther, 2011a and Lowther *et al.*, 2012) – hereafter referred to as the 'FSA NoV prevalence study'. This study reported a headline finding that 76% of oysters tested from UK oyster growing beds contained NoV. Whilst 52% of samples were below the 100 genome copies/g detection limit it is apparent that only the minority of oyster samples exceeded the subsequently proposed 1000 genomes copies/g harvest standard (Ref: EURL, 2014a). Furthermore, it was not possible to determine what proportion of NoV contamination detected by RT-PCR may have been viable and potentially infectious (see Section 3.1.3). An overview summary of the pooled results from all 39 sites is provided in Figure 1.3 which highlights the marked seasonal trend in NoV concentration.

Although only 1.4% of samples had >10,000 genome copies/g the NoV prevalence study also highlighted the variability of contamination between sites with some shellfish beds having consistent and elevated levels of NoV. In addition, some marked regional trends were observed as shown in Figure 1.4 which highlights the relatively low level of NoV in Scotland and relatively high level of NoV in SE England with median values of 82 copies/g and 226 copies/g respectively. This differential was believed to reflect the population density pressures experienced by the regions.

Figure 1.3: Monthly proportion of shellfish samples giving total No results in different quality brackets (copies/g) *(Source: Refs: Lowther, 2011a and Lowther et al, 2012)*

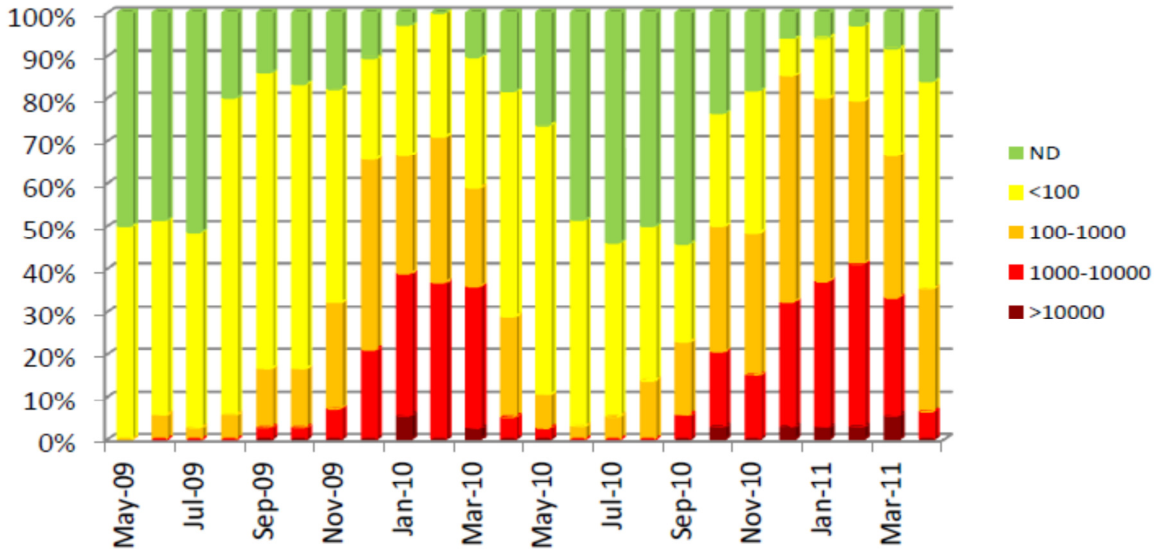
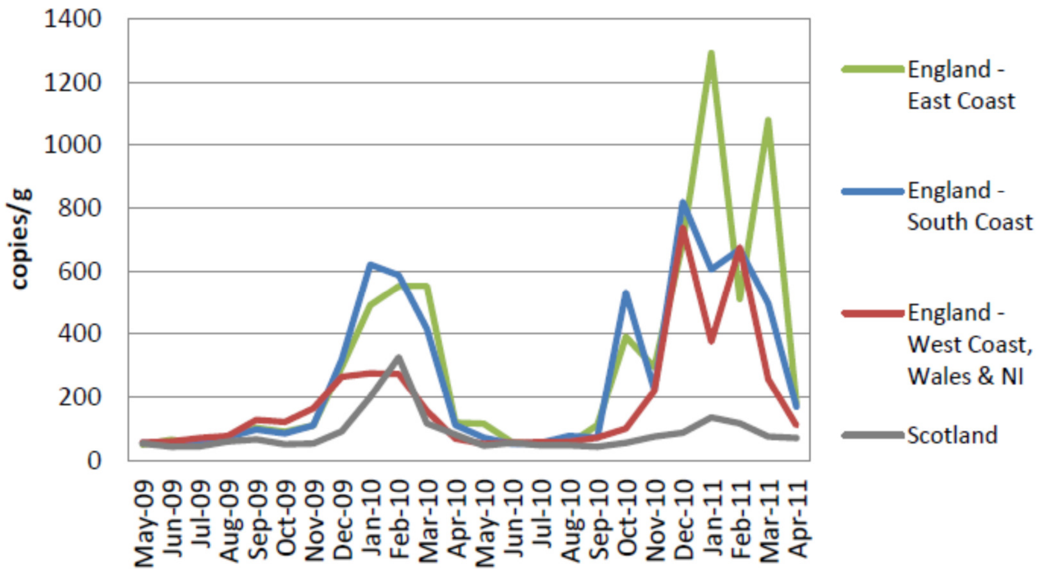


Figure 1.4: Monthly geometric mean NoV levels in shellfish for different UK regions *(Source: Ref: Lowther, 2011a)*



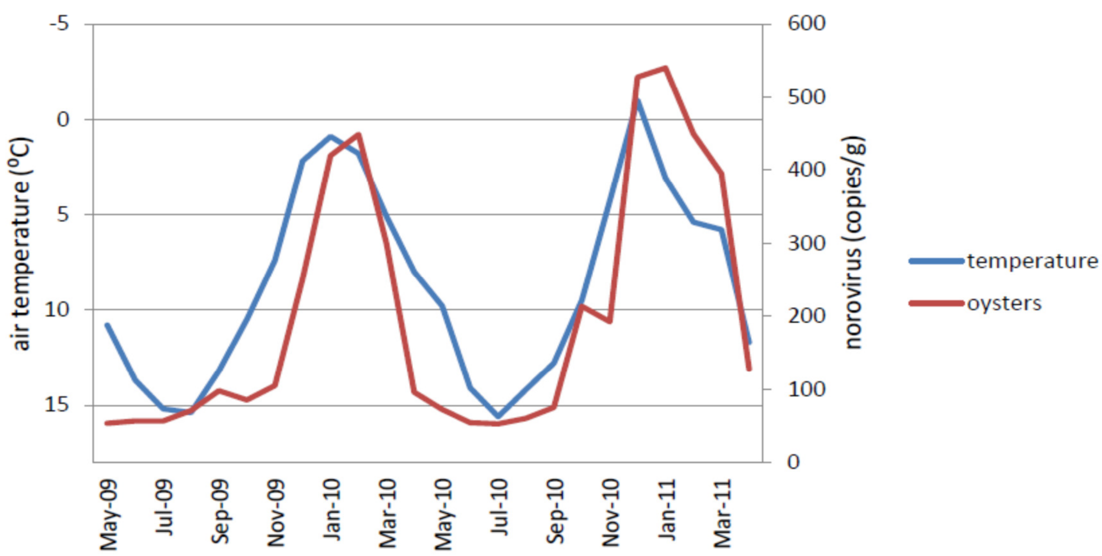
A statistically significant correlation was found between harvest area classification status and NoV levels – although individual sample *E. coli* data did not provide good agreement. Following some analysis and data screening (such as exclusion of the Scottish sites) it was possible to construct a risk matrix which is considered and developed further in Section 7.3.1.

Although a general pattern of seasonality was observed between oyster NoV data and lab outbreak reports, the relative magnitude of the two datasets differed. Oyster contamination was less in 2009/10 winter relative to that of 2010/11, whilst in contrast, PHE lab reported incidents were nearly double the level in the 2009/10 relative to that of 2010/11 - despite being colder in second winter. Lowther (Ref: 2011a) attributed this mismatch to possible industry management actions which may have reduced oyster related illness incidents.

Figure 1.5 highlights the very good inverse correlation (r=-0.877) between air temperature and oyster NoV levels. Lowther (Ref: 2011b) reports that air temperature relationship was somewhat more variable to site specific water temperature (r=-0.460 to r=-0.879). More recent re-analysis of this data has favoured the use of sea temperature (Ref: Campos Unpublished). Data from some sites with good correlation seemed to indicate that <10^{0}C concentration significantly increased NoV concentrations >100 genomes copies/g Dt. This factor is considered further in Section 7.3.2.

Figure 1.5: Relationship between air temperature and pooled NoV level for UK shellfish

(Source: Refs: Lowther, 2011a and Lowther et al, 2012)



1.3.5 European Food Safety Authority

The FSA NoV prevalence study, along with similar, but smaller and less systematic, studies in France and Ireland contributed to an EU wide report by EFSA (Ref: 2012) which provided an update on the present knowledge on the occurrence and control of NoV in shellfish. In common with the CODEX 2012 report, EFSA (Ref: 2011) focussed on the need to avoid harvesting oysters in the vicinity of sources of wastewater contamination. It was considered that only pre-harvest control measures (such as reduction in NoV load through wastewater treatment) could be effective in producing safe shellfish as post-harvest decontamination techniques (e.g. depuration and cooking) could not be fully relied upon.

Following the extensive work to develop detection methods for NoV in shellfish using RT-PCR (Ref: ISO 2013) EFSA published their opinion (Ref: 2012) that they considered the RT-PCR CEN method was suitable for use in a legislative context. CEN standardisation was subsequently completed in the summer of 2013 and is currently undergoing final acceptance of validated methodology. There is now scope for provision of a monitoring tool which could be suitable for regulatory use (Section 7.1).

DG Sanco proposal

The European Union Reference Laboratory (EURL - Cefas) released a discussion document outlining potential NoV management options at the request of DG Sanco (Ref: EURL 2014a Annex V). This document considered various options including potential:

- End Product Standards with 200 genome copies/g Dt (Digestive tissue)
- Harvest Standards 1000 genome copies/g Dt
- Setting new minimum depuration times for high risk species
- Tightening Class B status for oysters for >90%ile threshold
- Minimum closure period following outbreak.
- Use of exclusion zones around sewage discharges

In the expectation of the need to respond to the EU this project (FS513404) aims to explore one component of the potential management options.

1.3.6 Use of NoV Exclusion Zones - Regulatory Perspective.

The FSA's Advisory Committee on the Microbiological Safety of Food (ACMSF, 2014) has recently produced draft recommendations to FSA:

"The FSA should review risk management measures for shellfisheries (particularly oyster fisheries) in regard to point source human faecal discharges:-

- *Prevention of harvesting in areas in close proximity to sewer discharges, or regularly impacted by CSO discharges, is a sensible preventative measure and should be introduced.*
- *Policy should be formulated regarding preventative measures (e.g. bed closure periods, virus monitoring policy) following a known spill event or outbreak." (*Recommendation R6.6)

Annex V of the 2013 EURL annual workshop (Ref: EURL, 2014a) provided a 'discussion paper on live bivalve molluscs (LBM) and human enteric virus contamination: options for improving risk management in EU food hygiene package.' This discussion paper had been presented to Member States in EU Working Group to facilitate discussions on potential risk management options. Exclusion zoning around discharges was one NoV management option considered with a potential regulatory mechanism for implementation highlighted:

> *"Such buffer zones are not currently an explicit requirement of EU legislation but may be considered to be covered by the general provision in EU 854/2004 (Annex II, chapter II: C.1) that 'where the results of sampling show that the health standards for molluscs are exceeded, or that there may be otherwise a risk to human health, the competent authority must close the production area concerned, preventing the harvesting of live bivalve molluscs'."*

Wider European NRL consideration of exclusion zones is provided in Section 6.

1.4 Review Scope and Methodology

The literature review methodology had two key stages:

- Part 1: NoV exclusion zones. Where all NoV, zone and shellfish terminology terms were used (to inform Section 2)

- Part 2: NoV (and viral) environmental transmission stages relevant to the setting of NoV evidence based zoning. Where the wide scope of the subject area precluded an exhaustive review of terms (to inform Section 3).

White literature from academic peer reviewed sources were searched based on abstract sources within electronic scientific journal databases (EBSCO, PUBMED, etc). The Norocore search database (see Section 4.1) was also used. Grey literature from industry sources were located with the Google search engine. This section details the review terminology and scope limitations of the current project primarily with relation to the oyster species and proximity terms used (Part 1).

Part 2 database sources varied for components that were related to wider fields in public health, wastewater engineering and environmental science. Reference lists of papers selected for the review were scanned to identify any further papers. Review papers identified through the review were also used to inform additional keywords in further citation searching.

June 2014 was the date cut-off for the literature components of this report.

1.4.1 Norovirus (NoV) and Viral Terminology

Norovirus has been described by various terms within the scientific literature both as abbreviations and within wider group or generic descriptions. This report abbreviates Norovirus to 'NoV' throughout. Other terms and abbreviations in the literature include:
'NV', 'HuNV,' 'Small Round Structured Viruses' (or SRSVs), 'Norwalk-Like Virus' (or NLV).

A number of early (1970-90s) viral studies provide relevant data which informs our knowledge of decay and adsorption processes likely to apply to NoV. Scientific literature studies refer to a wide range of generic terms such as:
'viruses,' 'Gastroenteric viruses', 'enteric viruses' and 'Human Enteric Viruses' (or HEV).

With the designation of NoV into the 'Caliciviruses' group some references may refer to this wider group name. With the development of molecular techniques to differentiate various NoV Genogroups and Genotypes it has been possible to study which specific Genotype may be responsible for outbreaks and assess prevalence within wastewaters and shellfish. GI and GII are the two principal broad Genogroups generally implicated in human illness and shellfish related contamination and are broadly discussed within the current report as 'NoV' unless specific differentiation is required.

A number of animal related NoV Genogroups also exist such as the Murine norovirus, which is abbreviated within this report as 'MNV'. Although MNV is not considered infectious to humans it is relevant as a model virus as it can be assayed by tissue culture techniques unlike human NoV and may therefore be particularly relevant to informing viability studies.

Another important viral indicator group of relevance to understanding NoV environmental behaviour and considered within this report is the bacteriophages (viruses which infect bacteria) which present a further host of terms and abbreviations. Various 'phages' can be specifically described by the specific host cells they infect (e.g. MS2) or generic groups which infect enteric bacteria (e.g. coliphage) or grouped by how they infect host cells such as somatic phage (infecting via cell membrane) or F+RNA coliphage / Male Specific Coliphage (infecting via pili appendages). As with NoV a range of abbreviations are commonly used such as 'MSC' which is used within US FDA viral control measures.

1.4.2 Shellfish Species Selection

Oysters are the key group of shellfish selected within the scope of this study. This is primarily because it is widely recognised that oysters present an increased level of risk to consumers since they are eaten whole and raw. The native oyster (*Ostrea edulis*) and the Pacific oyster (*Crassostrea gigas*) are the two key oyster species harvested and produced within the UK. However, various international research papers on viruses in oysters will tend to utilise their own local species.

Some sub-sections of the report will consider other shellfish species as examples to illustrate general biological principals or patterns. For example, the New Zealand Case Study illustration (Section 4.3.1) considers quantitative NoV data from mussels as an example of a proximity relationship to a wastewater discharge.

It should be noted that the risk exposure profile for the Pacific oyster and native oyster will differ somewhat as the former are generally cultured using inter-tidal methods, whilst the latter are primarily fished sub-tidally (see Table A1, Appendix A).

1.4.3 Wastewater 'Discharge' Terminology

NoV contamination of shellfish waters can originate from a number of human wastewater point and diffuse sources. The focus of this report is upon wastewater point discharges which include treated continuous discharges and untreated intermittent discharges to both the marine environment and the freshwater riverine catchment.

Discharges are consented in the UK at defined positions with loading of known, or legally set, quality limits, volumetric limits and frequency. Three groups of consented point source discharges are considered in this report:

- Continuous discharges from settlements are treated to some degree. The level of treatment may be termed as primary (settlement), secondary (biological treatment) and tertiary (sometimes nutrient removal, disinfection or both) in order to meet the needs of the designation of the receiving waters, or the scale of the discharge. Waste Water Treatment Plants (WWTP) is the abbreviation used throughout this report in common with NSSP practice, although they are also widely referred to as Waste Water Treatment Works (WWTWs), Sewage Treatment Works (STWs) or Sewage Treatment Plants (STPs). The efficacy of NoV removal and inactivation athrough WWTPs is a major consideration reviewed in Section 7.2.1. The applicability of exclusion zones around continuous WWTP discharges in the UK is considered further in Section 6.1.

- Intermittent discharges are generally untreated from a microbial perspective. Two main types are considered Emergency Overflows (EOs) – normally a function of a system failure and Combined Sewer Overflows (CSOs) – a function of allowing surface water to enter the sewerage system leading to spills following rainfall events. The applicability of exclusion zones around CSO discharges in the UK is considered further in Section 6.2. Reactive monitoring of CSO intermittent discharges is considered in Section 7.2.2 and is a principal parameter within Active Management (see Section 7.1.4).

- Septic tanks provide a low level of on-site wastewater treatment to discharges from small populations or single occupancy dwellings. From a definition perspective septic tanks may discharge via a discrete point source to surface water, or they may overspill to a soakaway and therefore contribute to groundwater as a potential diffuse source. Diffuse septic tank and pleasure craft wastewater sources from a UK perspective are considered in Section 6.3.

Although diffuse and point wastewater discharges may originate through a riverine catchment it should be noted that the Cefas EURL recently defined riverine discharges into the sea as potential point contamination sources to shellfish waters (Technical Good Practice Guide – Ref: EURL, 2014b). Diffuse wastewater discharges from vessels are also a recognised contaminant source (Section 6.3.2) with a number of overseas authorities using zoning around marinas or ports (see Sections 2.1 and 2.2). From a UK context this is illustrated by the Technical Good Practice Guide which proposes buffer zones in association with marinas.

1.4.4 Exclusion, Prohibition and Buffer Zone Terminology

The principal objective of this project is to review exclusion zoning as a means to separate wastewater discharge derived contamination from shellfisheries. Despite blending all three terms (‘exclusion’, ‘prohibition’ and ‘buffer’) with ‘Norovirus’ (even with equivalent French and Spanish keywords) no hits were obtained to indicate evidence for direct NoV based zoning.

However, zoning does exist elsewhere to provide separation between designated commercial shellfisheries and wastewater discharges, although this is not expressly based on NoV criteria.

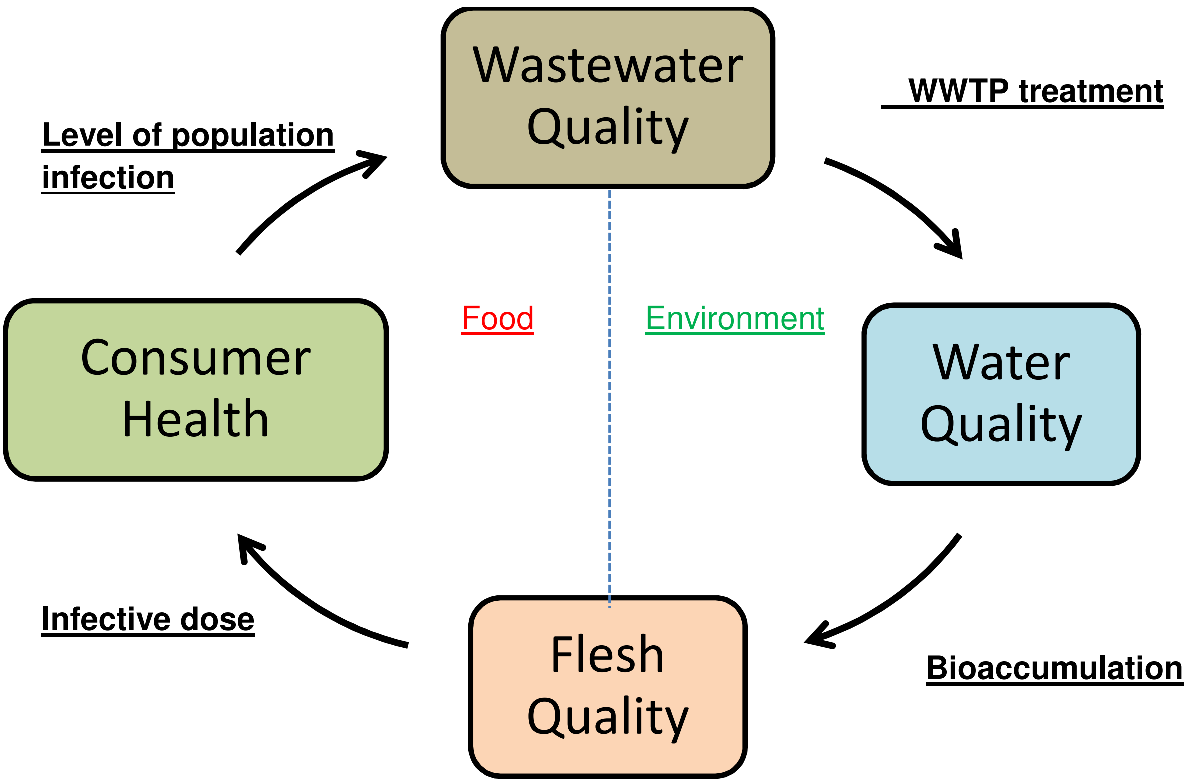
- Section 2.1 considers examples of ‘buffer zones’ which have been utilised in an *ad hoc* basis from certain European countries based on geographical distance criteria.
- Section 2.2 considers examples of ‘prohibition zones’ which are systematically used in countries employing the US affiliated NSSP system based upon bacterial water quality criteria (Ref: NSSP 2009). Although these countries do not currently have NoV based prohibition zoning this issue is under development and reviewed extensively in the US Case Study (Section 4.1)

Section 3 provides a review of literature relating to potential criteria which would be required if evidence based criteria were to be developed for NoV exclusion zones.

1.5 Project Overview - Summary

NoV is primarily a community borne illness spread by person to person contact. The contribution of NoV illness resulting from shellfish consumption to overall levels of foodborne illness may be limited relative to that originating from potential contamination through food handling. However, it is widely accepted that NoV is the principal disease implicated for food illness attributable to shellfish consumption (see Section 1.2).

Figure 1.6: Relationship between food hygiene and environmental processes affecting shellfish



Technical and regulatory advances in recent years have guided national and international authorities to address the potential NoV risks posed through shellfish consumption. A NoV prevalence study of oysters within the UK has demonstrated a widespread incidence of NoV with a strong regional and seasonal pattern of contamination with coincident observed illness over the winter. At an EU level potential NoV standards are currently under consideration as a result of which the UK is exploring alternative management options.

The FSA are examining a series of risk based management options to help reduce threats to human health of which shellfish 'exclusion zones' around wastewater discharges are one consideration.

The scope of this study is primarily to review literature and worldwide experience of wastewater zoning in relation to shellfish (Section 2). The terminology employed within this study is an important consideration in the selection criteria as summarised below:

- **Wastewater discharges** are considered to include: continuous treated discharges, intermittent untreated discharges and diffuse catchment / vessel sources.
- **Shellfish species** have been specifically related to oysters in recognition of their increased risk profile due to consumption practices
- **Shellfish areas** are considered to include both production and harvest areas

In the absence of specific NoV related zoning the potential zone requirements for an evidence based approach are also considered in Section 3. Examples of zone implementation in other countries, (not based on NoV), is explored in depth in the context of differing risk profiles and legislative approaches in Section 4.

Secondary considerations are to assess how possible management tools may incorporate zoning measures (such as computer modelling – Section 5) and their potential applicability to the UK (Sections 6 and 7). Whilst this study is not intended to provide a comprehensive impact study it is hoped that it will help provide the foundations for further work.

It should be noted that it is beyond the scope of this study to assess the significance of the threshold for any proposed NoV standards and how this might relate to any potential zoning. However, the wider issue of human health impact from shellfish flesh quality and its relation to NoV water quality is a critical consideration for any future development of evidence based zoning and management measures. Recommendations for further work are made within Section 8.

Owing to the complexity of technical issues surrounding NoV exclusion zoning a lay 'Executive Summary' report has been produced in parallel to this 'Technical report'.

2 LITERATURE REVIEW - EXISTING SHELLFISH ZONING CONTROLS

This report provides a literature review to assess the current status of reported information with respect to the setting and management of NoV exclusions zones. Section 1.4 sets out the scope and definition of terms used within the literature review.

Section 2 considers existing zoning practice in relation to separation of shellfish waters from wastewater discharges. It should be noted that existing exclusion zoning around wastewater sources uses criteria other than NoV and no examples of specific NoV based zoning were located within the searches.

2.1 Geographical Fixed Distance Zoning

From a European perspective a number of countries already have national geographical based exclusion zones as considered further in the following sub-sections. At the 2013 EURL annual workshop NRLs noting that implementation of exclusion zones on a proximity basis as the simplest measure although further work would be required to develop criteria.

> *"NRLs agreed that the introduction of prohibition (buffer) zones around significant point source human faecal discharges (e.g municipal sewage discharge pipes) would improve health protection against enteric viruses and other anthropogenic pollutants. It was agreed that further work was required to develop criteria (e.g based on geographic or dilution approaches) for such zones."* Resolution 5 of the 2013 Annual NRL meeting (Ref: EURL, 2014a).

A review of European practice is provided in the following sub-sections.

2.1.1 Iceland

EURL 2014a reports that the Icelandic Competent Authority have general requirements for buffer zones with a working guideline implemented for harvesting areas of ocean quohog (Iceland cyprine). This requires growing and harvesting areas not to be situated within a 500m of a discharge outlet or pipe valid for potentially contaminated harbour areas. No further information could be obtain from the Competent Authority and it is apparent that commercial exploitation of the ocean quahog for direct consumption is been limited in recent years with most landings employed as cod bait (Icelandic Fisheries 2014).

2.1.2 Italy

Although EU regulatory guidance and programmes are provided at a national level real enforcement through local laws are made at the regional level. In consequence, whilst national guidelines on enforcement on bivalve molluscs production does not give any information about buffer zones some regions have implemented local measures. It is understood that in the 15 maritime regions (of which 13 support mollusc bivalves production) just 3 regions are known to have specific measures for buffer zones:

- In the Marche (around Ancona) regional law n° 136 (18 February 2013) set up buffer zones around x9 ports and x9 mouths of rivers. These zones ranged from 0.25-1.2 nautical miles (~460-2200 m) around ports and 0.3-0.7 nautical miles (560-1300 m) around river mouths.
- In Campania (around Naples) a regional guideline states "*In the absence of data on current patterns, bathymetry and the tidal cycle to allow the evaluation of the circulation of pollutants, water bodies located within the range of 500m from each source of contamination can not be classified for the purposes of production and relaying live bivalve molluscs*"
- In Veneto (around Venice) the regional law n. 2432 (1 August 2006) set up buffer zones in general called 'areas of compliance' around potential sources of contamination (towns, canals, estuaries, drains, etc.). Default zones for urban and industrial areas are 500m and 1000m respectively. Potential 'souces of danger' including pollutant sources are superimposed using GIS mapping with shellfish production areas to assess the area lost as a result of the buffer zones.

2.1.3 The Netherlands

The EURL annual report 2013 identified that authorities in the Netherlands already operate geographically based exclusion zones around wastewater and potential contaminant sources to provide enhanced protection for shellfisheries.

Of the 17 production areas in Holland there are 3 coastal areas extending to the 12 mile limit whilst the bulk of shellfish aquaculture production is clustered within near-shore areas to the north within the Wadden Sea, or to the south in the Schelde estuary or nearby (e.g

Oosterschelde). With nearly half of the Netherlands population living in the central coastal Randstad region, which encompasses Amsterdam, Rotterdam and the Hague, much of the treated wastewater load is discharged to non-designated coastal waters where shellfish are not produced. All areas are Class A under the Shellfish Hygiene Directive with most designated for production of all types of bivalves molluscs.

National legislation to regulate these areas is set out in within the Commodities Act for live bivalve molluscs (2014) which lay down rules concerning the production areas for bivalve molluscs. Article 2 (Annex I) of the Commodities Act defines boundaries for each production area with an extensive list of major ‘exceptions’ around specific identified ports, marinas, pumping stations or sewage discharges within each area. The size of these exception areas are zones with radii generally of 100m or 300m with a minimum radius of 50m for a surface water discharge pipe and a maximum radius of 1500m from the mouth of the port of IJmuiden and from the Maas estuary.

It should also be noted that through the Commodities Act Dutch authorities also operate other enhanced management measures through the differentiation of sub-zones which are designed to prevent the placing of bivalve molluscs on the market which do not meet health standards. Article 3 (Annex II) shows how the Dutch Food and Consumer Authority has designated a number of special production plots termed ‘dilutive areas’ for mussel beds and ‘oyster wells’. Although these areas are not officially termed as relaying areas (all areas are already Class A), these special plots within the Oosterschelde are located below the low water limit with clearly marked buoyed off areas to demark plots to ensure pristine polishing sites.

Preliminary viral testing in oysters and mussels from the Eastern Scheldt in 2007-2008 detected several enteric viruses including NoV GII detected in 5% of samples from this site (Pol-Hofstad *et al.* 2013). Although this is a low incidence of NoV relative to that the UK it should be noted that this relates to a Class A area and demonstrates that even additional management measures and good bacterial indicator quality cannot guarantee NoV free shellfish.

2.1.4 EU Zoning Proposals for US Trade Harmonisation

US affiliated shellfish trade countries adopt very different shellfish regulatory approaches and considerations with respect to zoning (see Section 2.2 and 2.3). Member States (MS) of the European Union (EU) are currently in negotiation with the US FDA with regards to the potential for shellfish trade export to the US to meet the requirements of the National Shellfish Sanitation Programme (NSSP). The European Reference Laboratory (EURL) has prepared a Technical Good Practice Guide (EURL 2014b) which considers possible measures to adapt the current EU Classification scheme to make it fit for purpose to meet the needs of the US FDA. These measures include the adoption to ‘prohibition zones’ around wastewater discharges and within marinas to exclude shellfish operations based on the NSSP approach.

It should be noted that the EU Shellfish Hygiene Directive defines a ‘Prohibited’ Classification based on shellfish flesh bacteriological quality at a threshold not related to the NSSP ‘Prohibited zone’ which is based on bacteriological water quality. In consequence, the two terms are not interchangeable and NSSP ‘Prohibition Zones’ are likely to apply to EU Class B and C areas.

2.2 Dilution based zoning

Pleasure craft as a source of wastewater with a potential to impact upon the water quality in marinas and ports has been well documented (Section 6.3.2). There are examples of European national regulations for the use of exclusion zones as highlighted in Section 2.1. Furthermore, the Cefas GPG (EURL 2014b), considered as part of US trade harmonisation, also included guidance for a 300m exclusion zone. In the US itself there has long been a requirement to undertake simple dilution based zoning to establish the predicted level of bacterial contamination to set zoning for waters both within and adjacent to marinas.

Within the US NSSP Model Ordinance (NSSP 2009) Chapter IV. Shellstock Growing Areas @.05 Marinas outlines the requirements for marina modelling. These include:

- A dilution analysis shall be used to determine if there is any impact to adjacent waters.
- The dilution analysis shall be based on the volume of water in the vicinity of the marina.
- The dilution analysis shall incorporate the following:

  - A slip occupancy rate for the marina; (in the absence of site specific survey data FDA use 15-30% occupancy in calculations).
  - An actual or assumed rate of boats which will discharge untreated waste;
  - An occupancy per boat rate (i.e., number of persons per boat);
  - faecal coliform discharge rate of 2×10^9 per day
  - The assumption that the wastes are completely mixed in the volume of water in and around the marina.
- If the dilution analysis predicts a theoretical faecal coliform loading >14 faecal coliform MPN per 100 ml, the waters adjacent to the marina shall be classified as:
  - Conditionally approved;
  - Restricted;
  - Conditionally restricted; or
  - Prohibited

These zone descriptions relate to the bacterial water quality and the predictability of reduced quality as described more fully in Section 4.1.2 (see Table 4.2). The responsible authority has a requirement to assess all marinas on this basis although the choice and complexity of the modelling methods is in their discretion as appropriate for the setting (i.e can be simple volumetric model or a more complex computer hydrodynamic model (see Section 5.2). The US NSSP marina dilution modelling approach has been used to assess marina impact in previous UK assessments (Ref: FizGerald 2007). Bacterial loading calculations using the NSSP marina assumptions has been undertaken in the Scenario studies in Section 6.5.

Further more detailed consideration of dilution zoning with respect to continuous wastewater discharges is provided in the following Section 2.3.

2.3 Dilution and time based zoning

2.3.1 Existing NSSP Zoning Requirements

As with the previous Section 2.2 this sub-section should be read in conjunction with the US/Canada case study in Section 4.1. As described in Section 4.1.2 The US affiliated countries all operate their own adaptation of the NSSP which is reviewed every couple of years within the ISSC where amendments and updates are considered for inclusion. The last couple of ISSC conferences have considered viral issues in terms of both viral testing for early lifting of ‘event’ closure conditions and prohibition zoning considerations. The following sub-section is drawn on the recent ISSC 2013 prohibition zone proposals (Ref: Goblick, 2013):

Delineation of the Prohibited Zone around a Wastewater Treatment Plant
Establishing the size of the prohibition zone is dependent on a number of factors

- The distance to ensure that there is adequate dilution when the WWTP is operating as normal. (“Normal” means that the WWTP is operating fully within the plant’s design specifications, including design flows, treatment stages, disinfection, as well as compliance with all permit conditions. If operating outside ‘normal’ conditions it is deemed to be malfunctioning)
- That the collection system has no malfunctions, bypasses or other factors that would lead to significant sewage leakages to the marine environment.
- That there is adequate time when any malfunction occurs to ensure that all harvesting ceases and closures are enforced, so that contaminated product does not reach the market.

Prohibition zone classification adjacent to the WWTP outfall should taking account of the following factors (Ref: Goblick, 2013):

- *The volume flow rate, location of discharge, performance of the WWTP and the bacteriological or viral quality of the effluent;* Peak hourly flow, design flow or whichever is greater is used for assessment. FDA studies have determined that when WWTP peak hourly flow rates exceed design flow the virological quality of effluent typically degrades beyond what is considered as normal treatment. Furthermore, FDA bioaccumulation studies indicate that shellfish can accumulate significant levels of viral pathogens when exposed in durations of less than one hour.
- *The decay rate of the contaminants of public health significance in the wastewater discharged*; No decay is assumed.
- *The wastewater's dispersion and dilution and the time of waste transport to the area where shellstock may be harvested;* 100,000:1 dilution for Approved Zones, 1,000:1 dilution for Conditional Zones as well as the prerequisite notification time to close

the conditional area during a WWTP malfunction or period of degraded effluent quality, prior to the conditional area receiving the impact from the WWTP effluent. Time for wastewater plume transport is to be based on site hydrographic measurements of peak ebb or flood flows.

- *The location of the shellfish resources, classification of adjacent waters and identifiable landmarks or boundaries*.

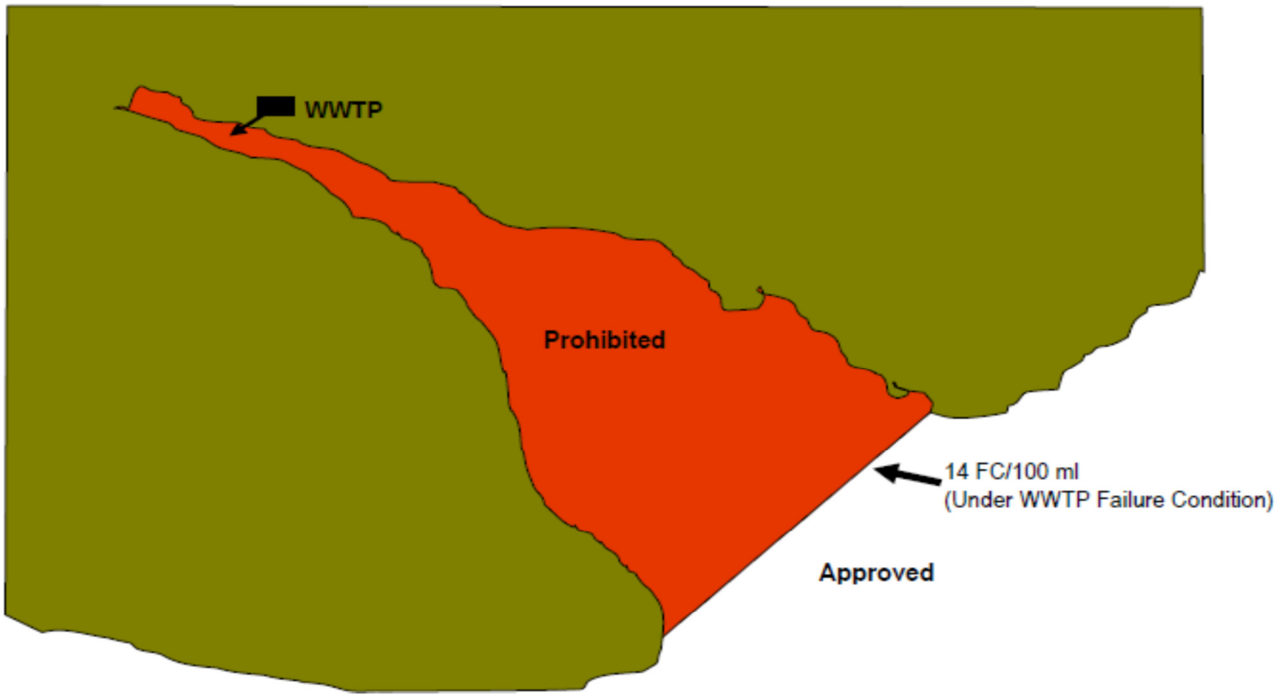
Rationale for Dilution Guidance (Ref: Goblick, 2013):

- *Approved Zones.* For Approved Zones the 100,000:1 dilution zone follows the NSSP recommendation that a worst case raw sewage discharge be assumed (see Figure 2.1 Scenario 1). This is based on the accepted NSSP level of $1.4x10^{6}$ FC/100ml found for disinfection failures requires a 100,000:1 dilution to dilute the non-disinfected sewage to meet the approved area standard of 14 FC/100ml.
- *Conditional Zones.* For Conditional Zones the 1000:1 dilution is the minimum level of dilution needed around a WWTP outfall to mitigate the impact of viruses and has been recommended by the FDA since 1987 (see Figure 2.1 Scenario 2). It should also be noted that if shellfish harvesting occurs within the zone of influence from a WWTP then these areas are also subject to a WWTP Management Plan as defined in Section II Chapter IV @. 03 C.(2)(a) of the MO.

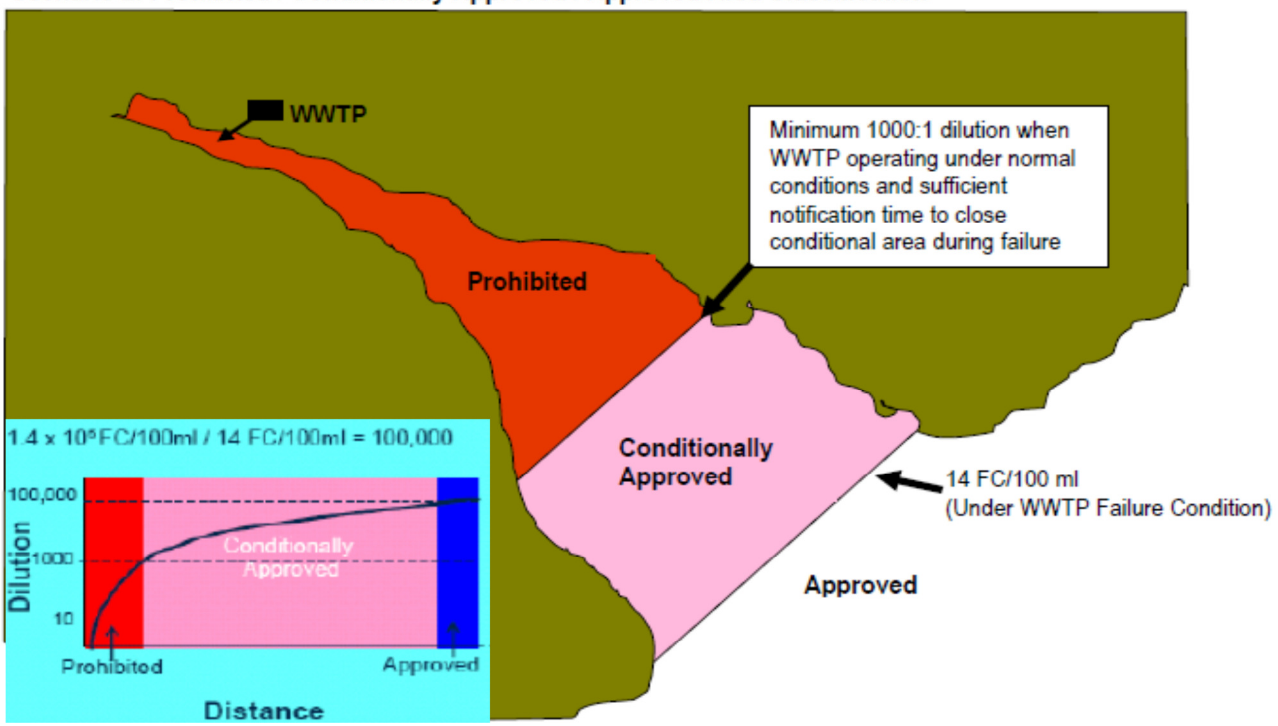
Figure 2.1: Scenarios for sizing prohibition buffer zones

(Source: Ref: Goblick and Carr, 2010)

Scenario 1: Prohibited / Approved Area Classification



Scenario 2: Prohibited / Conditionally Approved / Approved Area Classification



2.3.2 US/Canada Considerations of Zoning for Viruses

This sub-section should be read in conjunction with the other adaptation aspects discussed in Section 4.1.3.

US FDA Dilution Studies
From 2008-2012, the FDA performed a series of investigations to assess viral impact on shellfish from wastewater discharges (Ref: Goblick 2013). Studies were undertaken in Mobile Bay in Alabama (for detailed description see Ref: Goblick, *et al*., 2011); Hampton Roads in Virginia; Yarmouth (Ref: Goblick and Carr 2010), Maine; Coos Bay, Oregon and Blaine, Washington. The 2008-2012 FDA dilution factor studies evaluated WWTP effluent dilution factors against NoV levels in shellfish and the viral indicator Male-Specific Coliphage (MSC – N.B see Glossary as these are bacteriophage). The purpose of these studies was to provide a better understanding of viral impacts on shellfish which are summarised below:

- *NoV Results*. The FDA used a RT-PCR value of 300 genome copies/100g of digestive gland as a significant threshold based upon levels found in meal remnants which had been linked with reported shellfish related illnesses. (N.B. NoV methodology not EU CEN RT-PCR method.)
    - *Normal Conditions:* There were no cases in which conventional WWTPs operating under normal conditions produced results greater than 300 genome copies/100g of Dt (Digestive Tissue) in oyster sentinels when dilution levels at the associated sentinel stations were greater than 1000:1. However, with dilution levels of <1000:1 NoV levels exceeded the 300 genome copies/100g reaching a peak of 8,000 copies/100g in one case.
    - *Malfunction Conditions:* The US FDA dilution studies revealed that on a number of occasions when WWTPs malfunctions occurred oyster samples frequently exceeded 300 PCR copies/100g even when dilutions were greater than 1000:1. These results demonstrate the need for sufficient early warning systems to notify shellfish closure in the event of WWTP failure.
- *MSC Results.* As described in sub-Section 3.3.4 since 2005 the NSSP has used a MSC 50PFU/100g as a viral threshold to indicate unacceptable faecal contamination risk. Key findings (Ref: Goblick 2013) were:

- *Normal Conditions:* For conventional WWTPs operating under normal conditions, there were at least four occasions when dilution levels were between 700:1 and 1000:1 and MSC levels in shellfish exceeded 50 PFU/100g, but there were no occasions in which MSC levels exceeded 50 PFU/100g and dilution was greater than 1000:1.
- *Malfunction Conditions*: When flow rates exceeded the design capacity or during a treatment stage bypass, MSC levels in shellfish exceeded 50 PFU/100g in at least 13 instances in which dilution was greater than 1000:1.

Alternative Options

The ISSC 2013 prohibition zone proposals (Ref: Goblick, 2013) provides scope for alternative options where a less stringent 1000:1 dilution zone could be utilised in the event of appropriate wastewater control or enhanced disinfection is put in place. For example "*It is reasonable to expect a potentially higher reduction in viral load from a properly maintained wastewater treatment system employing ultraviolet (UV) disinfection with tertiary treatment operating under optimum design flow conditions*," (Ref: Goblick, 2013). However, the underlying principles of having recourse to provide Active Management to prevent shellfish harvesting in the event of system malfunction is still a prerequisite and any alternative approach would need to consider the time of travel for wastewater plume to the shellfish harvest site. In consequence, shellfish waters with large tidal amplitudes and/or swift tidal currents, the time of travel from the WWTP discharge to the shellfish harvest site may be the determining factor in sizing the prohibited zone. Alternative wastewater flow management measures such as use of emergency storage could be considered on a case-by-case scientific basis.

Canadian Dilution Zones

For Canadian delineation of no-harvest Prohibited areas in the immediate vicinity of WWTP discharges, regulators are currently applying an interim standard of 4 log reduction (i.e. 10,000:1) from raw wastewater through to prohibited boundary to mitigate risks from enteric viruses. It is understood that this is an interim guideline until the outcome of the joint US-Canada Health Risk Assessment on NoV in shellfish as outline in Section 3.1.1.

Use of Male Specific Coliphage (MSC) Standards

It should be noted that the Male Specific Coliphage (MSC), as termed in the US, is the F+bacteriophage referred to elsewhere within this report. In 2005, it was proposed to the

ISSC to introduce MSC viral measures (ISSC, 2005b) under the Model Ordinance (Section II, Chapter IV, @.03 A(5)(c)(ii)) used for re-opening harvest areas after an emergency closure due to raw untreated sewage discharged from a large community sewage collection system or a WWTP. The use of F+bacteriophage and consideration of a 50 PFU/100g threshold followed research work in the UK which suggested F+bacteriophage was a good viral surrogate which better reflected extended survival in the marine environment than the use of faecal coliforms. The use of this mechanism allows the potential fast-track return of a previously closed Conditional Area back into production in the event that viral contamination has not impacted or persisted.

Although the Mobile Bay (sub-Section 3.3.5) data seems to support the coincidence between attaining 1000:1 dilution and the F+bacteriophage 50 PFU/100g threshold it should be noted that other studies have not necessarily shown this relationship. The FDA dilution study on the James River (Ref: FDA, 2011) yielded poor correlation between microbial data and dye study findings although it was observed that wind influences on plume behaviour could significantly impact on trajectory leading to the conclusion that viral sentinel monitoring should be undertaken over an extended period of time.

It is suggested that future assessment work in the UK should aim to encompass F+bacteriophage (MSC) in addition to NoV to build on our knowledge of its use as a surrogate in the marine environment and the applicability of a 50 PFU/100g threshold.

2.3.3 US/Canada NoV Risk Assessment Process

The US and Canada are working together on a joint *Norovirus in Bivalves Food Safety Risk Assessment* with input from US FDA, Health Canada, Canadian Food Inspection Agency and Environment Canada. This encompasses expert input on shellfish, virology, wastewater, hydrology, risk communication, data management, probabilistic modelling and risk analysis.

Key objectives of the project are (Source: United States Food and Drug Administration, Health Canada, Canadian Food Inspection Agency, and Environment Canada, 2014):

- Evaluate the relative impact of selected factors on the risk of becoming ill with NoV from consumption of bivalve molluscan shellfish

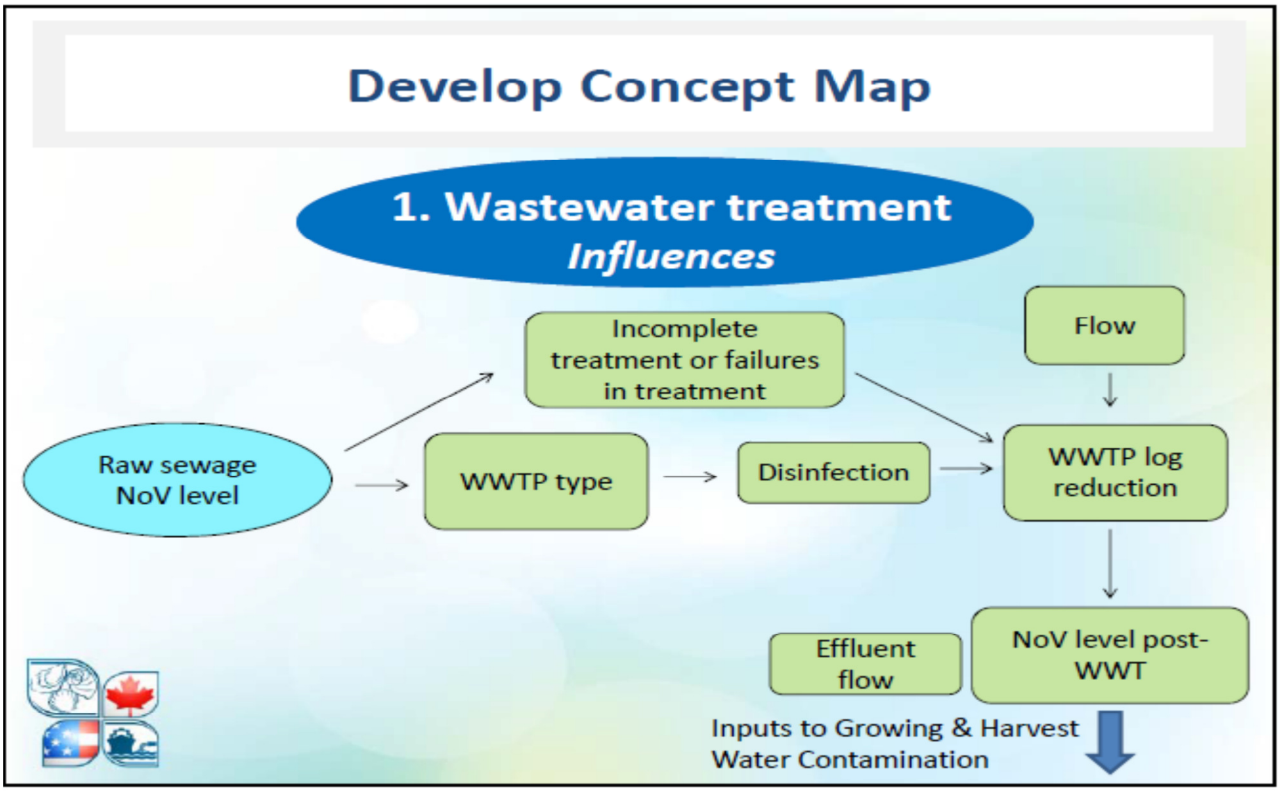
- Assess the impact on the level of risk resulting from specified control measures to mitigate risks from NoV contamination of shellfish growing waters
- Identify preventive practices and controls that could be used in the future
- Inform the development of a Food Safety Objective for NoV contamination in molluscs and/or a Performance objective for mollusc growing and harvest waters.

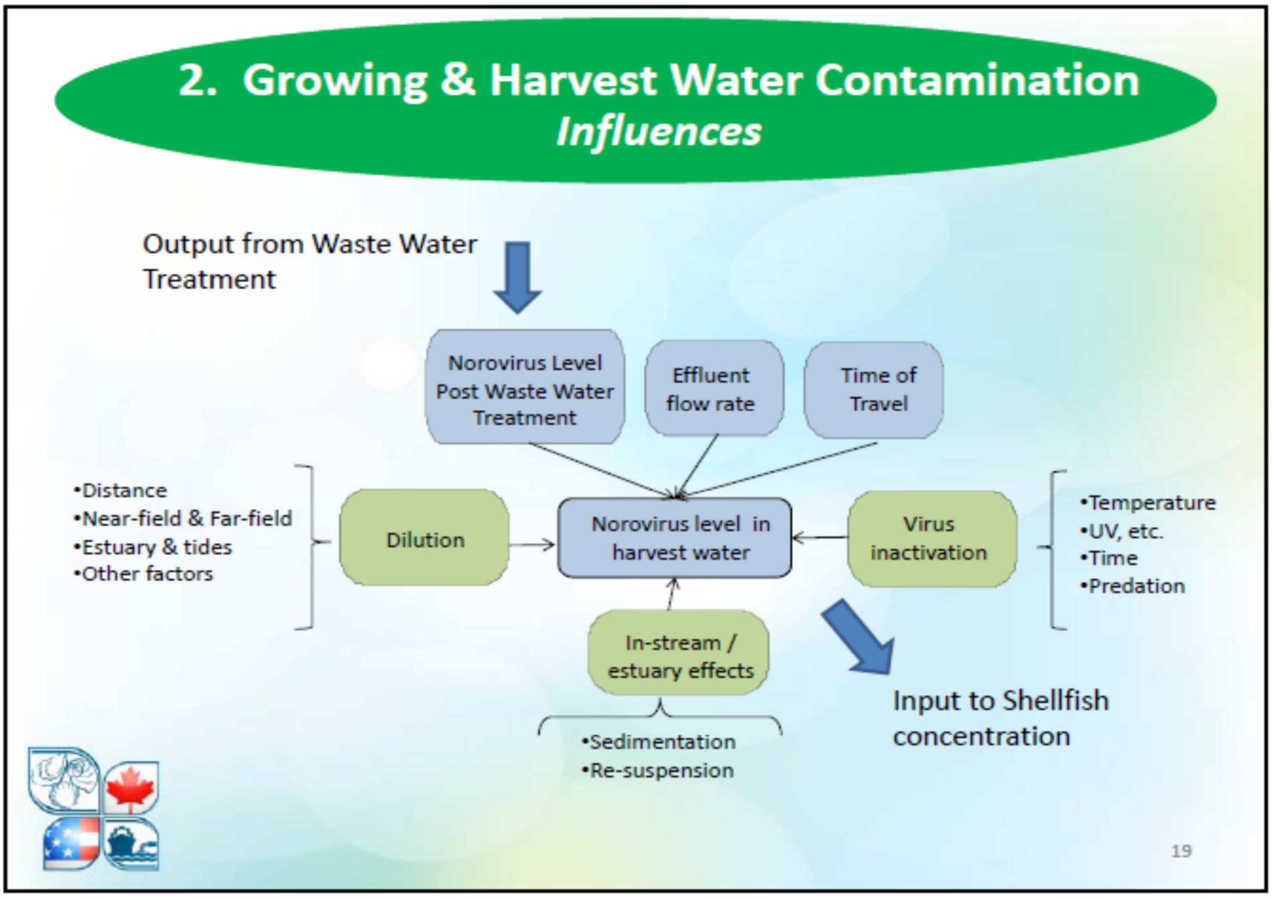
A central component to the project is the development of predictive risk modelling tools to help use science bridge between data and decisions and hence link events in food-supply system to public health metrics. Project calls for involvement during commissioning were issued in October 2011 and is currently working on Phase 3 'to develop and validate model'. It is understood that the project is two thirds of the way through.

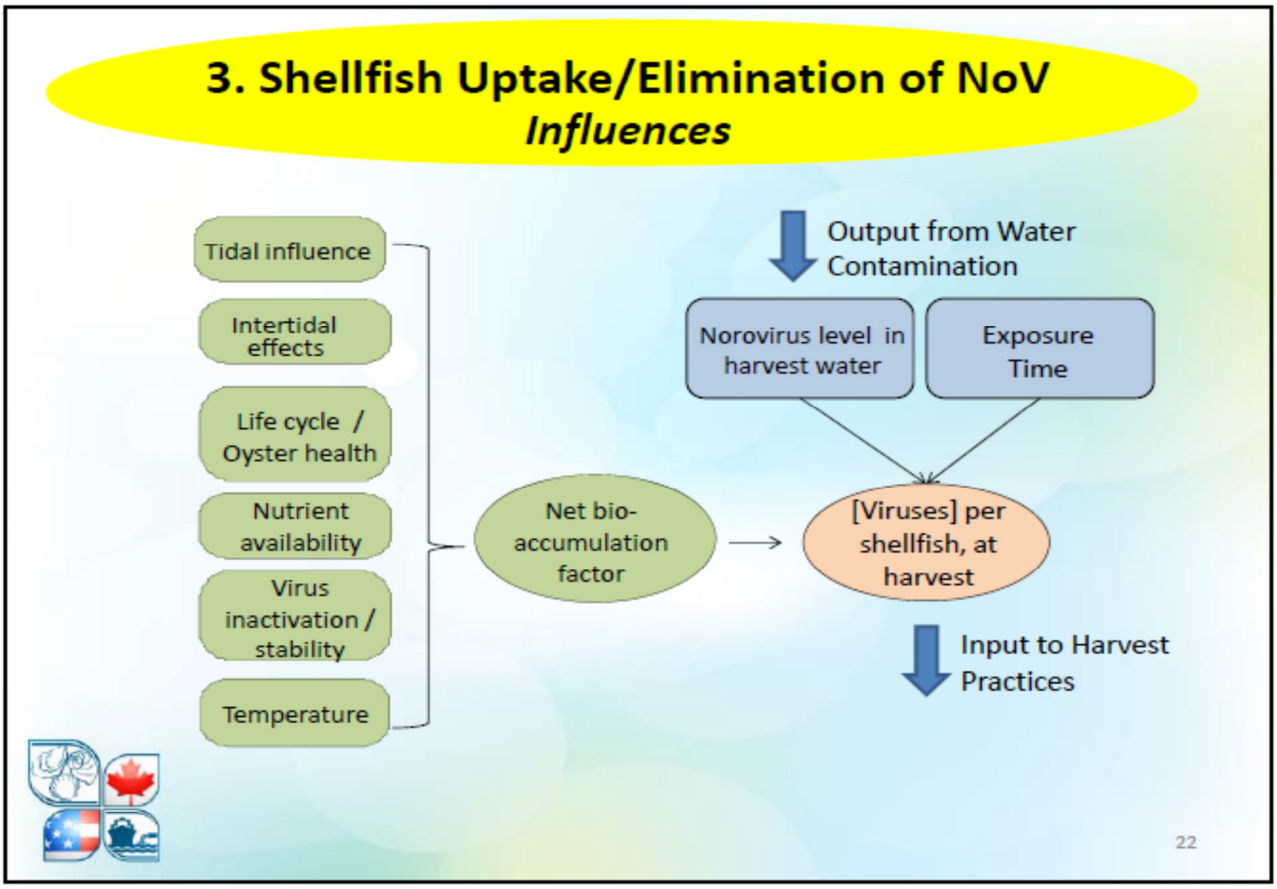
The Top Level Risk Assessment Model builds on key stages in environmental transmission of NoV:

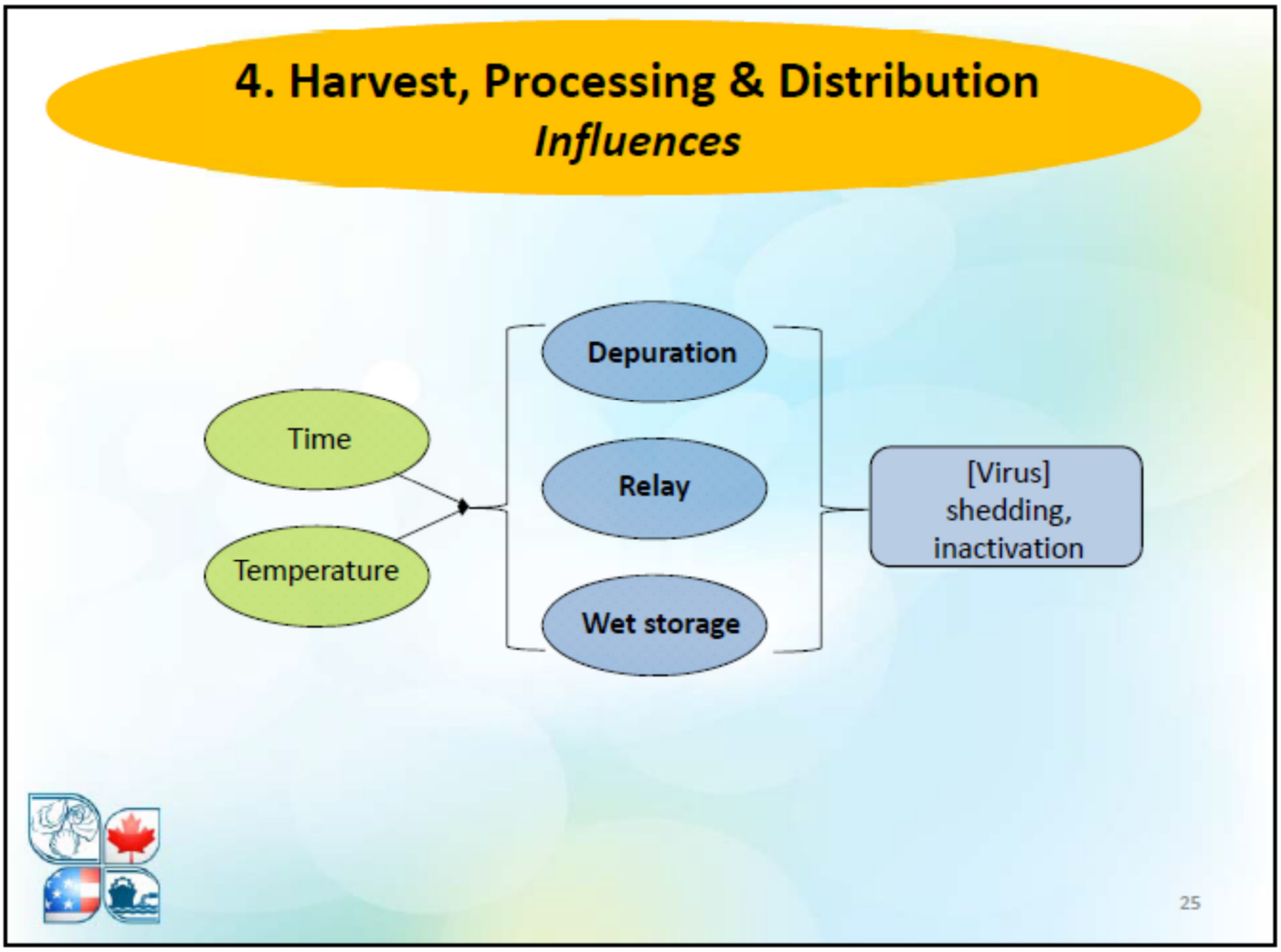
1. NoV in community
2. Raw sewage
3. Wastewater treatment
4. Harvest water concentration
5. Shellfish (at harvest)
6. Harvest, processing and distribution
7. Consumption characteristics
8. Exposure Assessment

Figure 2.2: US/Canadian risk assessment - NoV influence mapping
(Source: United States Food and Drug Administration, Health Canada, Canadian Food Inspection Agency, and Environment Canada, 2014).– Courtesy of Jane Van Doran)






3. Shellfish Uptake/Elimination of NoV
Influences
Tidal influence
Intertidal effects
Life cycle / Oyster health
Nutrient availability
Virus inactivation / stability
Temperature
Net bio-accumulation factor
Output from Water Contamination
Norovirus level in harvest water
Exposure Time
[Viruses] per shellfish, at harvest
Input to Harvest Practices
22


4. Harvest, Processing & Distribution
Influences
Time
Temperature
Depuration
Relay
Wet storage
[Virus] shedding, inactivation
25

Risk management options have been identified for Stages 3-6 which have been concept mapped to assess influences on the outputs from each stage as shown in Figure 2.2 Project workers hope to integrate the variables at these stages to optimise management options in order to develop a quantitative model. It should be noted that this staged approach has largely been replicated in this report (Section 3) and by some previously proposed ‘whole system’ models (Section 7.3.1).

It is recognised by the US/Canada risk assessment team that there is a need to link the probability of illness to the levels of contamination in shellfish and in water (Source: United States Food and Drug Administration, Health Canada, Canadian Food Inspection Agency, and Environment Canada, 2014). The next steps of model testing will encompass trying to characterise uncertainty which is likely to be strongly influenced by the data gaps at each stage of the environmental transmission pathway.

This programme is informed by the hydrographic assessment work undertaken by the US FDA from 2008-2012 to consider the suitability from a NoV perspective of the current 1000:1 dilution zones used to define Prohibition Zones separating wastewater discharges from Conditional shellfish waters (see Section 4.1.3). This work is pivotal to the principal objective of this report as it highlights that any zoning consideration needs to be in the context of the wider ‘whole system’ risk profile. In consequence, the output of this US/Canadian Risk Assessment is highlight relevant to the UK.

2.4 Existing Shellfish Zoning - Summary

Exclusion zones around wastewater discharges have not yet been developed around wastewater discharge on the basis of NoV impact on shellfish. However, examples of shellfish zoning in relation to wastewater discharge do exist in a number of countries using different criteria. Key findings include:

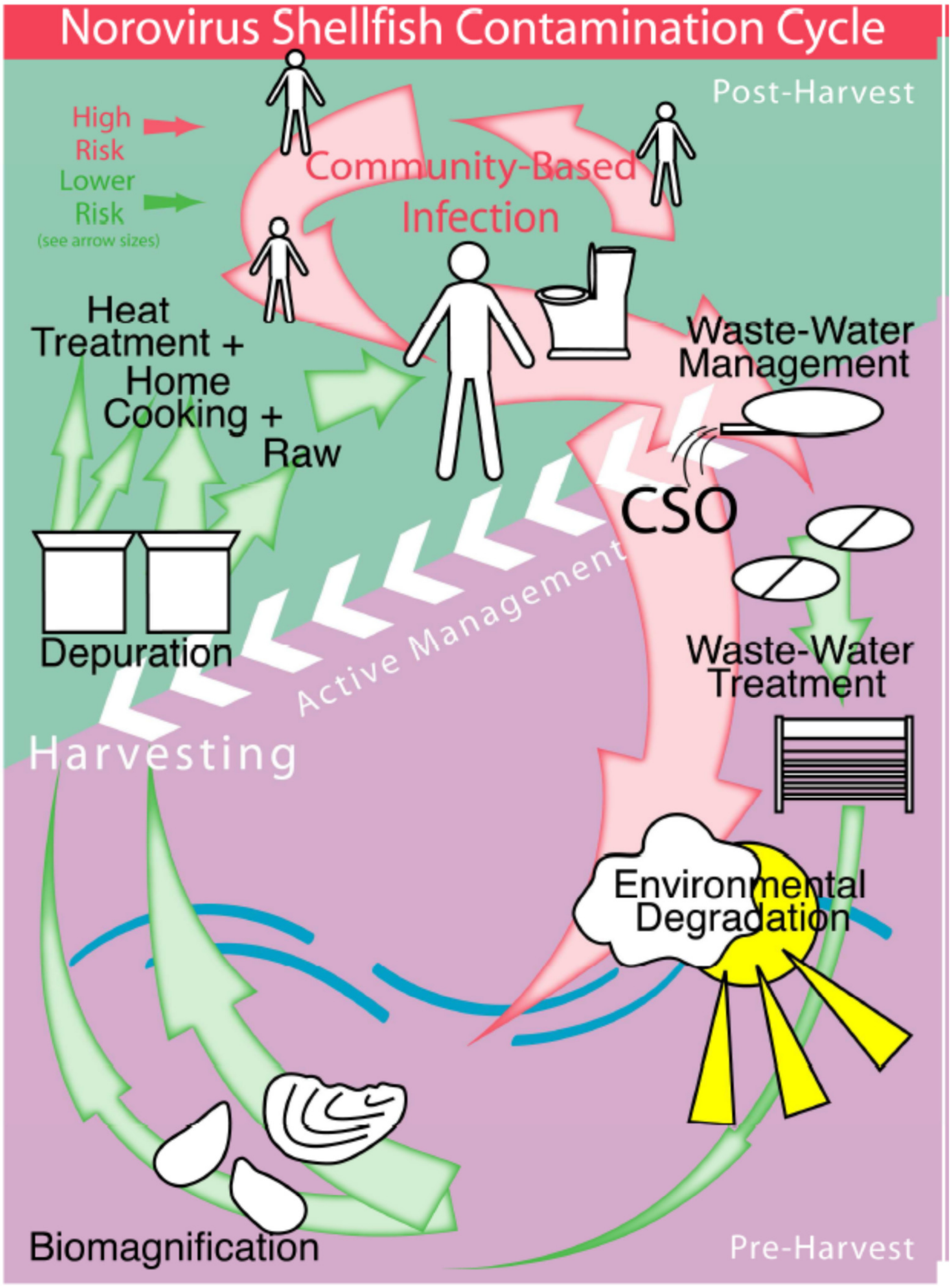
- Some European countries have adopted geographical based zoning to separate shellfish production areas from wastewater discharges. Recent consideration of

- exclusion zoning by EU NRL representatives favoured geographical proximity based zoning due to the ease of application. Examples included:
    - Iceland and Denmark (Section 2.1.1) – Limited measures to preclude shellfish harvesting from port and marinas.
    - Italy (Section 2.1.2) – Regional legislation in 3 of 15 marine regions requiring zones of ~500m to 1300m around potential contaminant sources including ports and freshwater inputs.
    - Netherlands (Section 2.1.3) – National legislation prescribing 50m-1500m zones around named wastewater discharges, pumping stations, marinas and ports.
- NSSP countries (e.g. US, Canada, Australia and New Zealand) use:
    - Zoning primarily based on bacterial water quality standards. Buffer zones around marinas are calculated on the basis of dilution to attain target bacterial standards (Section 2.2).
    - Zoning around WWTP discharges requires a 1000:1 dilution factor for Conditional areas and 100,000:1 dilution factor for Approved areas in order to attain bacterial standards (see Figure 2.1)
    - 'Conditional' zones also require a time component for zone consideration (Section 2.3.1). This is to provide early warning for responsive actions in the event of a contamination event such as a WWTP malfunction (also see Section 4.1.3 Case Studies for details).
- Zoning requirements in US/Canada are currently being re-evaluated as part of a wider NoV in Shellfish Risk Profile (Section 2.3.3). Preliminary feedback suggests an 'evidence based' approach for NoV zoning is under consideration (Section 2.3.3). The potential aspects under consideration (see Figure 2.2) are reviewed in detail within Section 3.

3 LITERATURE REVIEW - EVIDENCE BASED ZONING FOR NOV

In the absence of existing NoV zoning practice (Section 2) this section will provide a literature review of the principal components in potential environmental NoV transmission and how these might relate to future evidence based NoV zoning. Whilst wider community and food hander related NoV infection are considered in Sections 1.2 and 1.3 the stages of potential environmental NoV transmission from wastewater discharge to shellfish product are illustrated in Figure 3.1.

Figure 3.1: NoV environmental transmission pathway
(Source: Ref: FitzGerald et al., 2010b)



A holistic ‘farm to fork’ assessment is consistent with HACCP risk management practice. Such an evidence based approach with a similar staged breakdown is currently under consideration in the US as described in Section 2.3.3 (Figure 2.2) and is used in New Zealand for QMRA computer modelling assessment of wastewater impact on shellfisheries (see Section 4.3 and 5.1). Principal stages reviewed include:

- Catchment health – Section 3.1
- NoV in crude sewage – Section 3.2
- NoV in discharge (treatment NoV removal) – Section 3.3
- Dilution and dispersion – Section 3.4
- Environmental degradation – Section 3.5
- Bioaccumulation – Section 3.6

Section 7.3.1 also considers the use of science based evidence to compose a whole system risk scoring matrix to inform potential responsive zoning.

3.1 Catchment Health – Infection, Illness and NoV Viability

The focus of Section 3 is to review the stages in environmental transmission between wastewater discharge and shellfish uptake. It should be noted that ‘catchment health’ is a principal factor influencing NoV concentration in crude sewage. Some consideration is required for related issues of NoV viability and infectivity. This sub-section reviews these aspects to provide context to show how even low titres of viable NoV can potentially impact shellfish product safety and how this might relate to zoning.

The term ‘*catchment health’* has been used throughout this report on the basis of the following definition:

> *Catchment health describes the relationship between the level of infectious illness within a community and the magnitude of shed pathogen flushed into the connected sewerage system.*

3.1.1 Population Health

Infective Dose

NoV is highly infective as demonstrated by attack rates in outbreak studies and human challenge studies. Initial analysis of human volunteer studies indicated a very low infective dose of 10 viral particles (Ref: Tenuis *et al.,* 2002). This was then revised taking into account aggregation of viral particles to a re-calculated NoV infectious dose of 18 particles (Ref: Teunis *et al.,* 2008).

Volunteer infection studies have demonstrated a high probability of infection (~50%) at low NoV doses (Ref: Teunis *et al*., 2008). Further assessment of infection probability according to selector status (Ref: Thebault *et al.,* 2012) used previous outbreak data and showed that although susceptible blood groups have a significant potential for infection from a single infective NoV particle, non-susceptible blood groups have a minimal risk. Recent risk analysis work reassessing data from a number of studies and also taking into account recent research on variable susceptibility amongst hosts has highlighted there is still a degree of uncertainty about infective dose-response at low environmental concentrations <100 particles and advises caution about the use of data below this threshold as no subjects were exposed to this concentration (Ref: Messner *et al*., 2014). A dose-dependant response allows differentiation between infection (excretion of NoV in faeces) and illness (symptomatic). This highlights the difficulty in trying to establish 'safe' product thresholds and the need for a population wide determination of an acceptable level of significant impact. The balance between asymptomatic and symptomatic population dynamics has a profound impact on NoV shedding rates and the resulting wastewater loading to the environment (Section 3.2.2).

In England, Environment Agency representatives have indicated that it is necessary to know the infective dose of NoV within shellfish with an analytical method to indicate infective viability. Only then is it possible to consider how this relates to water quality and derive an appropriate Environmental Quality Standard for NoV (EA official, personal communication). Progress in this field is further complicated by the lack of a means to assess viability (see Section 3.1.3). Researchers in this area have also indicated that further human volunteer work is needed to validate new porcine mucin viability tests and that dose-response is likely to vary between GI and GII Genogroups.

NoV Impact on At-Risk Groups

To further complicate matters the dose-response relationship is also likely to be shaped by an individuals' immuno-regulatory health which itself is influenced by a range of factors including sunlight exposure (Vitamin D levels) and historical microbial exposure profile. At-risk groups such as the old, young and immuno-compromised may present increased sensitivity to infection with a potential for serious health impact if infected. Around 80 deaths a year attributable to IID infection in those >65 years old have been estimated using model analysis of 2001-2006 data (Ref: Harris *et al.*, 2008). Studies of two NHS wards including a paediatric primary immunodeficiency ward highlighted the difficulty in combating widespread parental visitor NoV contamination and the potential risk posed by even low NoV shedding asymptomatic carriers (Ref: Gallimore *et al.*, 2008).

Immuno-deficiency is an emerging problem in western society with an increasing incidence of auto-immune conditions and chronic inflammatory diseases (Ref: Rook, 2012) which may be treated by immuno-suppression drugs rendering patients more vulnerable to infection from diseases such as NoV. This places NoV impact into a wider societal context where the impact of the disease is linked with a diverse range of public health measures including uncontrolled use of antibiotics, diet and lifestyle issues.

3.1.2 Threshold Levels of NoV Contamination in Shellfish

It is beyond the scope of this report to consider zoning criteria based upon a level of population impact. In essence there is no agreed threshold upon which to base any standards.

- *Infection level* – The potential to ingest an infective dose is considered in the QMRA modelling (see Section 5.1) whereby a minimal dose (>18 NoV particles) within a shellfish meal is consumed. Monte-Carlo modelling allows population predictions to allow a threshold to be set (e.g. 5% infection).
- '*Significant' impact levels* - The potential NoV shellfish standards which have been proposed by EURL Cefas (i.e. an End Product Standard of 200 genomic copies/g Dt) balance practical RT-PCR reporting limits against food outbreak reports.

Further work is required to fill the knowledge gaps with respect to NoV (see Section 2.5), whilst the political attitude may well be shaped by financial implications highlighted in Section 6.7.2 and perhaps by the case studies experience from overseas (Section 3).

Fundamental to the consideration of 'acceptable' NoV levels is the determination of viability (Section 3.1.3) and how a shellfish NoV quality may relate to wider foodborne NoV impact on population health (Section 1.3.2).

3.1.3 NoV Viability

The viability of a NoV particle is a function of its capsid integrity (can it get into a host cell) and its genomic integrity (can it replicate once inside a host cell). Whilst infectivity is a better term from a pathogenic perspective, viability is a more appropriate term when considering viral behaviour and decay within the environment which is the focus of this report. Viability has multiple implications throughout the cycle of transmission including:

- Efficacy of UV disinfection systems (see Section 3.3.3)
- Decay in the marine environment through dark deactivation and sunlight deactivation processes (see Section 3.5.1 and 3.5.2 respectively).
- Potential for infection from food consumption and its implications to the setting of any potential End Product Standard or bed monitoring standard (see Section 7.1.1)

Viability remains one of the most hotly debated issues with respect to any implementation of NoV control measures. The continued inability to develop a live cell culture system to directly measure infectivity has been a fundamental analytical problem. This has led to the development of indirect analytical techniques and use of viral surrogates to help model potential behaviour but which remains challenging.

The FSA recently commissioned an extensive critical review of NoV viability issues (Ref: Knight *et al.,* 2012), although Rodriguez *et al.* (Ref: 2009) also provides a good review of PCR techniques with respect to the assessment of viral infectivity.

Nov Viability - Long Range PCR work

Some researchers have tried to develop a method to relate the degree of genomic damage to viral infectivity. Pecson *et al* (Ref: 2011) studied the capacity of UV 254nm to inflict singe genomic lesions upon MS2 bacteriophage and found heterogeneous genomic impact leading to the development of a framework upon which to assess infectivity which was proposed as a potential tool for NoV.

Rodríguez *et al*. (Ref: 2013) studied adenovirus inactivation through UV 254nm at varying dose rates up to 20 mJ cm^{-2} whilst assessing the impact of base pair length and showed that 1kb sites were almost as good 6kb site. This work stressed the importance of a rapid analytical turnaround with <1 day for PCR as opposed to 7 days for culture methods.

Nov Viability - Using Culturable Virus Models

Work using MNV models, similar to human NoV, has provided perhaps the best indication that UV should be effective at treating NoV. JungEun *et al.* (Ref: 2008), working with MNV from laboratory experiments showed that UV at 254nm with a 25 mJ/cm^2 dose provided 3.3-3.6 log reductions using cell culture techniques (but no significant difference with RT-PCR techniques). Studies in New Zealand (Ref: Wolf *et al.,* 2009) progressed exploration of UV efficacy upon MNV by comparing culture systems with LR-PCR providing a good estimation for UV disinfection efficacy in wastewater systems (see Section 3.3.3).

NoV Viability - Human Epidemiological Studies and use of NoV Model Surrogates

The value of MNV as a representative model of NoV inactivation behaviour is critical in the absence of a human NoV viability test. Wolf *et al.* (Ref: 2009) compared MNV RT-PCR (as a comparator to standard short attachment NoV RT-PCR by CEN method) against cell culture against long range PCR and demonstrated that MNV was an effective NoV model. Comprehensive work was undertaken to compare MNV and NoV in the efficacy of High Pressure Processing which was followed by human challenge testing in the US which demonstrated that MNV was a good surrogate for NoV.

NoV Viability - Capsid Integrity

Some workers have tried to move away from direct RT-PCR measurements in order to assess viability. Sano *et al.,* (Ref: 2010) have proposed a chemical method to determine the breakdown of non-culturable viral particles as an alternative to establish the viability of viral RNA. The method was tested on chlorination of astrovirus and relies on the production of carbonylated viral particles following oxidative damage. A similar principal was studied by Rule Wigginton *et al*. (Ref: 2010) who looked at the reaction of oxygen with the capsid proteins on MS2 bacteriophage when trying to assess the impact of UV disinfection. Tian *et al.,* (Ref: 2012) used of selective receptor-binding capture and magnetic sequestration (RBCMS) techniques which require intact capsid integrity as an indicator of NoV viability.

3.2 NoV in Crude Wastewater

The link between population health with resultant wastewater viral loading in environmental water viral and subsequent shellfish contamination has been well documented (Refs: Ueki et al., 2005, Iwai *et al*. 2009 and Anthony *et al.,* 2010.). This sub-section reviews literature data relating to the differing viral profiles and considers potential direct and indirect methods to evaluate catchment health. The modelling, prediction and early warning systems associated with increased NoV wastewater loading are likely to be major components in future risk management for shellfish.

Although initial studies using PCR techniques assessed viral incidence by frequency of presence or absence, an increasing number of studies have been conducted in the last 10 years which have attempted to quantify the level of NoV in wastewater and associated removal performance of WWTPs. Research groups have sought to characterise this risk in UK, France, Ireland, Netherlands, Italy, Sweden, New Zealand, US, Japan and Brazil.

Data from these various studies are summarised in Tables 3.1 and 3.2 which are reviewed in terms of the crude wastewater loading (Section 3.2.3) and removal efficacy (Section 3.3.1 to 3.3.3).

3.2.1 Seasonality and Regional Viral Profiles

Viral content in wastewater is a function of the level of infection within the contributing sewerage catchment. In turn the level of infection within the population is a function of multiple factors including environmental conditions, immunological status and exposure to outbreak triggers (Section 3.1.1).

The presence or absence of NoV (or a particular viral pathogen) in wastewater will not only reflect the health of the catchment but will also influence the risk profile for secondary contamination as an environmental vector (i.e. if there is no NoV in the wastewater, there is no shellfish contamination potential or corresponding foodborne risk). Ironically, the higher the prevalence of community based infection the greater the resulting risk of secondary environmental contamination and corresponding shellfish risk. It is therefore important to understand the pattern and intensity of catchment based infection and the potential for NoV loading seasonality on a regional basis.

In Northern European countries, NoV is commonly referred to as the ‘winter vomiting bug’ - a stark indicator of an observed seasonal epidemiological pattern of infection. Long term studies in Japan sampling a number of WWTPs show a similar seasonality of GI and GII with winter peak (Ref: Katayama *et al.*, 2008). In contrast, a long term study at

Gothenburg WWTP in Sweden demonstrated a seasonal winter peak for GII NoV but a summer GI NoV peak.

This regional difference in viral profiles was also highlighted by Petrinca *et al.* (Ref: 2009), who studied viral profiles (presence or absence) on a seasonal basis at three different Italian WWTPs finding low, or no detectable levels of adenovirus, and low (<10%) levels of NoV. In contrast, La Rosa *et al.* (Ref: 2010) studying 5 WWTPs in and around Rome found 96% prevalence of adenovirus and 92% and 72% prevalence of NoV GI and GII respectively, despite sampling over Summer-Autumn 2007 period. The prevalence and seasonality of these profiles are quite different from Northern European countries. A US wastewater study (Ref: Simmons and Xagoraraki, 2011) from 5 treatment plants showed absence of GI NoV and only 10% of occurrence of GII NoV.

In addition to regional patterns of viral contamination in wastewater is also influenced by catchment size. In general, larger source catchments have a higher probability of at least a small proportion of the population being infected. In consequence, major WWTPs are more likely to exhibit a smoother seasonal profile with increased level of persistence. In contrast, smaller WWTPs are more likely to be variable in NoV loading patterns and quantity with a lower overall level of persistence. Concentration profiles vary by virus type and possibly on a regional basis as demonstrated by Petrinca *et al.* (Ref: 2009). This study showed that some viruses had high levels of prevalence in larger WWTPs, whilst NoV prevalence exhibited no clear relationship to WWTP size. Similarly Hewitt *et al.* (Ref: 2011) showed that within New Zealand whilst adenovirus and enterovirus concentrations were greater and less variable for larger WWTPs, NoV GI and GII demonstrated high variability regardless of catchment size.

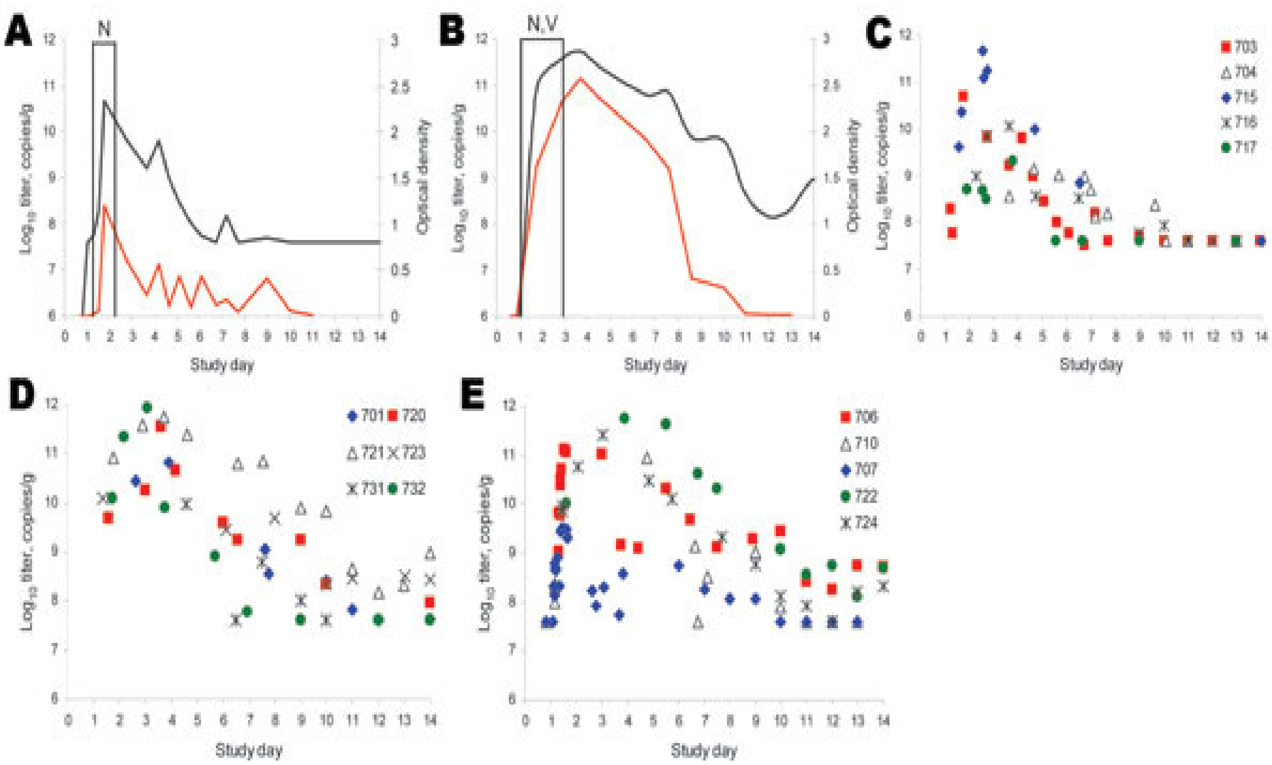
3.2.2 Theoretical NoV Concentration from Stool Loading

Faecal coliform levels remain constantly high within the human gut regardless of personal health, which is why *E. coli* works well as an overall bacterial indicator organism to highlight general faecal-oral risks. In contrast, NoV levels in the human gut are highly variable and dependent on a number of key factors:

- *Patient Health*. The health and age of the patient have been shown to correlate with NoV stool concentration. From patient studies Lee *et al.* (Ref: 2007) found that NoV concentration was related to severity of NoV symptoms and patient age.
- *Patient Blood Group*. Blood group is another patient specific factor which has been shown to have a marked impact on individual susceptibility, suggesting a differing dose-response relationship for blood groups (Ref: Tian *et al*., 2007).
- *Stage of Infection.* An infected patient will shed differing levels of NoV through the course of infection when symptomatic and during recovery whilst asymptomatic. A trial with volunteer subjects who were infected with NoV was undertaken by Atmar *et al,* (Ref: 2008) which depicted these features well (see Figure 3.2).

Figure 3.2: Shedding of NoV in faeces.

(Source: Ref: Atmar et al, 2008)



Key: **A.** Asymptomatic Patient, **B.** Symptomatic patient, **C**. NoV titre no clinical gastroenteritis (qRT-PCR), **D**. Nov titre patients with vomiting only, **E**. NoV titre patients with vomiting and diarrhea. *Key*: Black line qRT-PCR, Red line ELISA, N = nausea, NV = Nausea/Vomiting)

Theoretical estimations of NoV wastewater concentration can be made based on assumptions of stool NoV load, per capita flow rates and assumed levels of population infection.

- *Stool NoV Content*. A number of healthcare related studies have assessed the NoV concentration in stools. Lee *et al.* (Ref: 2007) with a 40 patient study obtained a mean faecal NoV DNA concentration of 8.93 $\log_{10}$ copies/g stool (inter-quartile range 8.22–10.24 $\log_{10}$ copies/g stool). Atmar *et al.* (Ref: 2008) with a 14 subject volunteer study obtained a higher mean faecal NoV DNA concentration of 10.98 $\log_{10}$ copies/g stool (ranging from 8.70–12.21 $\log_{10}$ copies/g stool). For the purposes of calculation, 10 $\log_{10}$ copies/g stool (1×10^{10}) would therefore seem a reasonably conservative compromise for a NoV concentration at the peak of infection.
- *Stool Mass*. European faecal production rates are quoted as 100-200g/day (Ref: Feachem *et al.*, 1983). A symptomatic person with NoV and diarrhoea has reduced capacity to absorb water through the gut which leads to production of a watery stool of elevated mass of >1000g/day (1500g/day used in Saranson *et al.*, (Ref: 2006) model assessment). In consequence, a symptomatic NoV sufferer will contribute >13 $\log_{10}$ copies/ day. It should be noted that this level of NoV loading is 5,000 times higher than faecal coliform shedding rates which are modelled at 9.3 $\log_{10}$ (or 2×10^{9}) copies/person/day.
- *Per capita Wastewater Volume.* UK Water Industry use a 150L/day per capita flow rate for engineering purposes - although groundwater infiltration rates vary on a catchment basis. With 150L/day/person to dilute the NoV loading a resultant peak wastewater content of 6.7×10^{9} copies/100ml or 9.82 $\log_{10}$ copies/100ml is possible with 100% infection. It should be noted that wastewater treatment schemes assume a faecal coliform concentration for crude of ~1×10^{7} counts/100ml.
- *Population Infection Rates*. Population infection rates will have a significant bearing on the theoretical NoV wastewater content. Annual NoV illness in the US (Hall *et al.*, 2013) is estimated as 21 million cases/year which is ~7% of the population. Whilst not all cases will be concurrent, the capacity to shed viruses for around 28 days following infection could mean that a winter seasonal peak may have the potential to generate a ~1% infection rate. For example, a 1% infection rate = resultant wastewater content of 6.7×10^{7} copies/100ml or 7.82 $\log_{10}$ copies/ 100ml - which is still in excess of the faecal coliform concentration. These theoretical NoV

levels are consistent with the NoV levels directly measured from wastewater (see Table 3.1).

3.2.3 Indirect Estimation of NoV in Wastewater

The use of surrogates to model NoV loading risk could provide a potentially useful tool to assess catchment health. Dore *et al.* (Ref: 2007) attempted an alternative approach to estimate catchment health by assessing pharmacy sales of diarrhoea products. Although this was an attractive concept the data was not thought to be representative as summer sales in conjunction with holiday times were believed to influence purchase patterns. A possible solution might be to directly measure the active ingredient content (loperamide chloride) in crude wastewater. However, in the absence of research data the PHE are not currently considering the use of chemical surrogates to assess symptomatic health (PHE officials, personal communication).

The FSA NoV prevalence study, for the FSA, highlighted a close correlation between NoV contamination in shellfish with temperature which has been mirrored by population studies (Ref: Lowther *et al.,* 2011). Lopman *et al*., (Ref: 2009) modelled NoV incidence in the population to assess the influence by both environmental and disease dynamic factors including temperature, humidity, population immunity and emergence of new strains. This study based on UK data found a high degree of inverse correlation between outbreak incidence and daily temperature average over previous 7 weeks (where previous weeks temperature had the greatest impact). A 1℃ increase in temperature corresponded to a 15% decrease in NoV reports. Also correlation with humidity with a 1% increase in relative humidity (over previous 5 weeks) corresponding to a 2% decrease in NoV reports. The relative magnitudes of these 'Attribution factors' were:

- 60% temperature
- 18% humidity
- 13% immunity levels
- 5% new strain emergence

NoV transmission risk has been related to increased humidity and rainfall (Refs: Astrom *et al*. (2009) and Greer *et al*. (2009)) in addition to storm wastewater overflows with associated reduction in salinity (Ref: Maalouf *et al*., 2010). The potential impact of weather events upon NoV outbreaks led Wang and Deng (Ref: 2012) to propose the use of satellite remote sensing to detect an array of water quality changes (e.g. low sea surface temperature, reduced salinity), which might correspond to storm periods and increased NoV risk.

More recent analysis of shellfish NoV data in relation to environmental variables has indicated that sea temperature may be a better indicator or NoV risk than air temperature. (Cefas official, personal communication). The recent Cefas ‘risk factors’ study also did not determine a relationship between rainfall and NoV content in shellfish (see Section 6.2.1).

Whilst statistical analysis of meteorological and NoV incidence datasets may indicate broad associations some caution may be needed in interpretation especially when outbreak reporting time lag and covariation of parameters are considered. Recent PHE focus has moved away from attempts to model NoV behaviour through an overly simplistic approach. However, there maybe scope for further development with gathering of more robust databases perhaps linked with wastewater catchment health surveillance (PHE officials, personal communication). Recommendations for further research are provided in Section 8.1.

3.2.4 NoV Illness Early Warning Systems

Early warning systems developed for public health resource management may also be of value to the shellfish industry. Loveridge *et al.* (Ref: 2010) observed that 4% gastroenteric symptom related calls in the preceding 2 weeks provided a good warning for a step increase in NoV outbreak incidence. Although the NHS Direct Syndromic Surveillance System used this approach until November 2013 this surveillance system was replaced by a new PHE system which is linked to the Hospital Norovirus Outbreak Reporting System (HNORS). This reporting along with clinical laboratory reports is available as a weekly update on the web with comparative regional and seasonal data (Ref: PHE, 2014).

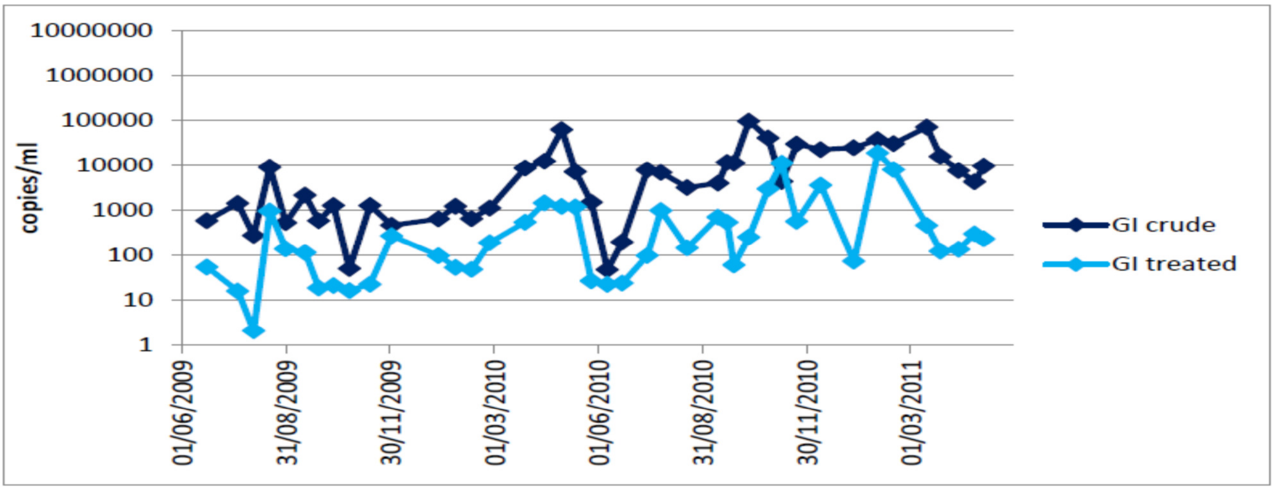
Although some workers (Ref: Dore *et al.,* 2007) have experienced difficulty in trying to obtain health care feedback health reporting directly from health care professionals could be a useful early warning tool. Before any mechanism could be developed the relationship and timing between catchment health and NoV loading would need to be better understood. Similarly surveillance monitoring using direct wastewater NoV analysis could inform an early warning system, although here too time lags would be critical.

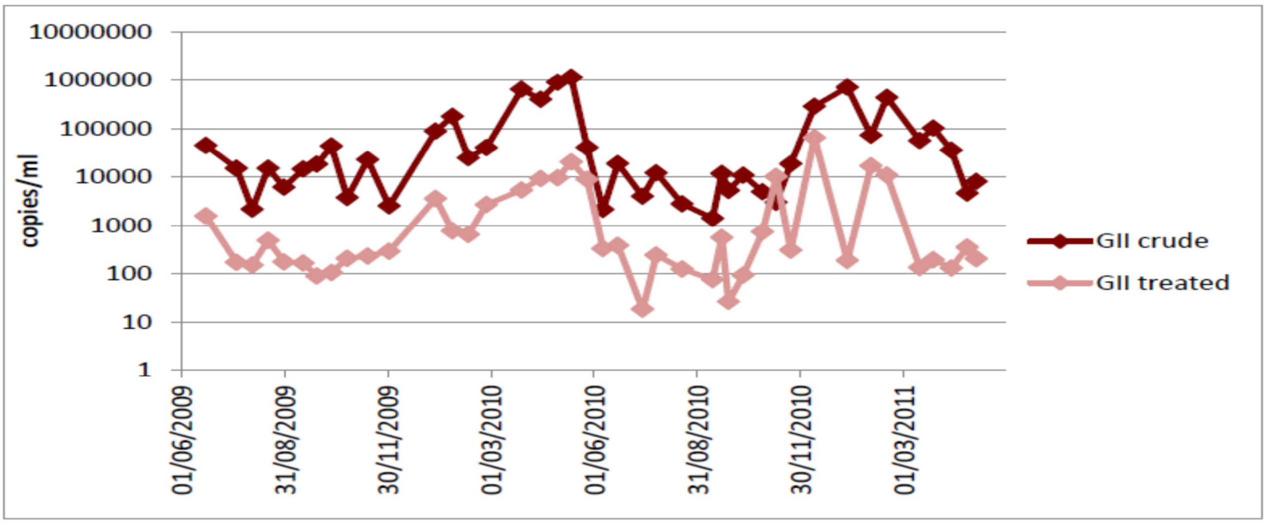
System response would dictate whether early warning systems are most suited for for public health purposes (i.e. NoV in wastewater increases before symptomatic epidemic spread), or for shellfish management purposes (i.e. symptomatic spread before increase in NoV wastewater loading to environment).

3.2.5 Direct Measurement of NoV in Wastewater

Direct analysis of NoV concentration in crude can be a useful technique to assess catchment health and the risk posed by wastewater spills. With known wastewater treatment efficacy it may also provide an indication of potential load from treated WWTP final effluent discharges. Figure 3.3 provides an illustration of how seasonal crude and treated NoV GI and GII load can vary on a seasonal basis.

Figure 3.3: NoV GI and GII in crude and treated wastewater (*Source: Lowther, 2011)*





Comparative crude wastewater data from the US FDA dilution studies (Ref: Calci, 2013) show the relatively constant faecal coliform level of 6-7 $\log_{10}$ copies/100ml whilst NoV levels at the US study sites used were significantly lower and more variable than UK examples.

Unlike bacterial indicators which demonstrate a relatively constant load, NoV content in crude wastewater is directly a function of what proportion of the catchment population is infected and shedding NoV. As highlighted in the previous section stool NoV content from an infected person is reported as high as 10^{12} copies/g (Ref: Atmar *et al.*, 2008). At periods of low infectivity such as in the summer when catchment health is good, many studies have shown that NoV may be undetected (at reporting limits) in crude wastewater. De Silva *et al.* (Ref: 2007) reported that wastewater NoV concentrations increased over 4 $\log_{10}$ at three of the four monitored WWTPs before corresponding NoV outbreak reports were provided from medical surveillance during the 2006 winter period. The pattern and concentration of genotypes led De Silva *et al.* (Ref: 2007) to suggest wastewater monitoring as a potential public health surveillance tool. Although PHE do not yet routinely undertake NoV surveillance monitoring of wastewater to assess catchment health the value for sentinel monitoring for key pathogens and emerging strains (Ref: Allen *et al.,* 2014) is recognised and is likely to receive more attention in the future (PHE officials, personal communication).

Table 3.1 provides a literature summary of crude wastewater NoV data from various studies around the world providing maxima, mean and minima values. As can be seen under peak loading conditions NoV concentrations can exceed faecal coliform levels (e.g. 10 $\log_{10}$ copies/100ml) although the 'peak' and 'mean' NoV concentrations in Table 3.1 when averaged are 6.45 $\log_{10}$ copies/100ml and 5 $\log_{10}$ copies/100ml respectively. This extreme variation between a 'sick' and 'healthy' catchment is one of the key difficulties in developing effective risk management to account for the 'worst case' conditions whilst not being over-zealous under 'normal' conditions. Recommendations for further research are provided in Section 8.1.

In summary, catchment health is one of the most important risk factors which will influence potential impact of wastewater loading upon shellfish quality. However, further work is needed to better characterise this principal risk factor and to assess whether direct assay or surrogates (such as temperature) can be effectively used as a monitoring tool to inform risk management.

3.3 NoV in Treated Wastewater

NoV loading from treated wastewater sources is an important Critical Control Point from a HACCP perspective. The CODEX 2012 document calls for improved controls to prevent pre-harvest contamination of shellfish, with wastewater treatment is seen as a principle mechanism to achieve this end. The draft CODEX called for a 4 $\log_{10}$ reduction of NoV through wastewater treatment, although this was subsequently revised to a ‘significant’ reduction (Ref: CODEX 2012).

The FSA’s Advisory Committee on the Microbiological Safety of Food (ACMSF, 2014) have recently recommended to DEFRA:

“There is a need for further research into the effectiveness of sewage treatment processes in reducing the norovirus concentrations in sewage and the effectiveness against norovirus of disinfection treatments.” (Recommendation R6.3)

3.3.1 NoV Removal Through Secondary Wastewater Treatment

Secondary biological treatment is a very important component in the reduction of NoV loading to the environment. Although this sub-section considers a secondary treatment from a general perspective most data available relate to Activated Sludge processes and Membrane Bio-Reactors (MBRs). MBRs are considered further in sub-section 3.3.2.

Biological treatment processes utilise microbial growth to metabolise carbonaceous waste, and in some cases nitrogenous waste, resulting in the production of secondary sludge. This microbial growth results in the removal of suspended solids, BOD and ammonia from wastewater. In the process of secondary biological treatment most indicator organisms (and potentially pathogenic organisms) are also removed.

For schemes where microbial quality requirements are in place for sensitive receiving waters (i.e bathing waters and shellfish waters), wastewater treatment efficacy is assessed on the basis of removal of bacterial indicators, such as faecal coliforms, rather than direct measurement of transitory pathogens. While indicator organisms provide a valuable consistent tool to assist in regulation and compliance monitoring they do not fully model NoV behaviour and therefore may not reflect risk.

There are a large number of papers published looking at various WWTP reduction efficiencies using a range of different indicator and pathogen target bacteria and viruses. Many studies indicate that each target microbe has its own individual prevalence and reduction characteristics, although generally reduction of bacteria is often more effective than that of viruses. Table 3.2 provides a literature summary of NoV and *E. coli* reductions from Activated Sludge and MBR processes.

It is widely recognised that viral adsorption onto suspended solids is the principal mechanism of NoV removal through wastewater treatment. This results in the retention and degradation of NoV within sludge systems. Arraj *et al.* (Ref: 2005) explored the removal of various viruses in a pilot activated sludge plant which demonstrated rapid removal with 1-2.2 $\log_{10}$ reduction of viruses from the liquid phase in just 10 minutes of suspension. Comparison between the removal mechanisms of Activated Sludge and fixed biofilm (e.g percolating filter) systems was explored by Kim and Unno (Ref: 1996). This study showed that whilst bacterial flocculation may cause adsorption in both processes, in the latter predation of viruses by protozoa and metazoa was also an important process leading to degradation of viruses. GI and GII NoV adsorption onto fixed biofilm was also demonstrated by Skraber *et al.* (Ref: 2009) who highlighted the relatively stable retention of NoV with no significant drop in levels over 49 days (from a genomic perspective).

The absorption of viruses onto secondary biological sludge solids implies concentration within sewage sludge where some degradation may occur. Once associated with sewage sludge, rates of viral degradation appear to vary ranging from 100% viability with adenoviruses to 16.7% viability for hepatitis A (Ref: Schlindwein *et al.,* 2010). There are no current techniques to directly assess NoV viability within wastewaters or sewage sludge. However, initial work by Tian *et al.* (Ref: 2012) using enhanced selective extraction techniques has shown that wastewater stored for an extended period (4 weeks) at ambient temperatures appeared to have vastly reduced viability suggesting degradation processes do occur. Consideration of sewage sludge as a potential diffuse NoV source is considered further in Section 6.3.3.

Table 3.1: Summary of crude wastewater NoV concentrations (Log_{10}/100ml)

Study	**Country**	**Peak**		**Mean**		**Minima**	
		GI	GII	GI	GII	GI	GII
Laverick *et al.*, 2004	UK			6.26			
Van Den Berg 2005	Netherlands			4.43			
Lodder and de Roda Husman 2005	Netherlands			2.71			
Haramoto *et al.*, 2006	Japan	4.41	2.38			1.23	5.28
Da Silva *et al.*, 2007	France	8	6.78				
Nordgren *et al.*, 2009	Sweden	5.3 (summer)	6.0 (winter)	4.51	4.61	3.0 (winter)	3.48 (summer)
Iwai *et al.*, 2009	Japan	5.36	6.85			1.66	2.58
Victoria *et al.*, 2010	Brazil	4.73		3.86	3.38		
La Rosa *et al.*, 2010	Italy	10.2	10.76	8.97	8.41	8.28	6.79
Sima *et al.*, 2011	France	7	7				
Simmons, Xagoraraki, 2011	US		5.04		4.63		3.72
Hewitt *et al.*, 2011	New Zealand	3.64	4.46			1.11	1.19
Palfrey *et al.*, 2011	UK		5.82		2.02-4.55		
Lowther 2011	UK	6.98	8.06	5.57	6.41		
Flannery *et al.*, 2012	Ireland	5	5	3.12 (summer) 3.94 (winter)	3.2 (summer) 4.61 (winter)		
Tian *et al.*, 2012	US			2.89	3.63		
Campos *et al.*, 2013	UK	5	6.87	4.18	5.69		

Table 3.2: Summary of Log_{10} reductions through wastewater treatment

Reference	Season	Period	N=?	MBR			Activated Sludge		
				E. coli	Phage	NoV	*E. coli*	Phage	NoV
Myrmel *et al.*, 2006.	Annual	Oct 01 -Oct 03	24-47 sets	-	-	-	-	2 100% - 84%	43% -26%
Da Silva *et al.*, 2007	Annual	Dec 05 -Dec 06	12-28 sets	-	-	*(Note 1)* GI 73% - 18% GII 100% - 0%	-	-	*(Note 1)* GI 45%- 21% GII 86%-17%
Victoria *et al.*, 2010b	Annual	Jan 05 -Dec 05	24 sets	-	-	-	-	-	GI 0.6 GII 0.32 66%-42%
La Rosa *et al.*, 2010	Summer Seasonal	May 07 -Sep 07	5 sets *(Note 2)*	-	-	-	-	-	GI 0.67 GII 0.43
Sima *et al.*, 2011	Winter Seasonal	Oct 09 -Jun 10	31 sets	4.9-5.7	-	3.3-6.8 GI 90% - 44% GII 84% - 31%	-	-	-
Palfrey *et al.*, 2011	Winter Seasonal	Nov 10 -Jan 11	4 sets	4.44	3.12	1.84	2.71	1.92	2.57
Lowther, 2011	Annual	Jun 09 -May 11	41 sets	-	-	-	-	-	GI 1.26 GII 1.64 100%

Flanney *et al.*, 2012	Annual	Jun 09 -May 10	38 sets	-	-	-	-	2.13	GI 0.8 GII 0.92 100%
Campos *et al.*, 2013	Winter Seasonal	Oct 12 -Mar 13	9 sets	-	-	-	-	-	GI 2.13 GII 2.63

Note 1 = insufficient data

Note 2 = 5 sets from 5 sites

Although comparative studies of WWTP NoV removal efficiency have generated mean results for different types of secondary biological treatment it should be noted that spot samples have yielded highly variable data (Ref: Palfrey *et al.*, 2011). NoV association with suspended solids may yield variable final effluent NoV content as a function of hydraulic and process factors. For example, if increased suspended solids were lost from final settlement tanks at times of peak flow there would be a higher NoV content at this time regardless of crude NoV concentration – resulting in an apparent lower log reduction. In addition, the residence time lag through the process makes it very difficult to obtain direct comparable data due to the dynamic nature of WWTPs. A few studies (e.g Ref: Nordgren *et al.*, 2009) have reviewed the viral log reduction rates for various treatment systems and assessed the degree to which this variation is a function of WWTP performance or differences in sampling and analytical methodology. Table 3.2 provides a summary of literature viral reduction data for Activated Sludge and MBR WWTPs.

In Republic of Ireland, the STRIVE project (Science Technology Research and Innovation for the Environment - funded by the Irish Government under National Development Plan 2007-2013) was a comprehensive project which assessed a number of NoV related components including NoV behaviour in wastewaters. Wastewater specific findings were reported in the scientific literature in Flannery *et al.* (Ref: 2013) and within the general STRIVE report (Ref: Dore *et al.*, 2013). The STRIVE report Dore *et al.* (Ref: 2013) indicated similar reductions of NoV and FRNA bacteriophage removal using RT-PCR with 1 $\log_{10}$ reduction through secondary treatment. Researchers highlight much higher levels of reductions for phage through infectivity assay with >2 $\log_{10}$ through secondary treatment. Concerns over the use of RT-PCR techniques for monitoring WWTP performance were also expressed in relation to UV performance results (Section 3.3.3).

The WRc study for Defra (Ref: Palfrey *et al.*, 2011) provided an initial overview of NoV removal from a range of treatment processes in the UK. WWTPs studied included percolating filters, Activated Sludge plants, Biological Aerated Filter (BAF) plants and a MBR plant. GII was frequently observed whilst GI was only detected on 4 occasions at the sites studied. However, the WRc study was of limited duration (~4 months) from 2009-2010 with only 4-6 sample occasions. Overall the WRc study showed best NoV removal rates for Activated Sludge plants (~2.6 $\log_{10}$ removal on average) and lowest performance for fixed biofilm plants (0.85 and 0.89 $\log_{10}$ removal on average for BAF and percolating filter WWTPs respectively). In contrast, Cheng *et al.* (2012) studied four WWTPs in

Repulic of Ireland and showed better effluent quality for percolating filters relative to that of Activated Sludge plants.

Campos *et al.* (Ref: 2013) reported the wastewater treatment efficacy findings for the recent Defra study (Ref: Cefas, 2013) which looked at an activated sludge site which demonstrated reductions of 1.5-2 $\log_{10}$ and ~2.5 $\log_{10}$ for NoV and *E. coli* respectively. It should be noted that this study also highlighted the limited removal influent-settled storm wastewater with log reductions of <0.5 NoV (similar for *E. coli)* which indicates NoV load from CSO discharges are little different to that of crude wastewaters.

In summary, studies in the UK and abroad have demonstrated that NoV removal through standard secondary treatment is variable and less effective than for the bacterial indicators against which WWTPs are designed. It is also apparent that direct measurement of NoV content using RT-PCR techniques maybe under-estimating WWTP performance as there is no account of the reduction in viability.

3.3.2 Removal Through Tertiary Treatment

Some tertiary treatment systems using physio-chemical processes do exist which are generally utilised downstream of secondary biological processes. In the UK a few MBR filtration plants are also operational but these use a physical separation process rather than a disinfection technique (see next sub-section 3.3.3) which is favoured for most schemes with microbial drivers.

Membrane Bio Reactors (MBRs)

MBRs are often considered a tertiary treatment addition to conventional Activated Sludge where conventional Final Settlement Tanks are replaced by membrane units with sufficiently small pore size (0.45µm) to prevent passage of bacterial flocs. Although membrane pore size is still too large to directly prevent passage of viruses (NoV at ~30-40nm is 10 smaller than pore size) the biofilm condition of the membrane is reported to have a significant impact as to the effective pore size and the scope for NoV removal. Recent research using bacteriophage (Ref: Lu *et al.*, 2013) showed that operational influences such as the membrane cleaning regime (e.g. cross flow or air scoured) and periodic chemical cleaning influenced biofilm formation which enhanced ultra-filtration performance. This study showed that a ‘fouled’ membrane removed >1 $\log_{10}$ more virus than a ‘pristine’ membrane.

Notable differences in NoV log reduction rates are reported between studies:

- *Poor MBR performance*: WRc study (Ref: Palfrey *et al.*, 2011) with reduction rates of 4.44 log_{10}, and 1.84 log_{10} for *E. coli* and NoV respectively.
- *Good MBR performance*: Ifremer study (Ref: Sima *et al.*, 2011) with reduction rates of >5 log_{10} for both *E. coli* and NoV. Exact calculations of reductions rates were not possible as both GI and GII were frequently undetected in the final effluent despite high influent concentrations.

The variable reduction efficiency reported in the scientific literature suggests that MBR performance may be site specific and not consistently reliable. As MBR systems are designed to remove bacteria WWTPs operators will seek to improve pass forward flows and the membrane cleaning regime is therefore set without regard to NoV removal rates.

Receiving water studies in the US in the vicinity of MBR WWTPs have shown elevated F+bacteriophage and NoV levels exceeding US requirements even beyond the 1000:1 prohibited dilution zone, and on a couple of occasions even exceeding 10,000:1 dilution (Ref: Goblick, 2013). These results show that although these plants may be effective in achieving bacteriological requirements they cannot securely control virological risk to shellfish waters.

Treatment combinations of both MBR followed by UV are under consideration and are reported to provide a higher level of microbial removal efficacy. Advanced Wastewater Treatment Systems (AWTS) are required for cruise ships transiting sensitive shellfish waters (Ref: Washington State Department of Health, 2007). AWTS normally employ an integrated system of enhanced aerobic digestion and low-pressure membrane filtration to treat wastewater, followed by UV disinfection and are reported to provide 2.5-4 log_{10} through MBR followed by a further 4 log_{10} through UV. However, data sources for this very high level of viral reduction have not been verified. Options for optimised MBR with UV treatment combinations for sensitive shellfish receiving waters may provide an good technical solution for NoV removal and effective disinfection. However, cost-benefit considerations (Section 6.7.2) will need to be satisfied and widespread retrofitting of MBR at existing UV WWTPs is unlikely.

Enhanced Filtration Systems

Some continuous flow physical filtration systems are on the market such as DynaSand® which are likely to improve NoV reduction by removal of secondary treatment residual solids. Some systems such as the US Centra-Flo® system utilise the addition of chemicals for removal of nutrients or heavy metals. Researchers at Newmark Civil Engineering Laboratory in US have assessed a range of chemical additions such as the addition of iron oxide to enhanced adsorption processes to remove viruses (Ref: Bradley *et al.*, 2011)

New enhanced filtration systems offer hope for a potential technical wastewater treatment solution, although as with MBRs, the cost-benefit for a new shellfish driver within the WFD has yet to be tested. In short they may be considered too expensive relative to the benefit provided.

In summary, technological solutions for enhanced wastewater treatment for NoV removal are possible, although for many shellfish waters they are likely to be too expensive relative to the benefit or reduced NoV loading. The context of other contributory NoV wastewater sources is considered in depth within Section 6.5, whilst WFD disproportionate cost aspects are discussed in Section 6.7.2.

3.3.3 Deactivation Through Tertiary Disinfection

Where environmental waters require a microbial quality standard (such as shellfish waters) there is often a need for supplementary tertiary disinfection following biological secondary treatment. CODEX 2012 calls for: *"Whenever possible, sewage treatment should involve a tertiary treatment step such as UV or ultra-filtration treatment."*

Disinfection Techniques

UV is the principal tertiary disinfection technique employed in wastewater treatment works for both bathing water and shellfish water schemes in the UK.

Elsewhere in the world different disinfection techniques such as chlorination are popular. Workers in Finland attempting to use a combination of coagulation with disinfection using Peracetic Acid (PAA) obtained some NoV reduction, although less than indicator organisms removal rates (Ref: Pradhan *et al.,* 2014). Wastewater studies from the US (Ref: Simmons and Xagoraraki, 2011) provided overall viral disinfection data which suggest better apparent overall performance for chlorination relative to that of UV with reduction rates of 0.9 and 0.3 $\log_{10}$ respectively. Each technique offers a range of benefits and disadvantages principally as a balance between microbial efficacy and the potential for residual breakdown products in the discharge.

Tertiary Disinfection for Bacterial Removal

It should be noted that as with other environmental regulatory monitoring regimes disinfection techniques are judged by their capacity to inactivate indicator bacteria such as faecal coliforms. UV radiation at 254nm wavelength is particularly effective at disrupting cellular DNA of bacteria thereby preventing replication. UK shellfish schemes tend to utilise a UV dose of 25mW/cm^2 which can provide ~3 $\log_{10}$ reductions in faecal coliform counts. In the case of bacteria there is scope for some cellular repair mechanisms which can help reactivate faecal coliforms post UV particularly if environmental conditions are beneficial.

UV disinfection schemes are not designed and operated around viral or NoV reduction performance criteria. Furthermore, RT-PCR techniques detect short lengths of NoV RNA regardless of viability. In consequence, whilst UV is likely to provide some level of NoV disinfection it is not yet directly possible to ascertain its efficacy at inactivation. Although UV schemes are routinely judged against faecal coliform indicator performance a significant level of research has been undertaken to ascertain potential efficacy upon other viral pathogens and phage viral indicators.

Contribution of UV Disinfection to Overall Treatment Performance

The STRIVE report (Ref: Dore *et al.,* 2013) indicated UV yielded low levels of reductions for both NoV and FRNA bacteriophage with just 0.5 $\log_{10}$ reduction when analysed with RT-PCR techniques. In contrast, the phage infectivity assay indicated much better UV performance with almost 2 $\log_{10}$ reductions. Overall the STRIVE project concluded that RT-PCR was an unsuitable method to determine the extent of infectious NoV reduction through WWTPs and within the environment. FRNA phage was proposed as an appropriate indicator of infectious virus reduction for NoV and as the basis for any ongoing monitoring programme to determine removal efficacy.

Campos *et al.* (Ref: 2013) reporting on an activated sludge and UV treatment combination demonstrated overall reductions of 2.9 $\log_{10}$ and 5.2 $\log_{10}$ for NoV and *E. coli* respectively. As may be expected UV was effective for *E. coli* (>2.5 $\log_{10}$ reductions) within minimal reductions for NoV. However, this study also indicated that there was evidence of NoV removal in relation to applied dose. It is possible that intense UV dose may be sufficient to not only inactivate NoV but also sufficiently disrupt viral RNA to impact detection by RT-PCR. The issue of genomic lesion is considered further with respect to Long Range (LR) PCR techniques to assess viability.

UV efficacy studies using MNV surrogates to assess impact on infectivity (Ref: Wolf *et al.*, 2009) indicate a potential ~2 $\log_{10}$ reduction in NoV viability, despite little apparent change in RT-PCR concentration. A 2 $\log_{10}$ reduction in NoV viability (i.e. UV treated wastewater at 1% viable) has been assumed in the modelling work in Section 5.

UV Dose Requirements

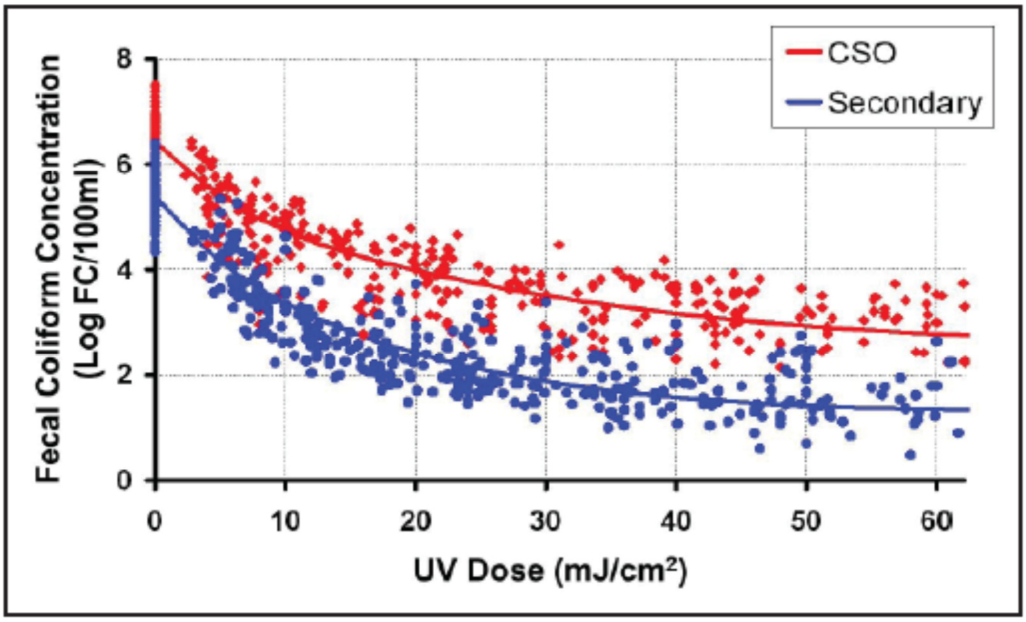
UV efficacy will be strongly affected by transmittance which is a function of organic content (e.g BOD) which can absorb UV radiation and suspended solids levels which can shield microbes from UV radiation. In consequence, there is a need to differentiate between laboratory studies where 'free' unbound viruses are subject to UV in controlled conditions from field studies in WWTPs where conditions are variable and confounding water quality parameters will reduce performance efficacy.

The inability to find a meaningful relationship between indicator organisms and human pathogens presents a challenge to managing an appropriate UV dosing regime. Jacangelo *et al.* (Ref: 2003) considered a New Zealand operating strategy for the Mangere WWTP (see Section 4.3.4) based upon raw crude enterovirus concentration to help guide UV dosing regime. This study showed that F+ specific bacteriophage was found to require approximately 20 mW/cm^2 per log removal, whilst much higher doses of 35 to 40 mW/cm^2 and 40 to 45 mW/cm^2 were required to reduce enterovirus and adenovirus to respective detection limits. In contrast, the USEPA 2003 Disinfection Manual cites multiple studies and proposes a much higher 50mW/cm^2 requirement to provide just 1.5 $\log_{10}$ reduction for viruses. Indications from initial studies (Ref: Lee *et al.*, 2008) have indicated significant UV inactivation with 3.3 $\log_{10}$ reductions at 254nm and 25mJ/cm^2.

It should be noted that UV dose requirements are ordinarily set according to required faecal coliform kill rates and take into account design criteria for particular types of wastewater. For example, recently a few UK schemes are considering UV application to CSO discharges which will be assessed against traditional indicator organism criteria. UV treatment of CSO discharges is already practiced in Canada (Ref: Muller and Len, 2011). To achieve an effective dose in such changeable and turbid wastewater UV systems are required to measure received dose allowing adjustment of lamp power output using variable output UV systems.

Figure 3.4 shows the differing dose curves for various wastewaters which highlight the vastly higher dose required in more dirty water to achieve the same faecal coliform concentration. For example, to achieve a 3 $\log_{10}$ faecal coliforms/100ml target microbial quality would require ~50mJ/cm^2 for a CSO as compared to ~15mJ/cm^2 for a secondary treated wastewater. To compound difficulties no such comprehensive understanding of UV impact on NoV viability currently exists. In consequence, it is difficult to assess the efficacy of current UK wastewater schemes in reducing NoV for CSO discharges.

Figure 3.4: UV Dose-response curves for faecal coliforms for low quality (CSO) and high quality (secondary treated) wastewaters
(Source: Ref: Muller and Len, 2011)



Use of Viability Studies to Assess UV Efficacy

Section 3.1.3 considers the difficulty in assessment of viability. Wolf *et al.* (Ref: 2009) indicated that UV is likely to provide ~2 $\log_{10}$ reductions in MNV viability which was proposed as a good model for NoV. Similar studies using LR-PCR techniques were also used to assess UV disinfection of adenovirus looking at impact of UV 254nm dose (Ref: Rodríguez *et al.,* 2013) and different UV wavelengths (Ref: Beck *et al.,* 2014). These techniques have great promise and are considered further in Section 3.5.2 where similar viability issues are considered from the environmental degradation perspective.

In summary, tertiary disinfection is likely to have a significant effect on reducing the NoV wastewater threat to the marine environment. Surrogate viral models such as MNV coupled with LR-PCR and bacteriophage data indicate that UV wastewater disinfection systems are likely to provide at least 2 log_{10} reductions in NoV. This means UV disinfect WWTP discharge may be only contain ~1% viable NoV. UV treatment of CSO discharges cannot ensure disinfection of microbial contaminants and would require a significantly larger UV dose to achieve similar efficacy levels. However, as current analytical techniques do not assess viability it is not possible with RT-PCR to demonstrate the probable reduction efficacy.

3.4 NoV Dilution and Dispersion

Dilution of wastewater plumes in the marine environment before reaching designated Shellfish Waters is an important component in achieving compliance to microbial standards for many areas. It is less certain whether these receiving water characteristics will be sufficient if judged against a NoV criteria. The US FDA are currently reviewing their 1000:1 dilution criteria for demarcation of Prohibition Zone around wastewater discharges for Conditional shellfish areas against potential viral standards (see Section 2.3.2).

Dilution of Faecal Coliform Indicator Levels

Wastewater discharges from continuous treated WWTPs to the marine environment within the UK are ordinarily discharged through an outfall which is submerged below Low Water to ensure adequate initial and secondary dilution to attain a 5.25 log_{10} reduction in faecal coliform levels between crude wastewater influent and the edge of the designated shellfish water.

For outfalls directly discharging into a Shellfish Water a tertiary disinfected WWTPs will deliver 4-5 log_{10} reductions through the treatment process so that the design criteria is met at the outfall location. Bacteriological quality in the receiving water will also benefit from an Initial Dilution of >10:1as buoyant wastewater ascents to the sea surface whilst mixing.

For some production areas WWTPs discharging biologically treated effluent at a distance from the Shellfish Water can still rely on sufficient secondary dilution and microbial decay to ensure that required reduction levels are attained before the Area boundary. Long sea outfalls of >1km are employed in many areas to allow discharge of high Population Equivalent treated discharges often through multiple diffuser ports (small outlets). This approach ensures low bacteriological levels at the shellfish water once discharged wastewater plume receives secondary dilution through mixing and dispersion.

Computer Modelling of Dilution and Dispersion

In many regions within the UK, computer models are used to demonstrate dilution and dispersion patterns from outfalls following ground truthing through comprehensive tracer studies and offshore monitoring surveys. This intensive level of work by the Water Utilities provides a good understanding on the plume trajectory which can be predicted under a range of environmental conditions (e.g tidal range and wind conditions). This can be used to evaluate time-of-travel between the outfall and the shellfish water allowing calculation of environmental degradation as faecal coliforms are inactivated (see Section 5.2).

Computer models are also used by some Water Utilities to assess the potential impact of CSO discharges upon shellfish waters. This has focussed upon designing schemes to meet the 10 'significant' spills a year design criteria. Examples of how modelling of NoV mixing could take account of a range of tidal and wind mxing characteristics is illustrated in Table 5.1.

Further development of these Water Utility models to assess potential NoV quantitative data from both CSOs and continuous treated discharges is explored further in Section 5.2. Consideration of whether more shellfish management use could be made with the tools developed by the Water Utilities is considered further in Section 7.2.4.

Dilution of NoV Levels

Detection of NoV in environmental waters grossly contaminated by wastewater have been reported (Ref: Pusch *et al.* 2005). However, it is difficult to assess dilution of NoV levels in environmental shellfish waters as:

- *Analytical Methodology* - It is difficult to use RT-PCR techniques consistently and effectively on a water matrix as suspended solids levels vary and no standard methodology exists.

- *Analytical Detection* - The potential water quality level which could give rise to levels in shellfish sufficient to infect a consumer are potentially so low it is not currently possible to detect with existing techniques (see Section 5.1.2).
- *Variable NoV Loading* - As NoV loading is so variable it is hard to successfully target sampling at times of peak NoV loading.

In view of difficulties in directly measuring NoV dilution in environmental waters it is more effective to use shellfish flesh as an indirect bioindicator of NoV uptake. Section 4.4 provides a series of site specific illustrations of how shellfish NoV quality varies in relation to wastewater discharge proximity. These case study examples offer differing environmental transmission pathways will yield shellfish NoV levels that are a result of physical dispersion and environmental degradation.

3.5 NoV Environmental Degradation

Viability is the key unresolved issue which undermines the use of the standardised RT-PCR tools generating uncertainty over the measurement of infectious or non-infectious NoV (Section 3.1.3). A number of different processes occur in the natural environment which can degrade both the surface features of NoV (which can impact its ability to attach to host cells) and NoV genomic features (which can impact its ability to infect host cells). The principal mechanisms by which NoV and other enteric viruses maybe degraded and/or removed from the water column are considered in the following sections.

3.5.1 Dark Deactivation

Micro-Ecology

Micro-ecology of viruses, bacteria, protozoa and metazoa and the inter-related food web relationship between these components and the larger multi-cellular detritivores which consume them is a subject in its own right. Within this micro-environment NoV is potential food for some bacteria and subject to enzyme secretions which can break down the capsid and inactivate the virus. The relationship between bacteria, protozoa, metazoa and their predation upon viruses was explored within Activated Sludge using a poliovirus model (Ref: Kim and Unno, 1996). Within natural sediments organic, nutrient content, Redox, pH and temperature will all influence bacterial activity and hence viral degradation. Furthermore, these sediment characteristics will also affect the degree of assimilation through predation by higher protozoa and metazoa. In the 1980s a number of researchers, primarily working with bacteriophages, to assess the decay of viruses resulting from microbial processes before the relative magnitude of solar influence was established.

Significance Relative to Solar UV

Flagellate driven decay of viruses using bacteriophage was determined at 0.15 h^{-1} when compared to decay rates in full sunlight of 0.4 to 0.8 h^{-1} showing that solar UV deactivation processes are likely to be more significant in summer (Ref: Suttle and Chen, 1992). Researchers looking at the balance of solar, chemical and biological influences within a range of different water bodies considered that the balance of dominance would vary according to the setting (Ref: Murray and Jackson, 1993) such that viral mortality in humic-rich coastal waters biological effects could be the most significant factor. Whilst humic-type substances may dominate solar UV absorption (Ref: Vantrepotte and Melin, 2006) suggesting lower solar UV efficacy, they may also have an important photosensitiser role thereby increasing disinfection (Ref: Suttle and Chen., 1992). Some studies have shown that increased salinity of the estuarine simulations also provided higher inactivation rates than the equivalent freshwater conditions (Ref: Sinton *et al.*, 2002), although it is hard to know to what degree these are a function of matrix/solar interactions.

Superficial Sediments

NoV inactivation by solar UV is reduced once suspended solids have settled to seabed sediment. Once deposited it is probable that although sediments may act as a microbial reservoir only the superficial recently deposited sediment pose a significant risk if resuspended as deeper longer buried sediment is less likely to retain viable viruses. This was demonstrated by Rao *et al*, (Ref: 1984) who showed that viable enteroviruses were present in 47% of fluffy superficial sediments as opposed to just 5% in compact bottom sediments.

Use of Viral Surrogates

The complexity of this subject is shown by the variation in decay performance for various viral surrogates. This is further demonstrated by the variable relative contribution of dark and light decay even between different strains of bacteriophages (Ref: Noble and Fuhrman, 1997) – which also highlights some of the dangers in the use of NoV surrogates. Whilst exact deactivation rates may vary between NoV and surrogates their use can perhaps give an indication of which parameters may impact upon deactivation processes. MNV surrogate studies (Ref: Lee *et al.*, 2008) have provided a good insight into environmental degradation processes with benchtop studies using stool suspensions on both temperature and salinity effects. Incubation at both –20℃ and 4℃ showed <2 $\log_{10}$ reduction in 40 days as compared to a >5 $\log_{10}$ reduction after incubation at 30℃ in 24 days. This study also showed that higher salinities are likely to provide a greater reduction in NoV infectivity with <0.3-, 1.5-, and 2.5 $\log_{10}$ reductions for distilled water and 0.5 (near full salinity of seawater) and 1 M NaCl respectively after 72 hours. However, it should be noted that when culture results are compared against those from molecular techniques then, as may be expected, the plaque-forming units (pfu) indicated inactivation, whilst the RT-PCR showed no significant change.

In summary, environmental degradation of viruses in the marine environment is an extremely complex subject. Decay rates have both light and dark components which vary considerably on a site specific and seasonal basis. Furthermore, suspended solids greatly confound investigations as they both adsorb viruses and reduce light based decay rates. It is unknown to what degree NoV bound to sediments may contribute to shellfish contamination through sediment re-suspension or uptake through filter feeding processes.

3.5.2 Sunlight Deactivation

Sunlight deactivation is likely to be a significant component in the reduction of NoV viability within many marine environments and as such solar influence probably dominates viral decay rates in most oyster production areas.

Deactivation Mechanisms

Sunlight is the natural equivalent of the UV disinfection discussed in Section 3.3.3. The principal differences between sunlight and UV are that UV systems operate:

- *Specific wavelength - whereas sunlight is a broad spectrum.* Natural UV light provides a different spectral output from UV lamps which are designed to optimise efficacy with peak output at 254nm.
- *Constantly dose - whereas sunlight varies*. Natural sunlight disinfection has often been cited as one of the major differences between UK and Mediterranean bathing water settings. Sunlight disinfection efficacy varies according to season, latitude, depth, water quality (suspended solids, organic compounds) and air quality

(weather, ozone levels). In essence, sunlight cannot be relied upon on a stormy winter's day.

Figure 3.5 shows the interplay between increasing genomic damage potential at lower wavelength and increasing intensity with longer wavelengths to yield a maximum solar impact at ~300nm. Flannery *et al.* (Ref: 2013) highlighted the use of longer wavelengths 290nm to emulate natural conditions.

Figure 3.5: Interaction between solar UV spectrum and DNA damage
(Source: reviewed by Ref: Murray and Jackson, 1993)

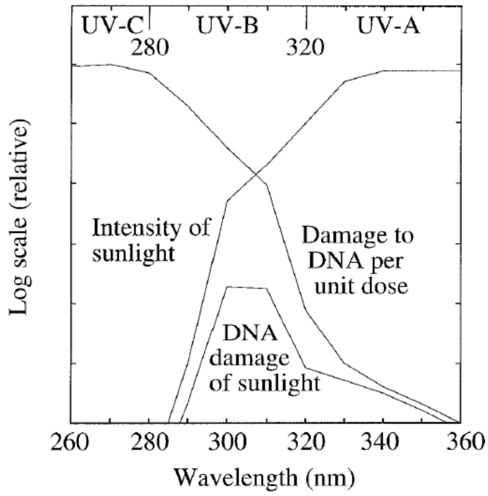
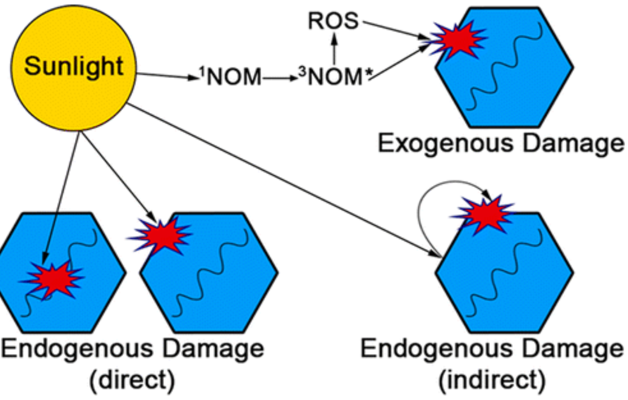


Figure 3.6: Illustration of sunlight viral inactivation mechanisms
(***Source: Ref: Silverman et al., 2013)***



NOM = Natural Organic Matter

Silverman *et al.* (Ref: 2013) recently put forward a more complex array of potential UV mediated viral deactivation mechanisms (Figure 3.6). In addition to the traditional direct endogenous impact on either viral capsid or genome an exogenous process is also presented. The three main deactivation mechanisms are:

- The absorption by solar UV-B causing direct DNA damage by pyrimidine dimer formation. The process is independent of oxygen and other marine environmental conditions.
- The absorption of UV-B and some shorter wavelength UV-A by cell constituents including DNA (called endogenous photosensitisers). The activated constituents react with oxygen to form highly reactive photo-oxidising species that damage genetic material within the viral particle.

- The absorption of a wide range of UV and visible wave lengths in sunlight by extra-cellular constituents of the marine environment (exogenous photosensitisers- notably humic material). The activated photosensitisers react with oxygen to form highly reactive photo-oxidising species. These damage the capsid host-binding particles on viral particles.

As can be seen part of the light decay mechanism is dependent on photosensitisers and as such is directly linked to water quality parameters. UV penetration of seawater is a field of research in its own right and will not be reviewed in this document although consideration of this issue may require further attention if modelling of deactivation rates is required (Section 5.2.5).

Rates of Deactivation

Decay rates are defined by T_{90} - the time required for 90% (i.e. 1 log_{10}) of the target organism to become inactive. Unfortunately, whilst most viral surrogates can be enumerated through culture techniques to indicate infectivity NoV analysis by RT-PCR cannot assess viability, hence most work on viral deactivation uses viral models such as F+bacteriophage as a surrogate.

Sinton *et al*. (Ref: 2002) undertook a series of dark and light inactivation tank tests using blended wastewater effluent in fresh and saline waters using a range of bacterial and bacteriophage microbial indicators. In all cases light conditions had greater inactivation rates than dark conditions (F+bacteriophage T_{90} light ~1day (27.5hr) and T_{90} dark of 6-7 days (165hr)). Similarly, work by Noble *et al.* (Ref: 2004), provided F+coliphage T_{90} of ~2 days for high light intensity conditions as opposed to ~4 days under lower light conditions. The magnitude of viral decay due to solar influence has led researchers to predict a strong daily signal in the concentration of infectious viruses with the assessment that a large proportion of the viruses in seawater are probably not infective (Ref: Suttle and Chen, 1992). This could be an important consideration when assessing shellfish RT-PCR results.

Recent work by the Marine Institute in Ireland under the STRIVE programme (see also Section 3.3.1) included work to assess NoV survival in the marine environment and is reported both in the scientific literature (Ref: Flannery *et al*., 2013) and within internal reports (Ref: Dore *et al*., 2013). The UV output reported in (Ref: Flannery *et al*., 2013) focussed upon fluence evaluation, whereas the STRIVE report provided T_{90} values of 14min and 2 hours under summer and winter respectively. It should be noted that beyond the uncertainties of using a bacteriophage viral model to estimate NoV performance this study used 'free' viral particles with very low turbidities reported. In consequence, this data is unlikely to represent performance in the natural environment. As indicated in Section 3.3.1 NoV related particles within wastewater discharges are readily adsorbed to suspended solids which shield them from UV degradation thereby extending T_{90} duration.

Dancer *et al.*, (Ref: 2010) undertook decay experiments using 'naked' NoV RNA in seawater under emulated North European winter temperatures and solar intensity (8℃, 1mW/cm^2 UV radiation). These results showed NoV was detected by qRT-PCR for up to 14 days with a genomic T_{90} of 141hr (5-6days). This study also incorporated a bioaccumulation component which showed limited shellfish uptake under the test conditions. This led the authors to conclude that although naked NoV RNA could persist in the environment its impact on shellfish flesh quality during winter conditions may be limited – suggesting that observed contamination levels might be a result of accumulating intact or bound NoV. Ideally, this data would have been compared against the breakdown of intact NoV, or NoV associated particles. Work output from Ifremer used RT-PCR data modelled NoV decay with a genomic T_{90} of 30 days (Ref: Pommepuy *et al.*, 2004) suggesting extended environmental persistence.

Viability – Difficulty in Assessing Decay in NoV

As with considerations of UV disinfection within wastewater treatment works (see Section 3.3.3) the principal research challenge with NoV decay in the environment relates to ascertaining viability.. Section 3.1.3 considers the difficulty in assessing viability which remains one of the principal problems in assessing NoV risk within environmental samples.

There are differing views within the scientific community as to how well RT-PCR viral data reflects infective risk. Laverick *et al.* (Ref: 2004) stated "*the NoV capsid is environmentally robust, it is likely that any naked RNA would be quickly inactivated…Hence detection of NoV cDNA in a sample strongly suggests that it can only have come from an infectious virion.*" This perspective has been supported by some other workers (Ref: Dancer *et al.*, 2010) who have defended the use of RT-PCR as being indicative of viable NoV. This was attempted by demonstrating through a combination of T_{90} decay and bioaccumulation experiments the relatively limited GII uptake of 'naked' NoV RNA compared to that of faecally derived NoV particles suggesting only intact (hence viable) NoV is taken up by shellfish.. In contrast, other researchers (Ref: Flannery *et al.*, 2013) have stated that RT-PCR significantly overestimates the survival of infectious virus and is therefore unsuitable for determining inactivation rates of viruses in seawater.

It is suggested that it would be useful to differentiate decay rates for viability from genomic integrity. The computer modelling work considered in Section 5.3.3. developed the use of a *viability* T_{90} decay rate alongside a parallel *genomic* T_{90} where NoV particles decay from one form to the other before eventually being undetectable by RT-PCR.

Variation in Decay Rates

A number of researchers have highlighted a differential between bacterial indicator and viral inactivation rates with longer persistence of viruses (as reviewed in Ref: Hijnen *et al.*, 2006). Another research challenge is that differential rates can be obtained from tank studies which can be criticised as being unrepresentative of the real world. Conversely, field studies may be problematic due to the uncertainty that measured values are solely the result of the target discharge. Waste Stabilisation Ponds (WSPs) do however provide a good indication of large scale processes for controlled water bodies receiving wastewater discharge (Ref: Sinton *et al.*, 2002). WSPs are a low technology tertiary treatment system commonly used in many countries including Australia and New Zealand were land use footprint is not such a critical limitation as for other developed countries. Comprehensive WSP studies on Christchurch WSP (Ref: CH2M Beca, 2009) is summarised in Table 3.3 below and demonstrates a markedly lower inactivation rate for viruses than indicator bacteria.

Table 3.3: Illustration of differential bacterial and viral inactivation rates in quasi-natural decay setting (from Waste Stabilisation Ponds)

(Source: Ref: CH2M Beca, 2009. n=6)

Group	*Parameter*	*Reduction Factor (log_{10})*
Bacterial Indicators	Faecal coliforms	2.47
	Enterococci	2.45
Viruses	Enteroviruses	0.38
	FRNA Coliphage	0.58

However, even different viruses can produce quite different decay rates under different conditions. A range of viruses have been studied including poliovirus type 3 (PV3), adenovirus type 2 (HAdV2), and two bacteriophage (MS2 and PRD1) which all behaved differently between each other and between different matrices (Ref: Silverman *et al.,* 2013). Caution must be exercised in the adoption of T_{90} data for NoV from viral surrogates.

Influence of Suspended Solids

As natural sunlight UV disinfection is a significant process influencing NoV decay, the degree of suspended solids adsorption/shielding from UV disinfection is likely to be of paramount importance. Solar inactivation rates appear to be reduced in more turbid waters due to reduced solar transmissivity (Ref: Chung and Sobsey, 1993). In consequence, mixing dynamics in allowing UV degradation throughout water column by allowing water to turn over is thought to be important influence for light penetration at some sites (Ref: Murray and Jackson, 1993). For example, thermal stratification in the water column could allow effective depletion of NoV at the surface but provide less decay at depth. In contrast, other researchers looking at a number of indicator organisms under a range of salinities, failed to find a significant influence of Total Suspended Solids (TSS) levels on light decay rates (Ref: Noble *et al.*, 2004). Walters et al, (Ref: 2013) working with FIOs noted not only a strong relationship between suspended solids concentration and UV efficacy but also a marked association with smaller particles ≤12 µm (suitable for shellfish – Section 3.6.2) associated with 91% and 83% of *E. coli* and enterococci. NoV interaction with suspended solids is considered further in the following sub-section.

In summary, solar UV deactivation processes will influence both genomic decay and viability decay within the marine environment. Decay rates will vary on a temporal and seasonal basis. Viral surrogates suggest a T_{90} of ~4-6 days although the lack of a NoV viability test makes it difficult to assess this feature. Section 5.3.3 considers the use of a computer model tool to allow sensitivity analysis for decay processes by allowing input of various T_{90} values for viability and genomic decay.

3.5.3 Sediment Adsorption

There is considerable data within the scientific literature describing viral associations with a range of particle types. This association can have a significant bearing on both physical removal mechanisms from the water column and *in-situ* environmental degradation processes. Despite research on sediment adsorption for both indicator microbes and pathogens has been relatively little work on NoV – as such much of the review in this section relates to other viral models. A good review of the survival of enteric microbes in aquatic sediments which covers much of the early viral sediment research is provided by Weaver and Sinton, (Ref: 2009).

Viral association with solids in the aquatic environment has been studied with enteroviruses obtained from turbid marine water (Ref: Sobsey *et al.*, 1977). Various virus-sediment associations have implications for both differing bond strength and potential for shellfish uptake.

There is potential for viruses to adsorb onto a range of different particle types. Each material offers differing levels of surface binding and stability as demonstrated for kaolin (clay), cellulose and carbon black (representing organic detritus) (Ref: Sakoda *et al.,* 1997). Kinetic studies of virus adsorption and inactivation indicated various types of virus-surface interactions for different materials ranging from quasi-equilibrium to direct irreversible adsorption (Ref: Grant *et al.*, 1993). Comparative viral : solids adsorption studies (Ref: Moore *et al.,* 1975) have highlighted virus specific characteristics which suggests that there is a need for new research focussed on NoV.

Principal associations considered include:

- Clay Particles
- Organic Detritus
- Plankton

Clay Particles

Fine clay particles may adsorb viruses although gill selection within shellfish is likely to eject material as pseudofaeces although there maybe scope for viral retention within the mantle or upon the gills.

Clay particles have long been recognised as an effective means of removing virus from water. Early work (Ref: Carlson *et al.*, 1968) termed this viral removal with clay as 'inactivation', although the mechanism was more likely to have been adsorption. It was soon recognised that the adsorption of viruses onto sediment could enhance survival in the environment (Ref: Gerba and Schailberber, 1975) and still allow viruses to remain infective (Ref: Schaub and Sagik, 1975). Hamblet *et al.* (Ref: 1969) working with Poliovirus (PV) undertook some comparative tests on viral removal using clean and turbid seawater (300mg/L of silt) and found that salinity had no impact on PV removal in clean water or for turbid fresh water, whilst there was around x100 fold reduction in PV for brackish (9ppt and 18ppt) turbid water. The researchers concluded that the PV only adsorbed onto the silt under certain salinities which is consistent with our current understanding of flocculation processes in an estuarine environment.

Organic Detritus, Cation and pH Influence

As with clay, organic detritus has been shown to adsorb viruses, although it is uncertain to what degree material would be passed into the pseudofaeces or taken into the gut for digestion. The chemical influence on sediment : viral adsorption is important in both aqueous and groundwater environments. Dissolved organic content, pH, cation concentrations and iron oxide coatings have a complex inter-relationship which determines whether viruses are adsorbed or desorbed from particles.

The impact of organic content of sediment and the presence of dissolved organic matter on viral adsorption seems to vary according to the type of organics and the physiochemical environment. In early work researchers demonstrated that despite effective adsorption with >99% enteric viral removal with estuarine sediment soluble organic levels resulting from secondary treated wastewater did not influence adsorption (Ref: LaBelle and Gerba, 1979). Furthermore organic levels were not shown to enhance viral survival (Ref: LaBelle and Gerba, 1982). More recently the reaction kinetics between bacteriophage binding to silica particles and the influence of dissolved organic matter and cation concentrations have been extensively studied by the research group at Newmark Civil Engineering Laboratory (Refs: Yuan *et al.,* 2008; Pham *et al.,* 2009). These studies have shown that the impact of organic compounds is influenced by the presence of cations and pH. Whilst organic compounds can limit binding through steric interference the presence of high divalent Ca^{2+} levels allows bonding between organic coating and viral capsid via cation bridges to carboxylate groups. However, the exact impact of organic compounds on particle : viral adsorption has been shown to differ between groundwater types and is thought to be influenced by the chemistry of the compounds involved (Ref: Gutierrez and Nguyen, 2013). Viral association with iron oxide coated sediments have also been noted (Ref: Murray and Parks 1980).

Cations at low pH have been shown to have little impact on viral aggregation or adsorption. However, when pH levels are greater than the virus isoelectric point (> neutral) cation concentration is important with divalent cations greater impact than mono-valent (Refs: Floyd and Sharpe., 1978; Wong *et al.*, 2012) – although even the cation type is important with Ca^{2+} having significantly more influence than Mg^{2+} (Ref: Mylon *et al.*, 2010). Each virus has a unique isoeletric point, even with differences between GI (~pH 6) and GII (pH 5.5-7) NoV (Ref: Goodridge *et al.*, 2004). In summary, pH and water hardness are likely to have a significant impact on virus adsorption characteristics in groundwater, riverine water and seawater. This has a bearing upon NoV behaviour in the aquatic environment after release from a wastewater discharge or from a diffuse groundwater septic tank discharge (Section 6.3.1).

Plankton

Plankton includes free drifting bacteria, phytoplankton and zooplankton, all of which are potential food particles for assimilation within filter feeding bivalves such as oysters. Gentry *et al.* (Ref: 2009) has provided one of the few environmental sampling studies where RT-PCR NoV levels in shellfish have been related to NoV levels in associated plankton. A key difficulty is the lack of established effective analytical methods for environmental matrices Gentry *et al.* (Ref: 2012).

Whilst adsorption of viruses onto plankton could influence shellfish uptake there is also evidence to suggest that living phytoplankton are unlikely to behave in a fashion similar to organic detritus and could actively deactivate viruses. Laboratory studies with sterile sewage with cultures of either algae or bacteria had no impact on poliovirus survival, whilst a mixed heterogeneous culture obtained from Waste Stabilisation Ponds provided appreciable inactivation (Ref: Sobsey and Cooper, 1973). Furthermore a number of recent studies have taken place to isolate virucidal polysaccharides produced by marine algae which have been shown to be effective against a number of viruses ranging from macrophyte extract effective upon herpes simplex virus (Ref: Harden *et al.*, 2009) to a marine diatom based sulphated polysaccharide with antiviral properties against herpes simplex, influenza and inhibitory effects on HIV (Ref: Lee *et al.*, 2006).

There is little information on this subject in the literature particularly with respect to NoV in this potential vital area of study. Recommendations are provided in Section 8.1.

Sedimentation and Resuspension

Once NoV has been associated with suspended solids there is a potential to be removed from the water column with settlement as sediment to the seabed. It has long been demonstrated that faecal coliforms and enteric viruses associated with wastewater discharges have been found in sediments in the vicinity of the outfall implying settlement and removal from the water column (Refs: Loutit and Lewis, 1985; Lewis *et al.*, 1986). As sedimentological processes may act as a net 'sink' to NoV with settlement at times of reduced current velocity stripping NoV from the water column (in a manner similar to the removal processes within WWTPs). Once associated with sediment NoV may be removed from 'sunlight deactivation' processes (Section 3.5.2) but subject to 'dark deactivation' processes (Section 3.5.1).

Enteroviruses were found to have a much higher incidence when within suspended solids samples (72% of samples) relative to that of estuarine waters (14% of samples) (Ref: Rao *et al.*, 1982). This suggests that within the marine environment viral adsorption to solids is a significant process which could have both positive and negative implications in both the water column and deposited sediments, as summarised in Table 3.4.

Table 3.4: Positive and negative impacts of viral adsorption

Phase	Negative	Positive
In Water Column	Viruses adsorbed onto suspended solids maybe more readily take up by shellfish	Viral levels likely to be reduced more rapidly as they are 'stripped' via adsorption to solids from the water column in a fashion similar to that found within a wastewater treatment process.
In Sediments	If adsorbed viruses are deposited to bottom sediments there is a potential for resuspension thereby providing a possible *in situ* 'source'.	Once associated with suspended solids there maybe good potential for deposition in bottom sediments which may remove viruses to a phase where dark deactivation microbial mediated process may inactivate them providing a 'sink'.

The 'protective' influence of sediments upon viral survival has been demonstrated both in the laboratory and in the field from a number of studies. These include 'thermostabilisation' (protection for elevated temperatures) (Ref: Liew and Gerba, 1980) and salinity and microbial moderated decay (Ref: LaBelle and Gerba, 1982). Enhanced polio virus survival due to sediment association was demonstrated by *in situ* studies at both polluted and unpolluted sites which exhibited reduced deactivation with T-99 (time for 99% deactivation) increasing from 1.4 - 6 days and 1hr to 4.25 days respectively. Enterovirus was also shown to survive for an extended period when adsorbed to sediment with infectivity increased from 9-19 days (Ref: Rao *et al*., 1984. Although sediment association enhances viral survival there appears to be differential rates for pathogens and phages at different temperatures and mixtures (Ref: Chung and Sobsey, 1993).

Although it is well recognised that sediment adsorption enhances microbial survival through the shielding from UV inactivation (Section 3.5.2) for both faecal coliform indicators and viruses with a positive correlation found by LaBelle *et al.* (Ref: 1980). The nature of the sediment is known to influence relative microbial survival with increased organic matter and nutrient content associated with wastewater thought to enhance faecal coliform survival (Ref: Gerba and McLeod, 1976). Enhanced survival in sediments contaminated by wastewater was also demonstrated for viruses by Smith *et al.* (Ref: 1978). This less hostile environment gives rise to the potential for sediments to act as environmental microbial reservoirs with elevated stocks of both indicator organisms and pathogens at levels orders of magnitude higher than the overlying water (Refs: Gerba *et al*., 1977; Goyal *et al*., 1978).

Whilst sediment under most conditions may act as a ‘sink’ there is scope for it to also occasionally act as a ‘source’ when resuspended. Release of NoV from sediment back into the water column may occur during energetic storm conditions as a result of increased current velocity (e.g elevated riverine flows) or with increased wave resuspension (e.g elevated wind speeds). Physiochemical conditions within the sediment (such as anoxic conditions) may lead to desorption of NoV from sediments. Resuspension with associated desorption of viruses and bacteria is likely to be a significant *in situ* source of contamination which although well studied as a process in tank tests has been little studied in the environment. Some early work on faecal coliforms resuspension through dredging river sediments was reported by Grimes (Ref: 1975). Laboratory studies highlighted that the chemical nature of the sediment (such as the presence of organic matter) was important to assess the potential for viral desorption (Ref: Gerba and Schailberber, 1975).

The potential health risk from resuspension of elevated microbial levels from a sediment based reservoir was also recognised from this early time (Ref: Matson *et al.,* 1978). Some researchers postulated that increased bacterial contamination levels in oysters may have been as a result of resuspension following rainfall events which were associated with increased turbidity levels. However, the strength of this relationship for FIOs did not correlate well with enterovirus shellfish flesh quality suggesting a more complex relationship between bacteria, viruses and sediments (Ref: Goyal *et al*, 1979). The potential for bioaccumulation of sediment associated viruses in oysters and clams was demonstrated by Landry *et al.* (Ref: 1983) who showed that contact with virally contaminated sediment had relatively little impact on shellfish flesh quality, whilst resuspended sediment was bioaccumulated. Despite this there is little published in recent years to ascertain the impact resuspension may have upon both Shellfish Hygiene Directive compliance for *E. coli* and public health risks from NoV.

In summary, there is considerable evidence within the literature that sediment adsorption is a highly significant process which will affect the behaviour and fate of NoV released from wastewater discharges – however, there is no substantive quantitative data to help evaluate the magnitude of this impact. It is possible that sedimentation of adsorbed NoV may remove and protect NoV in a seabed reservoir where dark deactivation processes may help inactivate NoV. Whilst this reservoir may also represent a potential resuspension risk it is probable that the resultant increase of genomic concentrations will not necessarily be wholly viable. Storm periods are likely to present an increased threat for reduced shellfish quality from both a bacterial indicator and public health NoV perspective.

3.6 NoV Bioaccumulation

Bioaccumulation is not a fixed magnification from the NoV content within the water column (i.e a water quality concentration) to NoV within oysters (i.e. a flesh quality concentration). Shellfish biological requirements will have a significant bearing on this relationship as outlined below:

- Water quality (Section 3.6.1) will influence whether oysters open their valves for oxygen exchange
- Food quality (Section 3.6.2) will determine what type and quantity of particles are filtered and consumed.

- Binding capacity between oyster gut and viruses (Section 3.6.3) influences the degree to which NoV may be retained.

Shellfish bioaccumulation factors (Section 3.6.4.), although widely studied over the years are poorly characterised with respect to NoV in oysters. The following sub-sections reviews literature on these issues.

3.6.1 Shellfish Uptake Potential – Water Quality Requirements

Bivalve shellfish as living organisms have species specific thresholds of temperature, salinity and dissolved oxygen which will dictate whether they open up and filter feed or shut their valves and wait for conditions to become tolerable. The capacity of bivalve shellfish to temporarily isolate themselves from unfavourable water quality conditions allows them to survive in the dynamic changing estuarine environments. This capability will also have a profound influence on oyster access to differing levels of microbial contamination. For example, in a drying estuary there is a considerable change in salinity between HW and LW and as microbial loads are often associated with riverine sources this will often translate to reduced microbial water quality at LW. Clearly, on a dropping tide as salinity drops (and often microbial quality deteriorates) there will come a point where shellfish feeding rates drop and eventually stop as oyster shut up until the following flood tide brings increased salinity (and potentially improved microbial quality). In consequence, the water quality profile for Shellfish Water does not necessarily reflect the conditions when oysters are activity feeding and open to bioaccumulation.

Different bivalves, and indeed individual oyster species, have unique biological requirements as shown by the depuration requirements for oysters in Table 3.5.

Table 3.5: Physiochemical biological requirements for depuration of oysters

	Pacific Oysters	Native Oysters
Temperature	8-18℃ *(Note 1)*	5-15℃ *(Note 1)*
Salinity	>20.5 ppt	>25 ppt
Dissolved Oxygen	>5mg/l	>5mg/l

Note 1: Depuration limit for lower temperature threshold, 'Conditioning' guidance for upper temperature threshold

As can be seen from Table 3.5 whilst both oyster species are happy in full salinity seawater the pacific oyster is much more tolerant of lower salinity estuarine conditions. Cefas have recently reviewed biological requirements with respect to chronic microbial contamination (Ref: Kershaw *et al.*, 2012) which focussed upon accumulation and clearance rates of FIOs in a range of shellfish species. This source summarises that Pacific oysters have a salinity range from 20-35ppt with an optimum of 25ppt. In contrast, the native oyster operates in 24-35ppt with feeding rate decreasing below 28ppt and ceasing at 16ppt.

The native oyster actively filter feeds at a lower temperature regime to that of the Pacific oyster which is a warmer water species. Although both oyster species can survive outside of the optimum depuration thresholds outlined in Table 3.5 uptake performance is likely to be impacted. The impact of species specific temperature requirements on microbial uptake was illustrated by Bernard (Ref: 1989) who undertook a series of bioaccumulation experiments on clams, mussels and oysters and assessed faecal coliform uptake rates at different temperatures. The output for oysters shown in Table 3.6 below highlights the most rapid uptake at 12℃ with a considerable reduction in uptake rates at 7℃.

Table 3.6: Impact of temperature upon faecal coliform bioaccumulation rate in Pacific oysters ***(Source: Ref: Bernard, 1989)***

Temperature	17℃	12℃	7℃
Time (hh:mm) to reach 300 FC/100g	01:16	00:41	03:08

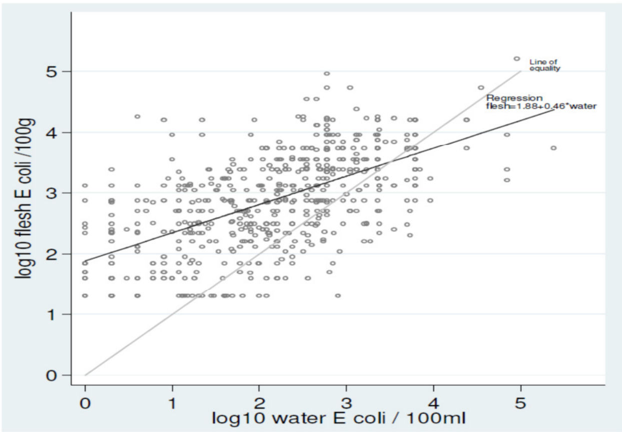
Dissolved oxygen levels rarely drop below biological requirements, although this can be possible at some estuarine sites following an algal bloom where elevated temperatures and increased biological demand overnight can lead to oxygen sag.

Kershaw *et al.* (Ref: 2012) consider FIO accumulation to be a function on pump filter capacity which in turn is influenced by biological features which influence feeding/filtering rate. This review highlighted that rapid uptake of FIOs can occur within 30 minutes of contaminant exposure. Unlike FIOs viral association and NoV binding in particular suggest a somewhat different equilibrium model.

Cefas have also recently analysed the relationship between the microbial quality of shellfish flesh and waters (Ref: Campos *et al.*, 2011), which also considered the interplay between the different biological requirements of shellfish species. This report analysed the relationship between these data sets from six production areas (Figure 3.7a) and concluded that only 18% of the variance in oyster flesh quality was as a result of changes in microbial water quality.

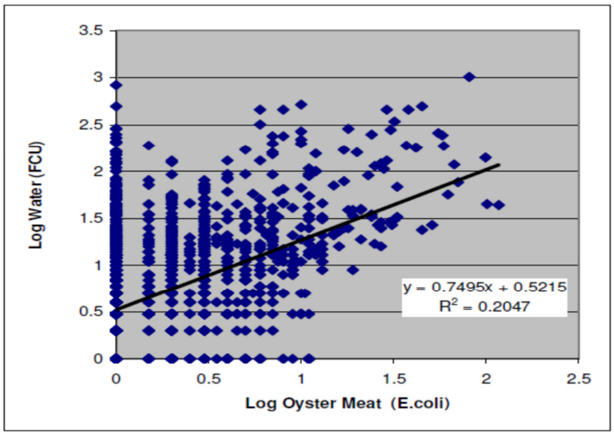
Figure 3.7: Scatterplot of *E. coli* levels in shellfish flesh and seawater.

a) (Source: Ref: Campos et al., 2011)



N= 602 paired data from 6 UK Production Area (all species)

b) (Source: Ref: Ogburn and White, 2009)



N = 1875 paired data from 5 NSW Production areas (oysters)

Regulatory shellfish water and flesh data from NSW from 5 catchment areas over a 5 year period was also used to perform comparative analysis (Ref: Ogburn and White, 2009). This study showed that not only was there a poor correlation between the two paired data sets (Figure 3.7b) but that the water test correlated with rainfall event impacts and as such was a better tool to assessing Conditional Status and concluded that water quality testing was a more meaningful regulatory tool than that of *E. coli* in shellfish flesh.

Whilst species specific selective operating requirements may account for some of this variation it should also be noted that differences in regulatory sampling regime will also impact upon the data set. In some catchment cases sampling mismatches occur between the two regulatory programmes (e.g obtaining shellfish flesh samples at LW and water quality samples at HW), although even synchronised sample programmes might struggle to take account of stratification which can provide different near-surface *water* quality from seabed *shellfish* quality. This could be particularly profound during rainfall events where riverine sources, often with reduced microbial quality, may form a salt wedge with cleaner high salinity seawater immersing shellfish on the seabed.

In summary, different biological requirements for shellfish species can have a profound influence on microbial uptake in response to physical conditions. The complexity of this interplay has been hard to determine for the relatively well understood uptake of Faecal Indicator Organisms (FIOs) such as faecal coliforms and *E. coli* but is even more complex for NoV.

3.6.2 Shellfish Uptake Potential – Food Quality Requirements

Assimilation of microbes and NoV in particular is likely to be strongly influenced by whether contaminants are 'free' within the water column or adsorbed upon potential food particles (Section 3.5.3) which maybe actively entrained. The composition and size of potential food particles will have a bearing on potential uptake and bioaccumulation:

- *Clay Particles.* Fine clay particles may adsorb viruses although gill selection within shellfish is likely to eject material as pseudofaeces although there may be scope for viral retention within the mantle or upon the gills. Silt particles range from 3.9-63 µm, whilst clays although <3.9µm and therefore below the optimum feeding uptake size can flocculate to form a larger 'apparent' particle size. Both inorganic particles are therefore within oysters' size sorting range. Hamblet *et al.* (Ref: 1969) studied accumulation and depuration of poliovirus (PV) in the Eastern oyster (*C. virginica*) which showed that viral uptake was retarded by increased turbidity. In this case the researchers showed that the PV was adsorbed to the silt under brackish salinities leading them to postulate that the inorganic silt was rejected in pseudofaeces and not retained as potential food by the oysters. In contrast, Metcalf *et al.* (Ref: 1979) also using PV found that kaolinite in bioaccumulation experiments on clams increased the level of viral uptake in tissues and the hepatopancreas in particular.
- *Organic Detritus.* In the right physiochemical environment organic detritus can adsorb viruses, although as discussed below it is uncertain to what degree material would be passed into the pseudofaeces or taken into the gut for digestion. Bioaccumulation tank tests using PV on clams and a range of suspended solids types showed an increased viral uptake with faecal matter relative to that of kaolinite suggesting a differential selection and retention mechanism for particles with adsorbed viruses.

- *Plankton.* Plankton includes free drifting bacteria, phytoplankton and zooplankton all of which are potential food particles for assimilation within the digestive tract of filter feeding oysters.

A recent review was undertaken on behalf FSA to review bivalve particle selection (Ref: Beecham, 2008). The same search criteria and database was used to build on this review to encompass recent development from 2008-2014.

Bivalves are well known for their capacity to filter large volumes of water and strip out suspended solids. Industrial water cleaning experiments have been conducted in China (Ref: Zhang *et al.*, 2011) which showed that pacific oysters were capable of being used to pre-filter abstracted water for aquaculture removing 1.08-1.32g/ind/day of suspended solids at optimum temperatures. This capacity to filter particles is so efficient that oyster reefs have been recognised for the provision of ecosystem services for the improvement in water quality in the filtration of solids and nutrients. Significant levels of seston removal has been recognised from field experiments with *Crassostrea virginica* reef studies showing both a reduction in planktonic nutritional quality (Ref: Powell *et al.*, 2012) and in chlorophyll a levels (Ref: Grizzle *et al.*, 2008). Considering viral adsorption onto suspended solids this represents a significant potential for oyster to bioaccumulate NoV.

The differentiation of food and non-food particles between the gut and rejected in pseudofaeces is likely to have an important impact on the potential for NoV uptake and retention in the gut. Planktonic food sorting is determined by a number of variables:

- *Particle Size*. The review by Beecham (Ref: 2008) highlighted that feeding mechanism in bivalves is an active filter process which allows the sorting of nutritious organic prey from non-edible particles rejected in the pseudofaeces (Ref: Beecham, 2008). Particle size sorting takes place in a couple of stages with initial filtering on the gills before sorting by the palps on the labia. Whilst the 75 µm size of the principal filament would seem to suggest a physical constraint to particle size, larger particles of up to 300 µm have been recorded with the indication that this selection is behavioural. Size selection and rejection and its implications to biotoxin uptake were studied using different phytoplankton mixtures (Ref: Mafra *et al.,* 2009) demonstrating broad selection between diatoms and flagellates but less differentiation between diatoms although a size sorting still allowed removal of algal cells exceeding ~70 µm. Tank studies of retention efficiency (Ref: Zhang *et al.*, 2010) indicated a minimum particle size selection of 6 µm regardless of food quality (as tested by silt addition). However, whilst the capture individual particles <1 µm may have a limited retention efficiency of <15% it has been shown that >70% of these suspended particles maybe incorporated at certain times of the year with flocculation of small particles into larger aggregates allowing ingestion of particles far below this size threshold. Ward and Kach. (Ref: 2009) working with both mussels and the Eastern oyster demonstrated that even nanoparticles of 100nm can form aggregates that are >100 µm in size with significantly increased rate of shellfish uptake. Furthermore, 100nm nanoparticles had a longer gut retention time than 10-µm polystyrene beads suggesting different particle size based digestive pathways.

- *Particle Surface Features*. In the same way as biochemical binding of NoV influences viral uptake it has been demonstrated that biochemical recognition mechanism mediated by lectins also operates to help select particle sorting in bivalves (Refs: Emmanuelle *et al.*, 2009; Espinosa *et al.*, 2010; Rosa *et al.*, 2013). This mechanism was demonstrated by artificially mucus coating particles and phytoplankton to influence selection. The importance of gill selection on food processing has been established using endoscope-directed sampling to establish qualitative selection sites and the influence of seston quality (Ref: Beninger *et al.*, 2008). Gill selection efficiency was shown to be directly proportional to seston quality and quantity and is able to increase ingested food quality when environmental food quality is low and / or when seston concentrations are high.
- *Particle Composition and Availability*. Seasonal variation in oyster food quality and selection criteria has been well documented. Site based plankton studies from France (Ref: Lefebvre *et al.*, 2009) including Pacific oysters have shown temporal variations with strong reliance on phytoplankton during times of bloom abundance switching to more opportunistic behaviour over winter when phytoplankton is more limited. Similarly field studies from Australia on Pacific oysters (Ref: Bayne, 2009) showed a strong seasonal variation in C:N ratios with the suggestion that oysters have different nutritional requirements throughout the year (e.g. increased C:N ratio in November to boost glycogen for overwintering). Comparative uptake rates of chlorophyll a and organic detritus for different bivalve species have shown the capacity to increase the mire nutritious consumption of phytoplankton whilst saturation levels limit the input of organic detritus. These relationships have been

shown to be species specific and capable of modelling using the ShellSIM model (Ref: Hawkins *et al.*, 2013).

In summary, oysters have the potential to actively filter suspended solid particles of clay, organic detritus and phytoplankton with any associated adsorbed NoV. The potential for ingestion into the gut is likely to be influenced by the particle type, nutritional quality and seasonality. There is very little current NoV specific data on this issue, although this is within the current study area of the author.

3.6.3 Selective Binding of NoV within Oysters

Once NoV bound particles have been selected by the host oyster the potential for bioaccumulation of NoV will largely be a function of whether specific binding sites can retain NoV to limit its expulsion through the pseudofaeces or to be passed through the gut in the same fashion as FIOs. It has long been known that viruses bind to sites within the shellfish gut resulting in retention beyond the clearance rate for FIOs. Hay and Scotti, (Ref: 1986) working with the Pacific oyster used a radiographic method with a labelled picornavirus surrogate for human enterovirus to show that principal binding to mucus in the digestive tract and to a lesser degree within the digestive diverticula tubules and mid-gut tissues but with no binding to gills, mantle or other body tissues. Subsequent depuration for 64hr failed to remove the label from gut tissues suggesting incomplete removal through depuration.

Over recent years a number of studies have been able to study the specific biochemical mechanism which allows NoV to selectively bind with certain ligands both within the human host and the shellfish intermediate (Refs: Le Guyader *et al.*, 2006; Tian *et al* 2007; Imai *et al.*, 2011; Maalouf *et al.*, 2011; Le Guyader *et al.*, 2012). Prolonged retention of NoV in oysters despite extended depuration periods has been attributed to this binding capacity (Ref: Ueki *et al.*, 2007). Detailed studies have shown a differential anatomical association within oysters with NoV GI selectively binding to Blood Group A antigen like ligands in digestive gland tissues and NoV GII binding to sialic-acid containing ligand present in a wider range of gut, gill and mantle tissues (Ref: Maalouf *et al.*, 2010). Bioaccumulation experiments comparing potential uptake by oysters using intact NoV from faecal samples and free NoV RNA, also looked at differential uptake of GI and GII (Ref: Dancer *et al.*, 2010). This study exhibited higher concentrations of faecally derived GII than GI in digestive tissue, although GII from free NoV RNA was poorly taken up and showed markedly lower concentrations of (2.9 $\log_{10}$ difference) relative to GI. Comparative studies with a range of viruses have shown variation in bioaccumulation and retention between different oyster species (Ref: Nappier *et al.*, 2008) highlighting how specific differing mechanisms may vary uptake rates.

The differentiation in viral binding characteristics within specific shellfish species is likely to be a principal reason why some viral types are depurated much more rapidly than others. A number of researchers have demonstrated PV removal in <48hrs (Refs: Hamblet *et al.*, 1969; McLeod *et al.*, 2009), whilst elimination of NoV and HAV demonstrated no significant decrease in contamination over comparable depuration periods. The binding capacity of NoV in oysters is likely to be critical in the determination of potential Bioaccumulation Factor (BF). Although literature and tank studies have demonstrated the equilibrium profile for both FIOs and PV the equivalent data has not yet been generated for NoV.

3.6.4 Bioaccumulation Factors (BFs)

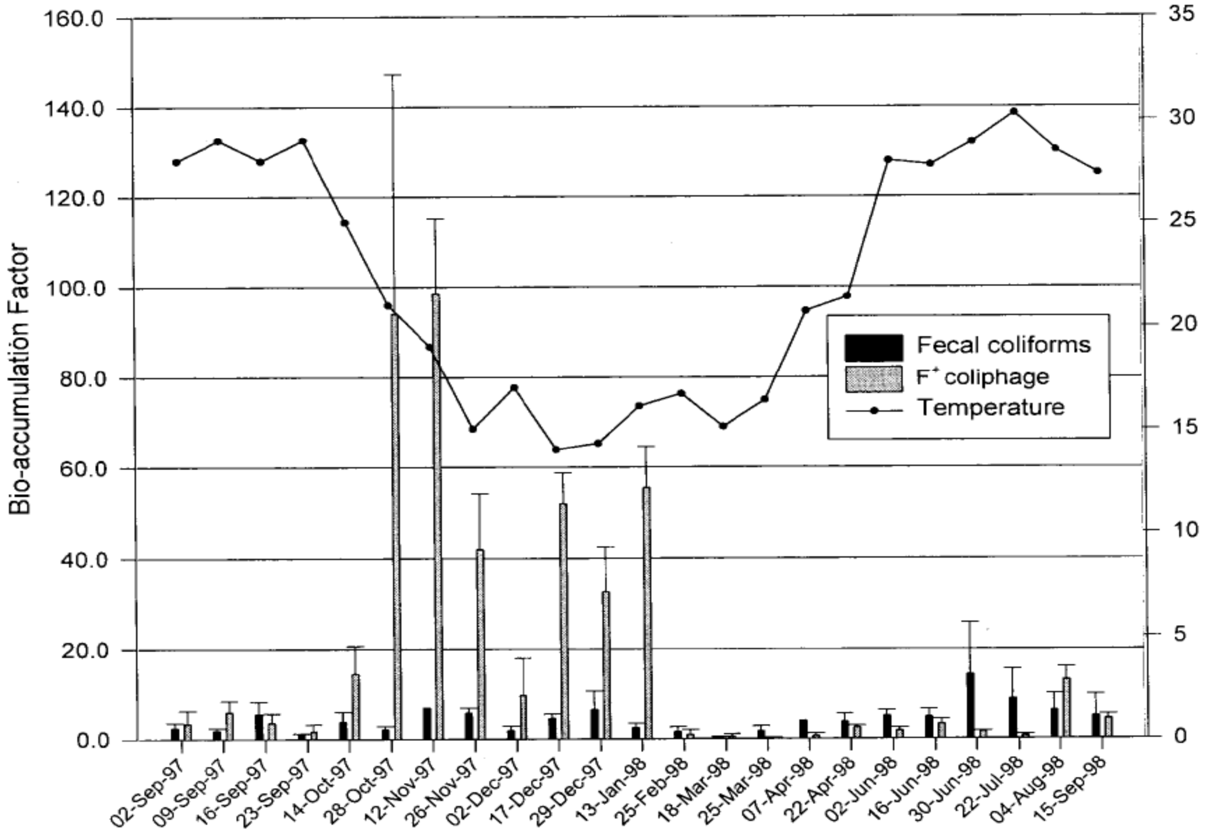
Burkhardt and Calci. (Ref: 2000) undertook pivotal research work which provided a range of BFs for different faecal indicators which demonstrated a vastly different performance for viruses (F+coliphage) than for bacterial indicators. Key results which are reproduced in Figure 3.8 showing a mean BF of 4.4 for faecal coliforms as opposed to 19 for F+coliphage, although for much of the time faecal coliform BFs exceeded those of the viral surrogate.

One of the most striking features of the Burkhardt and Calci (Ref: 2000) work was the demonstration that BF of viruses was not necessarily constant and that winter *hyperaccumulation* occurred. Results from October to January gave an F+coliphage BF averaged 49.9 with a peak BF of 99. This period of hyperaccumulation was not dependent directly on physical variables (temperature, salinity, DO) but on seasonality related to shellfish condition. The researchers speculated that seasonality in mucus production (with associated viral binding) corresponding to glycogen content, might drive this winter hyperaccumulation mechanism. Whilst this may be part of the reason we now also know that seasonality and shellfish condition also influences their feeding selectivity in particle type and size (Section 3.6.2).

Burkhardt and Calci. (Ref: 2000) applied their derived coliform BF to the NSSP 14/100g faecal coliform water quality standard and highlighted that even the upper 8.8 BF would only result in a peak shellfish flesh content of 118/100g (or 111/100g *E. coli*) below *E. coli* 230/100g regulatory requirements. However, the equivalent application of the F+coliphage BF to viral pathogens would result in more extreme flesh concentrations with potentially adverse impact. The researchers noted that this winter seasonal pattern of hyperaccumulation corresponded to observed period of increased gastroenteric illness and suggested three factors influencing the incidence of shellfish related illness:

- Seasonal load of pathogens – health of community lower in winter
- Lower temperature and light conditions influencing pathogen survival
- Seasonality in oysters BF with hyperaccumulation occurring during winter

Figure 3.8: Geometric mean Bioaccumulation of faecal coliforms and F+coliphage by *C. virginica* in Gulf Coast estuarine water assessed with respect to season and temperature *(Source: Ref: Burkhardt and Calci, 2000)*



Various viruses are likely to have different BF due to the binding and species selectivity as discussed in the previous sub-section. In consequence, the BF of 49.9 for F+coliphage in Eastern oysters (Ref: Burkhardt and Calci, 2000) are not directly comparable to the BF of

25-35 for PV in clams (Ref: Metcalf *et al* 1979). What does appear to be clear is that BF levels are potentially higher and more variable in viruses relative to FIOs.

The power of the Burkhardt and Calci (Ref: 2000) work was that it used seasonally conditioned shellfish, with corresponding real seawater and wastewater and at dilutions representative of those found in the estuarine environment. Although computer QMRA modelling (see Section 5.1) uses a mean BF of 49.9 (based on Burkhardt and Calci (Ref: 2000)) workers in New Zealand acknowledge the need for additional research to obtain relevant BFs for NoV. Considerable work is needed in this area as highlighted in Section 8.1.

Impact of NoV Strain, Level and Pattern of Contamination

BFs are somewhat influenced by the nature of the tank experiment with variables such as:

- The shellfish:water ratio
- The level of microbial dosing
- The condition and contamination level of the shellfish stock prior to BF trial
- Individual strain variation

Laboratory based bioaccumulation studies have recently been undertaken to assess the relative impact of chronic (repeated 'low' level) and acute (single high level) contamination events (Ref: Ventrone *et al.*, 2013). This study showed that when the same contaminant dose is introduced to oysters over an extended period (9 days) the same level of shellfish flesh contamination is produced as if all NoV were added in one major 'accidental' spill event. These results suggest that there was no scope for NoV reduction before the next daily bioaccumulation 'event'. It should be noted that biological activity in Pacific oysters at the cold test temperatures (8°C) would have been reduced representing a 'worst case' situation.

This bioaccumulation study reported bioaccumulation efficiencies of roughly 10 to 20% for seeded GI and 3 to 5% for seeded GII indicating that bioaccumulation was strain dependant. The authors suggested that bioaccumulation did not reach saturation, although the data indicates that incremental NoV addition became successively less over the study period.

Ventrone *et al.* (2013) spiked shellfish with diluted stool samples rather than a more artificial 'free' virus from a centrifuged sample. However the study used high initial NoV concentrations (>5 log/L) which are likely to exceed levels found in the marine environment around commercial shellfisheries. It would be interesting to repeat this type of bioaccumulation study at concentrations and temperatures more representative of those experienced in shellfish production areas.

The Ventrone *et al.* (Ref: 2013) work provides a somewhat different more complex dynamic pattern for potential NoV uptake in Pacific oysters at more representative 'winter' temperatures with much lower BF than the Burkhardt and Calci. (Ref: 2000) study.

It is likely that the potential for uptake and retention in the gut is strongly influenced by the nature and concentration of suspended solids in the marine environment. Hamblet *et al.* (Ref: 1969) showed a differential BF for PV of 18 in clean seawater and 5 in highly turbid silty seawater suggesting a greater potential uptake of 'free' viruses relative to those adsorbed and rejected on inorganic particles.

NoV concentration in the marine environment is likely to have a profound impact on BF with higher BF probable at lower ambient concentrations. This feature was apparent from recent work on chronic low level of microbial contamination on faecal coliforms (Ref: Kershaw *et al.*, 2013) which provided an average BF 11.7 for the Pacific oyster which ranged from BF 29 at 0.4cfu/100ml to BF 5 at 107 cfu/100ml. In consequence, some care maybe needed when assessing BF from different tank bioaccumulation studies that the concentration ranges studied are relevant to the potential application. Direct comparison between studies is therefore difficult.

The bioaccumulation of NoV within shellfish could have implications for the wider removal of NoV from the environment in settings where shellfish biomass levels are extensive. There may be scope for shellfish biomass to provide an *in-situ* filter for microbial contamination. This ecosystem services approach has been considered and trialled elsewhere from a nutrient perspective (Refs: Lindahl *et al.,* 2005 and Lindahl and Kollberg, 2008). Computer modelling of shellfish growth and microbial uptake is also outlined in Section 5.2.1.

In summary, NoV Bioaccumulation Factors are poorly understood and are a major evidence gap in understanding the link between water quality and flesh quality. This forms a disconnect between environmental processes and food product safety.

Recommendations for further work are provided in Section 8.1.

3.7 Whole System Microbial Performance

Section 3 aims to review the progressive stages of the NoV environmental transmission pathway from wastewater to shellfish.

3.7.1 Whole System Seaonal Implications to Zoning

The microbial reduction between the NoV concentration in a wastewater discharge and the resultant NoV concentration in the shellfish can be considered a ‘whole system’ reduction. This approach help understand the factors which might affect the scaling and design of any exclusion zoning within the marine environment. The various stages of the environmental transmission pathway could also inform a ‘whole system’ risk scoring scheme (Section 7.3.1) if required. Figure 3.9 provides a schematic illustration of the various stages of the environmental transmission pathway showing their relative magnitude between winter (worst case) and summer (best case conditions).

3.7.2 Relative Whole System Performance of E. coli and NoV

Current environmental regulations have been designed around delivery of $5.25\log_{10}$ reductions in bacterial indicator concentrations to attain a target water quality standard at the shellfish water. This is achieved by a combination of wastewater treatment and dilution (see Section 3.4). There is no such equivalent target for NoV. Furthermore, the research to date suggests that at virtually every stage of environmental transmission the performance for NoV is less than the *E. coli* indicator.

This section aims to compare the relative performance between *E. coli* and NoV. Figure 3.10 highlights the log reductions achievable for the various stages from wastewater to shellfish water (see also Figure 3.1 for context):

E. coli. Figure 3.10a provides an illustration of *E. coli* contamination levels at each stage of a potential environmental transmission route under best and worst case conditions. The 5.25 $\log_{10}$ reduction requirement is readily attained under most conditions. Of note is that even under worst case conditions *E. coli* levels are around the 100 counts/100ml shellfish water quality standard. This calculation is on the basis of 10:1 initial dilution (1$\log_{10}$) at the outfall (which is provided for most continuous discharges). The degree of environmental degradation and secondary dilution will be site specific and will provide an additional reduction level.

- NoV. Figure 3.10b provides an illustration of NoV contamination levels at each stage of a potential environmental transmission route under best and worst case conditions. For NoV there is a massive variation in the initial starting crude wastewater concentration, which coupled with reduced efficacy at most transmission stages has the capacity to generate elevated environmental concentrations of NoV under worst case conditions. In contrast, over the summer when NoV catchment health is good wastewater may have minimal NoV and present no significant environmental risk (as also illustrated in Figure 3.9).

Figure 3.9: Illustration of 'Whole System' process implications for zoning

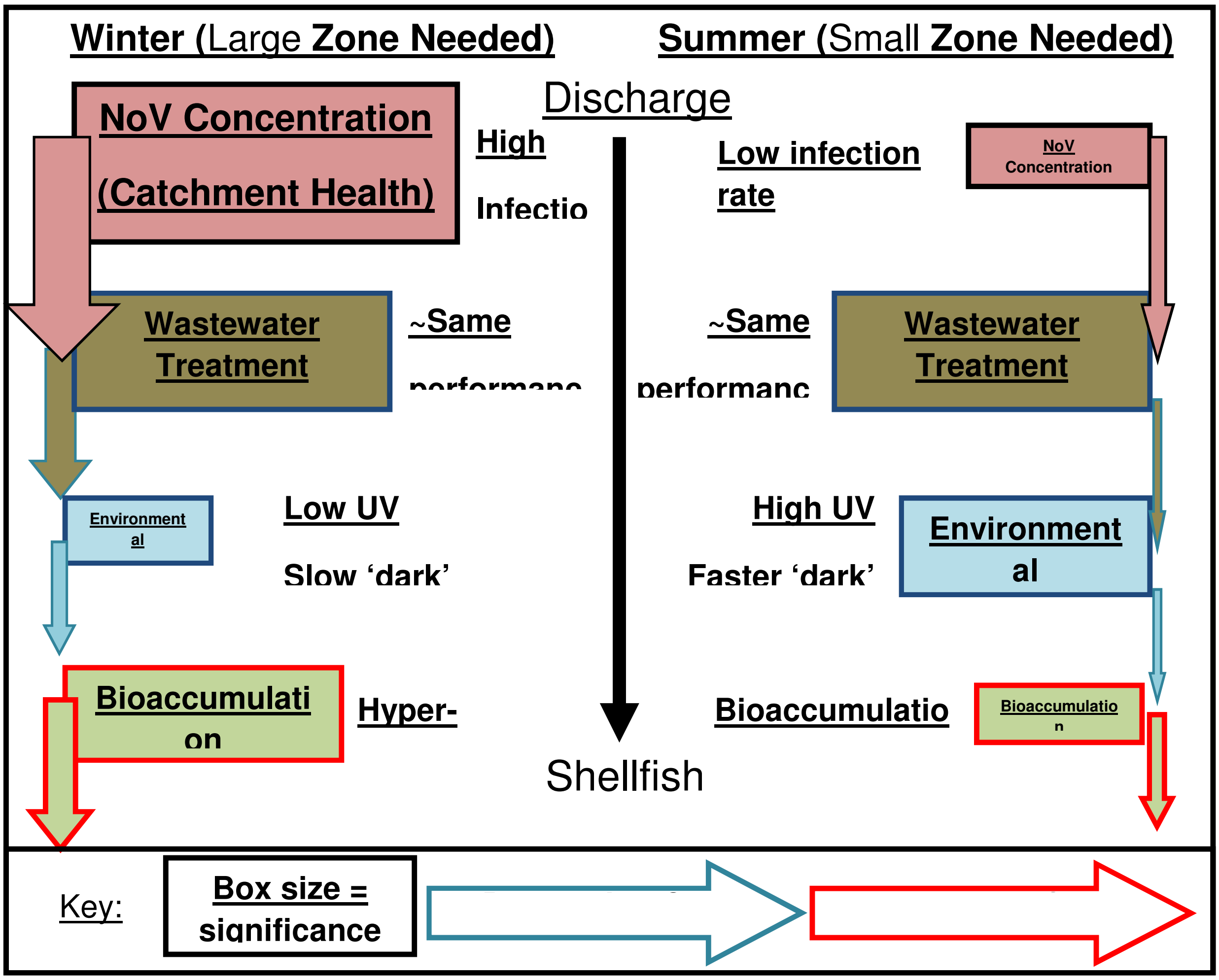
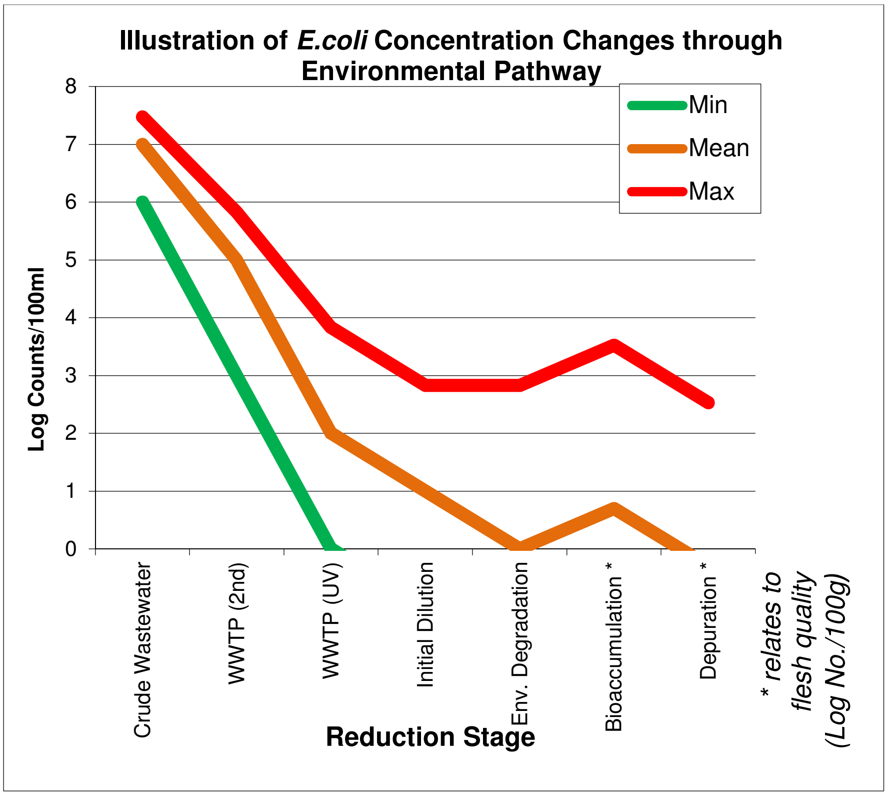


Figure 3.10: Summary of microbial contamination through environmental pathway



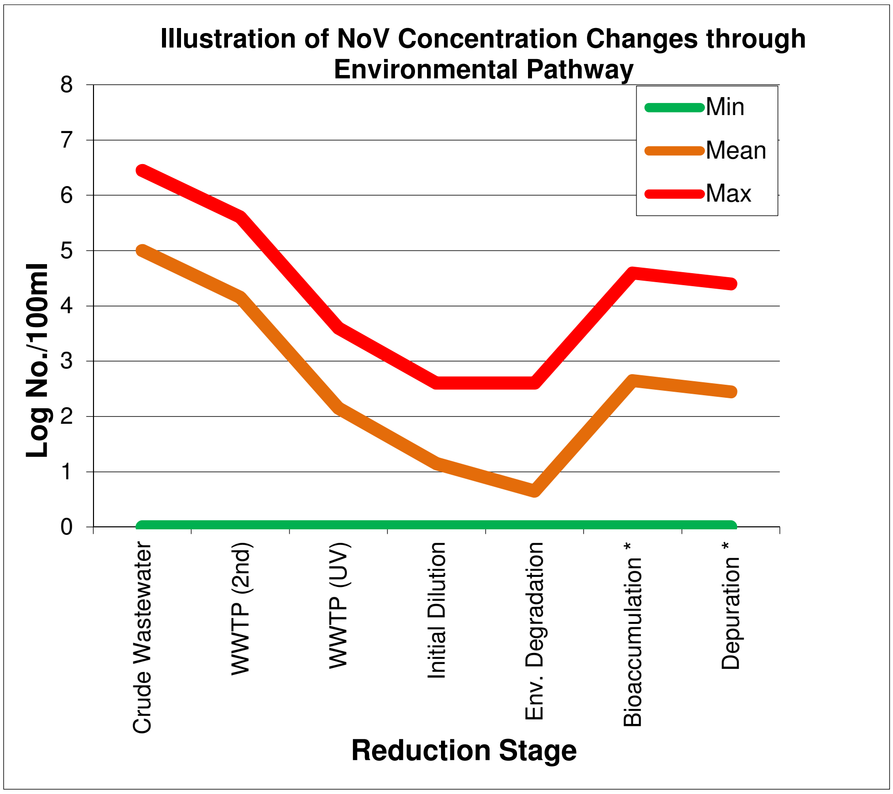
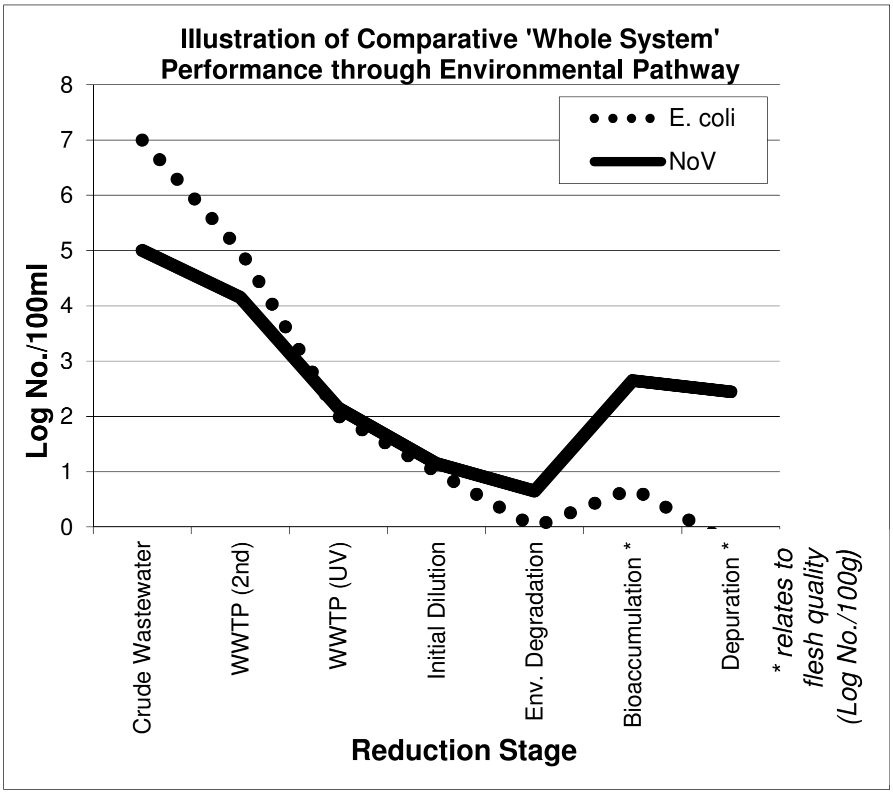


Figure 3.11 provides a comparative plot of 'mean' *E. coli* and NoV whole system performance.

Figure 3.11: Illustration of *E. coli* and NoV 'Whole System' performance



Note: Reductions using 'mean' values (see Figure 3.10a and b)

Figure 3.11 highlights the differential performance between the bacterial indicator and viral pathogen. The whole system performace of NoV is relatively less effective than for *E. coli*. From a zoning perspective there is clearly a need for additional levels dilution to attain even the same level of microbial reductions. With zoning to provide an additional 1000:1 dilution (such as proposed within US FDA guidelines – see Section 2.3) a further 3 $\log_{10}$ reductions could be achieved. In this way an exclusion zone could aim to maximise dilution to reduce NoV contamination levels before reaching shellfish stocks. As a final

3.7.3 Target Water Quality Standard for a Whole System Approach

In England the EA utilised a $5.25\log_{10}$ design standard for *E. coli* designed to deliver 100 counts/100ml as a geometric mean at the shellfish waters. Current scientific evidence of hyper-accumulation (Section 3.6.4) and a low infective dose (Section 3.1.2) would suggest a prospective NoV water quality standard lower than 100 copies/100ml (see Section 5.1.2). It must be stressed that any future consideration of a NoV water quality threshold would be as a theoretical *design* standard. There are two key problems:

- *Evidence Gaps*. Whilst some modelling studies have employed NoV water quality thresholds in their assessments (Section 5.1.2) there is a high level of uncertainty about setting a definitive design standard at this stage. Evidence gaps, such as bioaccumulation factors, are provided in Section 8.1.

- *Analytical Methodology*. Ideally a theoretical design standard could be verified by actual testing. Although Viruses can be detected in low levels within clean potable waters there is currently no standardised reproducible analytical methodology for determination of NoV in environmental samples. The difficulties of viral analysis from environmental waters was highlighted in the recent Viroclime study (Ref: Kay, 2013).

For illustrative purposes:

If a shellfish harvest standard of 1000 genome copies/g were adopted a worst case NoV water quality might equate to ~20 genome copies/100ml.
Based on the assumptions of:
1000 genome copies/g Dt target threshold in shellfish flesh
x6 oysters having a wet mass of ~100g and 2g Dt (= 2000 genome copies/100g in flesh)
100% of NoV bioaccumulating within Dt
And a x100 Bioaccumulation Factor (Section 3.6).

There is a need for more robust science based evidence for NoV in order to provide a stronger whole system understanding of the relative performance of each stage. This is important as it highlights how some stages are more significant than others which could shape management decisions. In addition, the outputs of these findings (e.g. environmental decay rates) can help inform other management measures such as computer modelling.

Recommendations have been provided in Section 8.1.

3.8 Evidence Based NoV Zoning - Summary

Section 3 details the literature status with respect to the principal controls on NoV within the environmental cycle of human community derived pathogen loading, potential contamination of shellfish with its potential link to foodborne infection. A number of key findings have been considered:

- Determination of dilution levels for exclusion zones should ideally be designed from a 'whole system' NoV perspective if a science evidence based approach is to be used. This would require comprehensive appreciation of all stages of environmental transmission pathway:
  - NoV crude wastewater load ('Health of Catchment') (Section 3.1 and 3.2)
  - Wastewater NoV removal efficacy (Section 3.3)
  - Mixing (Section 3.4)
  - Environmental degradation (Section 3.5)
  - Shellfish bioaccumulation (Section 3.6)

- The objective for this study is confined to look at processes between wastewater discharge and shellfish. It should be noted that coupled with this is a wider need to understand NoV viability and dose-response from a consumer/population exposure perspective if a holistic 'whole system' approach is to be effectively used.
  - *NoV Viability* (Section 3.1.3). This remains the single most important aspect to establish an agreed method (or range of methods) to assess NoV infectivity at all stages of environmental transmission from wastewater, to environmental waters to shellfish.
  - *Dose-response* (Section 3.1.2). This is vital in order to establish thresholds throughout the environmental transmission cycle relating to the threshold for an infective shellfish portion and an appropriate shellfish flesh standard. This in turn would govern a potential water quality standard use to target a design criteria and scheme driver for wastewater treatment.

- *'Health of catchment'* (Section 3.1 and 3.2) is a principal control on NoV loading in the wastewater system and a major source of variation. From a risk control basis this factor needs to be better understood and monitored. Consideration should be given to the assessment of indirect surrogates (e.g. temperature) which may help predict catchment health. At present it is not possible to differentiate the possible effects of seasonality as winter (air temperature) influence on rates of community infection (which will impact NoV wastewater loading) may correspond with shellfish hyperaccumulation (sea temperature). There is a need for more research in these areas (Section 8.1 and 8.2).

- *Wastewater NoV load* – (Section 3.3) Crude (CSO) and Treated. Reduction and removal of NoV through WWTPs is also one of the most Critical Control Points from a HACCP perspective. In consequence, we need a better understanding of:
    - Level of loading due to microbiologically 'crude' discharge from CSOs to shellfish catchments
    - Secondary treatment NoV removal rates. Variable although secondary treatment with Activated Sludge can reasonably deliver $2\log_{10}$ reductions.
    - Tertiary disinfection NoV inactivation efficacy A probable further $2\log_{10}$ reductions in viable NoV (i.e ~1% viable at discharge). However, this will not be evident from RT-PCR data.
    - Consideration should be given for development of a more consistent methodology and the assessment of indirect surrogates which may help predict catchment health.

- *Environmental Degradation.* (Section 3.5). This complex area is poorly understood which undermines any evidence based policy for setting wastewater treatment efficacy. Key work is needed on:
    - *Decay T_{90} rates for NoV.* (Sections 3.5.1 and 3.5.2). Technically difficult until the viability issue is resolved although preliminary profile of various shellfish waters in terms of potential water quality influences on T_{90} (e.g. TSS, organic compound and phytoplankton concentration) could be a useful exercise.
    - *Sediment : microbe interactions* (Section 3.5.3). This is a poorly understood relationship and likely to be critical for shellfish uptake (Section 3.6).

- *Bioaccumulation* (Section 3.6). There is considerable data to show different levels and patterns of bioaccumulation between viral and bacterial indicators. Some work using NoV surrogates have provided an indication of hyper-accumulation over winter months yielding Bioaccumulation Factors far in excess for *E. coli*. The principal study, most widely utilised, obtained Bioaccumulation Factors of ~100 and ~50 as a peak and over the winter respectively. This compares with equivalent factors of <10 for *E. coli.* There is a need for NoV Bioaccumulation Factors obtained from representative water quality (with appropriate form of NoV) in environmental concentrations reflective of shellfish waters. The focus of this work will need to encompass NoV binding to various solids types and evaluate concentration and speed up uptake variables in the experimental settings (see Section 2.3.2).

- '*Whole System' performance* (Section 3.7). The reduction in microbial levels between a wastewater discharge and the resultant concentration in the shellfish can be considered a 'whole system'. The current regulatory system in some regions is designed to deliver $5.25\log_{10}$ reductions between crude wastewater and the shellfishery. Most of this reduction is easily provided by WWTPs with UV disinfection and requires minimal dilution to attain the target 100 counts/100ml mean water quality standard. The reduction rates for NoV are lower and the potential water quality requirement may be more stringent. Preiminary calculations suggest a potentially lower water quality standard (~20 genome copies/100ml) to attain the proposed harvest shelfishstandard. This 'whole system' approach provides a scientific rational for determining dilution rates, although the evidence base needs to be stronger.

- Recommendations for research data gaps have been provided in Section 8.1.

4 CASE STUDIES

The objective of Section 4 is to review overseas experience with respect to exclusion/buffer zones from overseas settings in the context of developing viral risk management measures. Information has been obtained from North America (Section 4.1), Australia (Section 4.2) and New Zealand (Section 4.3). The US and Canada have been grouped, as both countries are currently working together on a joint NoV in shellfish risk profile. It should be noted European examples from Netherlands and Italy of geographically based exclusion zones are also discussed in Section 2.1.

In addition, Section 4.4 also reviews a series of examples of wastewater proximity profiles of NoV in shellfish from the Republic of Ireland and the UK.

4.1 North America (US and Canada) Case Study

4.1.1 Overview

Oyster production is a major aquaculture/fisheries industry in the US and Canada. An overview of the US oyster industry (Ref: Lutz *et al.*, 2012) indicates that in 2010, 28.1 million pounds (of meats) worth $117.6M were produced primarily between three quite different key regions as shown in Table 4.1.

Table 4.1: Summary of US oyster production
(Source: Ref: Lutz et al., 2012)

Region	Species	Production (2010)	Method
Gulf of Mexico (primarily Louisianna)	*Crassostrea virginica* (the Atlantic oyster or Eastern oyster)	~50-55% of production 15.5M pounds of meats	Wild harvested
The Pacific region (primarily Washington State)	*Crassostrea gigas* (Pacific oyster).	~35% of production	Primarily Cultivated beds
Chesapeake Bay region, (primarily Maryland)	*Crassostrea virginica* (the Atlantic oyster or Eastern oyster)	10-15% of production 16M oysters $5m (massive declines over last 30yrs)	Primarily Cultivated beds

Canadian aquaculture is dominated by salmon and mussel production. Although oyster production is significantly smaller than that of the US Canadian production still outstrips that of the UK. Canadian oyster aquaculture production was valued at $16M in 2009 with British Columbia, Prince Edward Island, New Brunswick and Nova Scotia contributing 40%, 31%, 25% and 4% respectively. As with the US, East coast production utilises *Crassostrea virginica* (the Atlantic oyster or Eastern oyster), whilst the West coast uses the non-native *Crassostrea gigas* (Pacific oyster). FAO estimates indicate that 80-85% of East coast production is from wild fishery landings. Aquaculture production has reduced somewhat in recent years from a peak of ~14,000T in 2003 to ~9,000T in 2009 (although price/kg has increased). Much of Canadian aquaculture production is exported to the US and as such the oyster industries are closely intertwined.

It should be noted that that US oyster industry is going through a time of change and volatility with a significant drop in production in key areas with production down from $136.5 in 2009. In Louisiana, the key Gulf production state, production in 2010 was half that of 2009 following an oil spill, coastal freshwater flooding and algal bloom problems. Meanwhile in the Chesapeake Bay area production which had primarily come from large reef areas has seen a massive decline over the last 30 years despite a number of restoration projects. In Canada, recent reports from British Columbia indicate massive mortality of 80-90% in Pacific oyster stocks impacting on newly hatched spat in parallel with extensive losses in scallops and no obvious disease vector leading to concerns about ocean acidification.

A number of new developments provide some hope for the future with increasing consideration of off-bottom culture in the Gulf region to boost East coast production and the use of high-tech Active Management systems in the Washington State area (see Section 7.1.4) which can help with shellfish management.

Water quality and microbial inputs from wastewater discharges also remain a significant challenge with high population density in certain areas. Section 4.1.4 outlines the wastewater consent permitting regime in the US which provides a significant level of protection to shellfish growing waters through the Clean Water Act. Furthermore, although CSO discharges are still a significant problem in older metropolitan areas particularly in the

North East of America newer urban areas have good stormwater separation preventing the exposure to untreated wastewater spills experienced for many UK shellfish waters.

4.1.2 North America Regulatory Requirements (Prohibition Zone Setting)

The US National Shellfish Sanitation Program (NSSP) Model Ordinance considered in this sub-section is essentially the source regulation before national implementation within the Canadian Shellfish Sanitation Program (CSSP), The NSW Shellfish Quality Assurance Program (Section 4.2.2) and the New Zealand BMSRCS (Section 4.3.2). To avoid repetition focus here will be upon differences for the US and current US FDA proposed developments.

Background

Following an oyster borne typhoid outbreak in 1924-25 the U.S. Public Health Service, and the shellfish industry initiated the NSSP to ensure the safety of shellfish for human consumption by preventing the harvest of shellfish from contaminated growing areas. This program has centred around the use of faecal coliform bacterial indicator water quality standards to define a series of classification zones with differing control and harvesting implications as outlined in Table 4.2.

Table 4.2: Summary of NSSP area zoning

Classification	Definition	Status	Shellfish Harvesting Activity
Approved	Sanitary survey shows that the area is not subject to contamination that presents an actual or potential public health hazard.	Open	Harvesting allowed
Conditionally Approved	Meets Approved criteria, but only during predictable and manageable periods	Open	Harvesting allowed except during specified conditions (rainfall, WWTP bypass or seasonal)
		Closed	Harvesting NOT allowed
Restricted	Areas that do not meet water quality standards for an	Open	Depuration and/or Relay harvesting only

	Approved classification, but the sanitary survey indicates only a limited degree of pollution		
Conditionally Restricted	Meets Restricted criteria, but only during predictable and manageable periods	Open	Depuration and/or Relay harvesting allowed except during specified conditions (rainfall, WWTP bypass or seasonal)
		Closed	Harvesting NOT allowed
Prohibited	Sanitary survey indicates that faecal material, pathogenic microorganisms, or poisonous or harmful substances may be present in concentrations that pose a health risk to shellfish consumers	Closed	No harvesting allowed or water use allowed for processing (administratively imposed precautionary closure)

The Interstate Shellfish Sanitation Conference (ISSC) was formed in 1982 to foster and promote shellfish sanitation through the cooperation of state and federal control agencies, the shellfish industry, and the academic community. The ISSC promotes cooperation and trust among shellfish control agencies, the shellfish industry, and consumers of shellfish; and ensures the safety of shellfish products consumed in the United States. The ISSC meets on a Biennial basis to review proposals to update NSSP Model Ordinance guidance to various State Shellfish Control Authority (SSCA) bodies. This output is reviewed in NSSP affiliated countries such as Australia and New Zealand.

Adaptation for Viral Threats

In 2005 preliminary proposals by US FDA to the ISSC (Ref: ISSC, 2005a) were made to attempt to revise the Conditional shellstock growing area and wastewater requirements with inclusion of a number of public health and viral components. The ISSC 2005 Task Force recommended no action as there was no viral standard. Subsequent ISSC work has allowed input of these changes towards a joint bacterial and viral objective for Prohibition and Conditional areas within the current Model Ordinance (Ref: NSSP, 2009).

Much of this sub-section has been summarised with reference to the most recent proposal by the US FDA for the provision of dilution guidance for prohibition zones associated with WWTP discharges put forward in ISSC 2013 (Ref: Goblick, 2013) which was made in January 2014. Proposals relate to Prohibited Areas (closure zones) based on the minimum criteria established under Section II, Chapter IV. @.03 E(5) of the Model Ordinance (Section E Prohibited Classification). If these most recent proposals are accepted they will be incorporated into the latest 2013 Model Ordinance.

There is increasing recognition that bacterial indicators are inadequate to predict the risk of viral illness for the following reasons (Ref :Goblick, 2013):

- Enteric viruses are resistant to treatment and disinfection processes in a WWTP and are frequently detected in the WWTP's final effluent under normal operating conditions
- Shellfish can bioaccumulate enteric viruses up to 100 times from water column
- Certain enteric viruses are retained by molluscan shellfish to a greater extent and for longer than the indicator bacteria

Currently, Prohibition Areas (closure zones) around WWTPs are based upon minimum criteria set out within a Modal Ordinance (MO) founded on bacterial indicators. The proposed NSSP MO (Ref: Goblick, 2013) requires all growing areas which have a WWTP outfall of *public health* significance within, or adjacent to, the shellfish growing areas to have a Prohibited zone established adjacent to the outfall. Therefore Sanitary Surveys are required to take a keen account of WWTP effluent dilution in relation to the level of enteric viruses in shellfish in the establishment of Prohibition zones. This is needed to inform the Management Plan within Conditional Zones which "includes bacteriological and viral quality of the effluent" (Ref: NSSP, 2009).

Consideration of the delineation of the prohibited zone around WWTPs and the development of future NoV zoning is provided in Section 2.3.3.

4.1.3 North America Site Specific Illustrations of Zone Investigations

As indicated in Section 4.3.4, the US FDA undertook a series of dye dilution and microbiological investigations between 2008-2012 to assess the relationship between

WWTP viral contamination and dilution rates. The Mobile Bay study was the first in this series of projects and has been reported in both the scientific literature (Ref: Goblick *et al.,* 2011) and in ISSC presentations (Ref: Calci, 2013 and formed a central component for a PhD thesis (Ref: Woods J S, 2010).

Dye and microbiological results, reproduced in Figure 4.1, show that the discharge from a WWTP with a major discharge (a PE of 200,000) it took ~8km to attain a 1000:1 dilution of wastewater levels required to demark a potential Conditional shellfish area. Overall the Mobile Bay data does illustrate a proximity profile of viral contamination in relation to discharge from a wastewater outfall. NoV and F+coliphage (MSC) data also indicate that at the most distant point ~5.5km from the discharge Station 4 still reflected occasional contamination of NoV and phage which exceeded threshold requirements.

At a closer inspection of the data there are some issues which could benefit from further investigation. The researchers undertaking the Mobile Bay study stated that overall the F+coliphage results compared well with NoV GII results (Ref: Goblick *et al.,* 2011). Whilst these results (reproduced in Figure 4.1) do generally show high levels in the vicinity of the outfall and lower levels further away from the discharge it is difficult to see a comparable trend with similar ratio between the two viruses. In particular Station 3, noted for its low dye and NoV levels, was thought to have 'missed' the wastewater plume, yet this site still exhibited elevated F+coliphage results. It is possible that whilst F+coliphage results may be a useful viral indicator in wastewater and in the near-field they maybe less representative of NoV in the far-field. Also notable is the absence of hyperaccumulation over the study which had been reported by Burkhardt and Calci. (Ref: 2000) (see Figure 3.8) although possibly the season for hyperaccumlation at Mobile Bay was extended and the September 2008 to March 2009 sampling period 'missed' the summer minima of viral bioaccumulation.

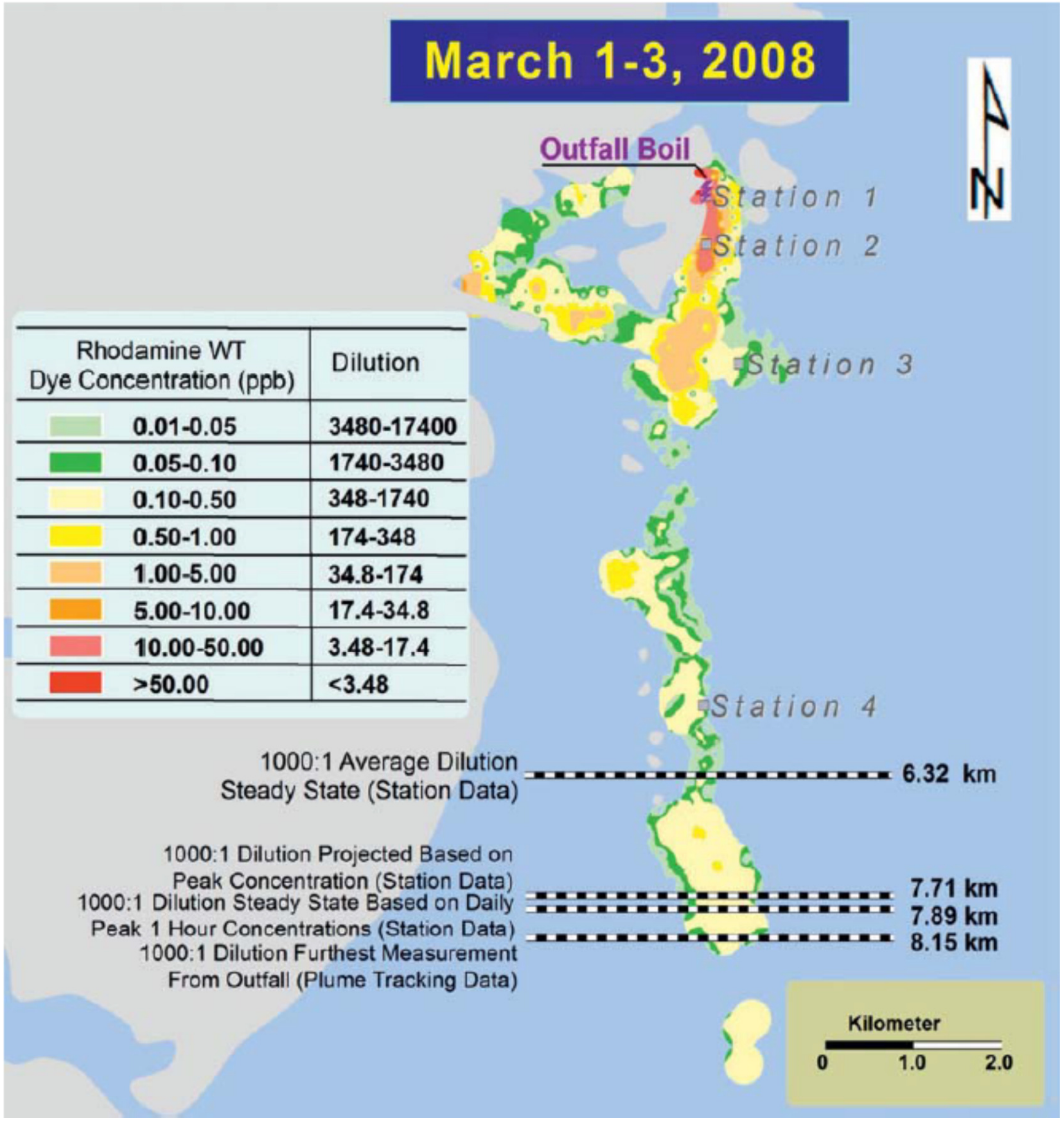
NoV in crude wastewater results for Mobile indicate that generally GI and GII levels were generally <4 $\log_{10}$ PCR units/100ml. These results are somewhat lower than literature data for many of the European studies (see Table 3.1) which raises the concern as to whether the 1000:1 dilution proposed by the US FDA would be sufficient to dilute wastewater from WWTP discharges serving a population with a higher pathogen loading.

The magnitude of the ~8km exclusion zone implied by the Mobile Bay study should be placed in context with the NoV impact zones considered in Section 4.4.

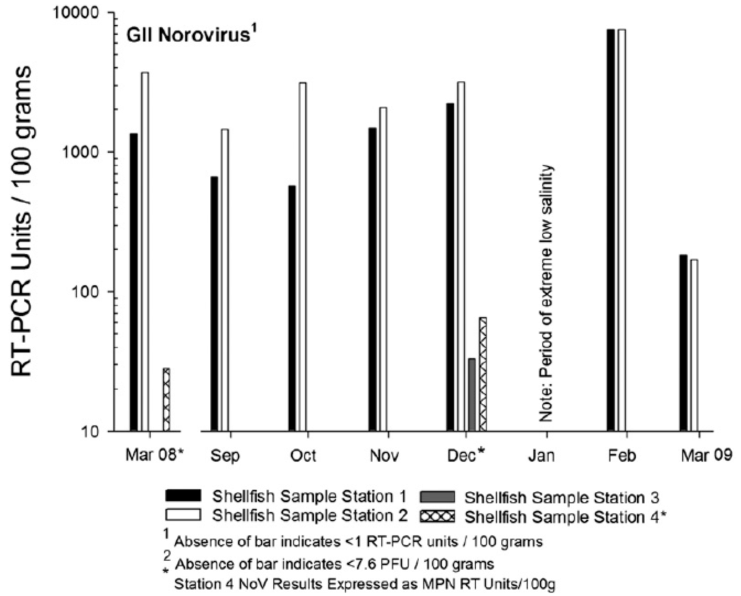
The FDA study on the James River in Hampshire Roads was undertaken in a similar fashion to that of the Mobile Bay study using a combination of dye dilution studies and viral (NoV and F+coliphage) sentinel monitoring (Ref: FDA, 2010). Results of this study provided notably lower NoV and phage results within the expected 1000:1 dilution zone following oyster cage deployment along the plume trajectory following a preliminary dye release. Altered wastewater plume trajectory as a result of different wind conditions was attributed to the mis-matched results highlighting the difficulty in undertaking definitive site specific studies in a variable marine environment. It is understood that further complication arises in shallow water estuarine settings where marked concentration gradients can occur, producing different shellfish uptake levels. This can present difficulties to investigators when there is a reticence to deploy sentinel cages near commercial shellfish production beds.

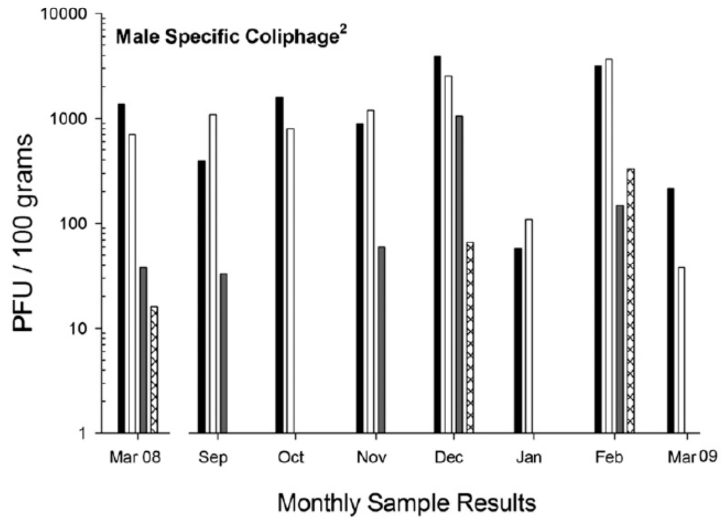
Figure 4.1: Mobile Bay Alabama - FDA wastewater dilution study *(Source: Ref: Goblick et al., 2011)*

Rhodamine WT Dye Dilution Results with 1000:1 Dilution Estimates



Microbiological Accumulation by Month (Stations 1-4)





4.1.4 North America Wastewater Discharge Controls and Requirements

US National Pollutant Discharge Elimination System

In the US the Clean Water Act authorises the National Pollutant Discharge Elimination System (NPDES) permit program has controlled water pollution by regulating point sources that discharge pollutants into waters of the United States since 1972. The NPDES administered by the Environment Protection Agency (EPA) regulates discharge consenting for WWTPs, CSOs and stormwater drains.

US CSO Impact

As indicated in the overview (Section 4.3.1) the US like the UK has Combined Sewer Systems in older metropolitan areas which can pose a significant risk for wastewater spills under wet weather conditions. EPA data indicate 800 regulated Combined Sewer Systems compared with 7,300 separate stormwater sewer systems reflecting the dominance of separated systems. Distribution maps on the EPA website highlight most CSO systems are found in the NE and Great Lakes regions.

EPA's CSO Control Policy through the NPDES permitting program provides guidance on how communities with combined sewer systems can meet Clean Water Act goals in a flexible and cost-effective a manner as possible. To ensure that controls meet local environmental objectives they should have:

- Clear levels of control to meet health and environmental objectives
- Flexibility to consider the site-specific nature of CSOs and find the most cost-effective way of control
- Phased implementation of CSO controls to accommodate a community's financial capability
- Review and revision of water quality standards during the development of CSO control plans to reflect the site-specific wet weather impacts of CSOs

From 1997 the ‘nine minimum control’ measures were brought in to reduce the prevalence and impacts of CSOs (EPA, 1995). Whilst many of these are operations requirements are comparable to measures in the UK there are two measures of note which differ:

- Public notification to ensure adequate notification of CSO occurrences and CSO impacts. Shellfish harvesting is a specific commercial activity cited whilst the notification measure adopted should be cost effective whilst providing reasonable assurance to affected public in a timely manner.
- Monitoring to effectively characterize CSO impacts and the efficacy of CSO controls. Impact of CSOs on shellfish beds causing closure is one type of use impairment noted in this report.

Communities with combined sewer systems are also expected to develop long-term CSO control plans that will ultimately provide for full compliance with the Clean Water Act, including attainment of water quality standards. NOAA 1992 calculated that 7% of US harvest shellfish acreage limitations were attributable to CSOs as opposed to 37% impacted by WWTPs suggesting that CSOs Prohibiting area impact on shellfish waters is likely to be less than that of continuous WWTP discharges. However, US experience could still provide useful insights for potential impact studies in the UK on the implementation of automated CSO monitoring and responsive Active Management (see Section 7.1.4).

Canadian Discharge Regulations and Early Warning Systems

In Canada, provincial and territorial governments issue operating permits for municipal sanitary wastewater systems and regulate the effluent quality most of which include faecal coliform limits in their regulations or permits. Environment Canada (EC) is in the process of implementing national effluent quality standards (via federal regulations) for selected contaminants of concern in order to achieve consistent minimum standards in all jurisdictions. Whilst these national standards are not microbial standards for shellfish water it should be noted that 85% of the sanitary wastewater systems discharging in the vicinity of active Canadian shellfish areas receive a minimum of secondary-level treatment. EC make appropriate recommendations on CSSP shellfish area classification to other regulatory authorities, namely Fisheries and Oceans Canada (DFO) and the Canadian Food Inspection Agency (CFIA).

EC classify adjacent areas to account for the most likely failure scenario for a wastewater system. Where the normal operation of the system is reliable, the failure detection and notification systems are robust, and closure response to such failures can be timely, the impact zone can be operated under a Conditional [harvest area] Management Plan (CMP) if there is sufficient interest from various parties. This allows harvesters to have access to adjacent growing areas outside of the prohibited zones under normal operation of the wastewater system, and special control measures are in place to prevent contaminated iproduct from reaching market. It is understood that In the event of a failure, an automatic broadcast service alerts harvesters and the area is immediately closed.

A good overview of the Canadian approach to wastewater management with respect to shellfish waters is provided by a recent review of the sanitary assessment process (Ref: Roberts *et al*. 2014). This presentation highlighted how 320 discharges from WWTPs the majority with secondary treatment to marine waters were in the vicinity of shellfish harvesting areas on the Pacific and Atlantic coasts. A number of techniques are used to assess potential impact including wastewater microbial monitoring, plume studies using drogues, hydrometric modelling and in some cases dye studies to assess dilution rates. It is notable that the wastewater profiling included determination of bacteriophage concentrations in addition to standard faecal indicator bacterial organisms in an attempt to help assess viral reduction rates through the treatment process.

4.1.5 North America Future Developments

In the US and Canada a number of groups and consortia are currently working on NoV related issues as outlined below:

NoroCORE

U.S Department of Agriculture (USDA) and the National Institute for Food and Agriculture (NIFA) have funded the $25M NoroCORE programme http://norocore.ncsu.edu/ with the objective to reduce the burden of food borne disease associated with viruses, particularly NoV. NoroCORE is an extensive collaborative project with input from multiple universities and agencies and administered through North Carolina State University.

The project has six core objectives:

- Develop improved methods of studying human NoV and their role in food borne illnesses.
- Develop and validate rapid and practical methods to detect human NoV.

- Collect and analyze data on viral food borne illnesses – including how they are transmitted – and provide risk and cost analyses.
- Improve understanding of how human NoV behaves in the food safety chain in order to develop scientifically justifiable control measures.
- Develop online courses and curricula for food safety and health professionals and food service workers, and provide information to fresh produce and shellfish producers and processors on the risks, management and control of food borne viruses.
- Develop a public literature database, build virus research capabilities in state public health laboratories, and develop graduate level-curricula to educate masters and doctoral students trained in food virology. (norocorelit.com)

NoroCORE hosts an annual Conference with useful presentations available via its website for 2012 and 2013. Although NoroCORE has a focus wider than just shellfish some components have a related theme (e.g. Task 4.1: "Characterize the occurrence of virus contamination in fresh produce and Molluscan shellfish production and harvesting"). This has allowed some resources to be directed at NoV in shellfish and open coastal waters. It is understood that the wider NoroCORE direction is in recognition of the major NoV burden through other foodborne vectors and infected food handlers in particular within the US.

NACMCF

National Advisory Committee on Microbiological Criteria for Foods (NACMCF) is a US expert group formed to provide impartial scientific advice to Federal Food Safety Agencies who 'charge' NACMCF to report on specific topics. The Food Safety and Inspection Service , the Food and Drug Administration , the Centers for Disease Control and Prevention , the National Marine Fisheries Service , and the Department of Defense Veterinary Service Activity have charged NACMCF to develop a unified approach to reducing illness from NoV. The NACMCF appear to differ somewhat from the UKs ACMSF with a more intense level of work on specific issues with full conference meeting each year in addition to sub-committee meetings. The NoV Control Strategies charge works through four Work Groups:

- WG 1 – Epidemiology and Attribution;
- WG 2 - Mitigation and Control;
- WG 3 – Methods and Surrogates;

- WG 4 –Risk Assessment

It is understood that the last round of reporting was due in March 2014.

Risk Assessment Process

The US and Canada are working together on a joint *Norovirus in Bivalves Food Safety Risk Assessment* which is considered in Section 2.3.4 within the zoning context. This group are expected to report within the coming year with output of interest to the other US affiliated Case Study countries.

4.2 Australia Case Study

4.2.1 Overview

A good review of historical evolution of the Australian oyster industry and production patterns is provided by Maguire and Nell. (Ref: 2006). Australia produced >13,000T of oysters worth A$71.8M (2003-2004) with a regional breakdown from three main regions as shown in Table 4.3.

On the whole oyster production in Australia has seen dramatic growth in recent years, although it should be noted that regional variations do exist and New South Wales (NSW) production peaked in 1970s and is relatively static now. Most recent figures from NSW Shellfish Program 2014 indicate that the 76 commercial shellfish growing areas between Eden in the south and Tweed Heads in the north with around 300 oyster farming businesses produced 9.2 million dozen oysters worth A$42M and representing 44% of Australia's oysters. This share of Australian production is somewhat down on the 2003-2004 (Table 4.3), although market value is virtually the same suggesting the relative growth of production from Tasmania and Southern Australia. The NSW Farmers' Association estimated that 20% of the oyster production in NSW is lost annually due to pollution closing estuaries (Ref: NSWP, 2012). Low levels of urbanisation in Tasmania and Southern Australia are reported to allow these States to avoid Public Health problems (Ref: Maguire and Nell, 2006). As NSW is still the largest production State in Australia with a higher population density and historical incidence of NoV related shellfish outbreaks this section will focus on developments in this State.

Table 4.3: Summary of Australian oyster production
(Source: Ref: Maguire and Nell, 2006)

Region	Species	Production (2003-2004)	Production Method
New South Wales (NSW)	Production based primarily on native Sydney oyster (*Saccostrea glomerata*) and the Pacific oyster (*Crassostrea gigas*)	~6,000T ~A$38M	Sydney oyster originally wild stock harvested and dredged with increasing use of intertidal and subtidal racks.
South Australia	Mainly Pacific oyster (*Crassostrea gigas*)	4,382T ~A$21M	Intertidal and subtidal culture systems
Tasmania	Mainly Pacific oyster (*Crassostrea gigas*)	3,243T ~A$12M	

4.2.2 NSW Regulatory Development

In NSW a number of NoV gastroenteritis outbreaks occurred in the 1980s and 1990s, followed by a Hepatitis A outbreak in 1997, which culminated in the implementation of the Shellfish Quality Assurance Programme in this State. From a legislative perspective Australia operates under equivalent shellfish regulations to the US NSSP system, although each State has its own parliament with powers to 3 nautical miles. The NSW shellfish industry is regulated by NSW Food Authority under the Food Regulation 2010. All oysters and mussels in NSW are harvested in accordance with the NSW Shellfish Program, which has adopted the Australian Shellfish Quality Assurance Program (ASQAP) as a minimum standard. All the requirements of the NSW Shellfish Program are contained in the NSW Shellfish Industry Manual (Ref: NSW Food Authority, 2010). Whilst ASQAP indicate that the State Shellfish Control Authorities (SSCA) are responsible for the identification, monitoring and assessment of hazards in shellfish growing waters, including enteric viral pathogens, it is accepted that this is through the use of faecal coliform indicator in shellfish and seawater (Ref: ASQAP, 2009).

The application of an epidemiological model to assess the suitability of the dual shellfish flesh and water regulatory monitoring program for NSW was recently conducted using paired data form 5 estuaries with 5 years data. The sudy indicated poor correlation between the data sets and potential contamination events with neither measure providing a reliable measure of public health risk (Ref: Ogburn and White, 2009 as shown in Figure 3.7b).

4.2.3 NSW NoV Impact and Case Studies

In recognition that the NSW Shellfish Program does not necessarily provide complete public health protection from shellfish borne viruses the NSW Food Authority recently commissioned a study which undertook eight case studies across five oyster growing areas to assess what key factors result in failure to prevent NoV contamination of oysters in growing areas and resulted in NoV illness outbreaks in consumers (Ref: Hay *et al.*, 2013) which are reviewed below.

The aims of the project were to:

- Identify the fundamental reasons why the current bivalve shellfish classification and management systems can fail to protect consumers from viral contamination in shellfish;
- Identify and evaluate potential frameworks for improved management strategies for shellfish growing areas, and their barriers to implementation, including any information gaps;
- Make recommendations on priorities for future work to develop and implement improved management strategies to protect shellfish from viral contamination.

The case studies provide a number of important findings many of which are likely to be relevant to the UK:

- *Insufficient Robust Sanitary Survey* The sanitary survey failed to adequately protect consumers from illness arising from NoV contamination as a result of:
    1. Insufficient reliable information gathered during the sanitary survey process to allow an adequate assessment of the risk of virus contamination of shellfish in the growing area.
    2. The design of the SQAP, which incorporates infrequent detailed sanitary surveys and minimal annual field observation in the catchment annually which may not pick up a changing viral risk profile.

- *Ineffective Implementation*. Failure to continue to manage the risk from sources of viral contamination previously implicated in earlier NoV illness events giving a potential for recurrence.
- *Viral Testing and Use of Surrogate Tools*. In the absence of a universal viral indicator, there maybe scope to utilise a combination of techniques:
    1. Viral indicators such as F+coliphage (MSC) that could be used as predictors of risk within a defined set of circumstances. Although a data gap may exist as to how well F+coliphage may reflect regional viral decay and behaviour
    2. Microbial source tracking applied close to the potential sources of contamination near growing areas, to determine potential present of human faecal contamination sources.
    3. Testing for targeted viruses of concern under specific circumstances (e.g. to identify whether shellfish are implicated in an illness outbreak, or to confirm that known contamination has cleared from shellfish in a growing area). Although a data gap with respect to NoV viability is highlighted.
- *Reluctance for Viral Shellfish Testing.* The absence of viral shellfish testing in commercial growing areas makes it difficult to place the results in context. This reluctance arises from regulatory uncertainty regarding potential infectivity arising from positive results.

The Hay *et al.* (Ref: 2013) study has provided a comprehensive series of recommendations relevant to the UK with regards the integration of shellfish and environmental monitoring policy and the need for science based policy development.

4.2.4 NSW Wastewater Discharging Controls and Requirements

The Hay *et al.* (Ref: 2013) report in Section 4.2.3 highlighted the many potential problems related to small poorly designed, operated and regulated on-site wastewater disposal systems in NSW. These issues led to an inquiry by the NSW Parliament (Ref: NSWP, 2012).

Regulatory problems with multiple potential wastewater contamination sources was highlighted within this Parliamentary report by a Case Study from Kalang River in Northern NSW when an outbreak of NoV in 2008 caused several people to fall ill after eating oysters. Following Prohibition the harvest area has remained closed leading to the dissolution of the local oyster farm. Investigation identified multiple contamination sources including the sewage treatment works, local caravan parks, boat sheds on the river and many on-site wastewater systems that were wrongly placed and mismanaged/unregulated.

Principal recommendations from the NSW Parliament were focussed upon improving guidelines and controls for small On-Site wastewater disposal facilities although wider NoV regulation proposals were made:

> *"The Committee recognises the significant stress placed on the State's oyster farmers given the frequency of contamination events, for which they often bear the costs and deal with the consequences. The absence of routine testing for viruses, which have been known to cause disease outbreaks, is of concern to the Committee. To this end, the Committee recognises the work of the NSW Shellfish Quality Assurance Program and encourages further and continuous testing of waterways where oysters are harvested including new testing methods, such as for viruses and heavy metals, the costs of which should be borne by the NSW Government to ease the burden on oyster farmers and local councils alike.*
>
> *Recommendation*
> *The Committee supports the work of the NSW Shellfish Quality Assurance Program and encourages further and continuous testing of waterways where oysters are harvested. In addition, the Committee encourages the exploration of new testing methods to consider different risks to oysters, including viruses and heavy metals." (Source: Ref: NSWP, 2012)*

Enquiries to ascertain the degree to which any subsequent adoption of viral testing has been implemented and whether this has been provided using public resources have indicated that no measures have yet been put in place. Anecdotal observations indicate that in the absence of a NoV outbreak, this issue has a relatively low political priority. It is understood that whilst there maybe scope to improve wastewater codes of practice implementation of regulatory monitoring by the Local Councils is likely to be resource limited. Furthermore, public resource availability for viral testing is not considered likely with all quality assurance funded by the shellfish industry based on bacteriological requirements.

Exhaustive investigations can help determine the level of human wastewater contamination contributions from surfacewater and groundwater sources (Ref: Lucas *et al.,* 2007) although they require considerable resources and may still be inconclusive. An intensive study on the Tilligerry Creek, which had suffered periodic drops in microbial quality to shellfish waters, used a variety of chemical testing techniques following sampling exercises which were linked into groundwater and rainfall monitoring. Despite previous public health issues in this catchment only non-human faecal contamination from surface waters were identified at the time of study highlighting that even complex and expensive surveys have difficulty in detecting transitory human wastewater contamination 'events'. However, other simpler and cheaper qualitative dye studies have been explored by NSW authorities to assess wastewater impact on shellfisheries (Ref: Baker, 2014).

It is possible that although there is conflict over the use of marine resources within NSW the low population density in Tasmania and Southern Australia may provide scope for the oyster industry to continue expansion elsewhere in areas not subject to contamination from multiple wastewater discharge sources.

4.2.5 NSW Future Developments

Many of the recommendations from the Hay *et al.,* (Ref: 2013) report (Section 4.2.3) should be picked up by a PIRSA-SARDI study currently underway. Felicity Brake, a PhD student, working with Dr Cath McLeod is conducting a series of studies including:

- A survey of NoV occurrence in Australian oysters from six production areas in the three main oyster producing States following sampling from 2010-2011. Results (obtained during a period with no reported NoV outbreaks) indicated a very low incidence (<2%) of detected GII and no detection of GI positive oyster samples. (Ref: Brake *et al*., 2014 a)
- A spatial and temporal study of NoV in oysters following a spill event. A spatial survey (Ref: Brake *et al*., 2014b) looked at the impact of a wet weather triggered 3000m^3 wastewater overflow upon NoV uptake in oysters. Following the event NoV was detectable in oysters close to the source for 6 weeks with an impact) as far downstream as 5.3km from the source with proximity concentration gradient at the seven monitoring sites.

- A risk profile for NoV in Sanitary Surveys.

The output from the ISSC and updates to the NSSP MO (Section 4.1.2) are eagerly awaited.

4.3 New Zealand

This case study provides an overview (Section 4.1.1) of the management of public health risks from shellfish consumption in New Zealand (New Zealand) from commercial and recreational sources with respect to the potential impact from wastewater sources. Shellfish (Section 4.1.2) and wastewater (Section 4.1.3-4.3.4) management and regulatory controls are presented and the sensitivity to continuous and sewage spill events explored. An illustration is provided (Section 4.1.5) of the potential proximity related impact of a continuous wastewater discharge to NoV in shellfish for an extended length of coastline.

4.3.1 Overview

New Zealand Aquaculture Industry

New Zealand has valuable aquaculture and fisheries sectors with I-inshore shellfish production in 2013 of 3,764T worth NZ$ 296 million (MPI, 2013a). Whilst New Zealand has rich primary resources the country has been successful in processing added value products and exporting around the world. The export value in 2012 was NZ$ 279 million and NZ$ 1,221 million for aquaculture and wild capture mussels (the principal BMS export species) respectively (MPI, 2013b). Although China forms the principal market for inshore shellfish fishery products (69%) aquaculture trade is dominated by exports to US (24%), EU (15%) and Australia (15%) (Ref: MPI, 2013b). Overall aquaculture production of 45,000 tonnes peaked in 2011 (Ref: MPI 2013b).

Aquaculture production is primarily composed of salmon, mussels and oysters worth NZ$ 65 million, NZ$35 million and NZ$8 million respectively (2011 prices from Ref: MFA, 2014). Aquaculture shellfish production in 2011 (primary product) stood at 101,311T of mussels and 1,804T oysters with most product exported (Ref: MFA, 2014)

Historically, landings of the dredge oyster (*Ostrea chilensis*) were important, although disease with *Bonamia exitiosa* in recent decades has significantly impacted this wild fishery. The Pacific oyster (*Crassostrea gigas*) is the principal oyster species used in commercial aquaculture production. Although low levels of horse mussel (*Atrina zelandica*) is landed, the Greenlipped mussel (*Perna canalicula*) is the principal mussel produced in commercial aquaculture.

Under the Common Levies Act aquaculture industry pays levy upon production which helps fund Aquaculture New Zealand and the New Zealand Seafood Industry Council Ltd to provide representation and support training and research. The planning process for aquaculture is somewhat different from the UK with well developed Coastal Zone Management at a Regional Council level which allows marine farms to be established under lease within Aquaculture Management Areas designated within the Resource Management Act (1991).

Commercial Shellfish Production

Shellfish production is focussed in a limited number of key areas as shown in Figure 4.2 with aquaculture production of Pacific oysters largely limited to the areas in North Island and the Marlborough region. Offshore dredge oysters are primarily fished in the Marlborough and Stewart Island regions.

Figure 4.2: New Zealand marine aquaculture commercial production areas (*Source: Ref: MFA, 2014*)

Commercial shellfish production areas are generally away from the principal cities and towns. The top 15 urban areas, which are supported by good wastewater treatment, contribute 75% of the countries human population. The wastewater treatment and sewerage system are considered to provide a good level of provision for commercial shellfish under routine conditions with contingency arrangements to address ‘events’ (see sub-Section 4.3.4).

Non-Commercial Shellfish Recreational Sources

Non-commercial recreational shellfish collection for personal consumption is likely to present an ongoing level of risk with surveys showing that enteric viruses occur frequently in stocks near sewage outfalls and following sewage discharge events (Ref: Greening *et al.*, 2009). As there is a strong cultural heritage of Maori artisanal shoreline foraging for mahinga kai (fish, shellfish and seaweed) which may be consumed raw there is a need for appropriate public health protection. Protection measures encompass the posting of guidance notices to notify users, whilst QMRA measures for wastewater discharges (see Section 4.3.4) address both designated commercial and non-commercial stocks.

Risk Profile of Production Areas

New Zealand has been unique in its ability to produce quality shellfish to meet the standards of both the US market (based on water quality) and the EU market (based on flesh quality). The foundation for this commercial success has been a combination of an extensive largely pristine coastline with a relatively small human population (4.4 million, 2011). The principal New Zealand water quality concerns relate to animal faecal coliform loading resulting from both natural (e.g. 80 million possums) and agricultural sources. For a country the size of the UK with less than a tenth of the human population there are ten times the levels of livestock loading. The dairy industry forms the largest primary export sector worth just under NZ$ 15,000 M/yr and has roughly doubled in value over the last 10 years (Ref: MPI, 2013a). It is understood that despite increased livestock faecal coliform loading, the receiving water characteristics are such that the vast majority of shellfish waters are the equivalent of EU Class A quality.

Although historically some NoV problems have been experienced with oyster stocks, enhanced risk management measures (see Section 4.3.3) have been effectively implemented to limit adverse impact. It should be noted that the risk profile for the New Zealand shellfish industry is quite different from that of the UK.

A risk profile for NoV in New Zealand shellfish was conducted in 2009 (Ref: Greening *et al.*, 2009) to inform a Risk Management Framework upon which regulation is based. This study encompassed an assessment of the NoV incidence in non-commercial and commercial shellfish stocks (see following sub-section) in the context of New Zealand shellfish consumption study. The study estimated 16% (~65,000 cases) of NoV infections attributed to shellfish transmission each year. Oyster consumption was recognised as the principal vector with continued outbreaks attributable to commercial product and widespread contamination in feral wild non-commercial shellfish.

4.3.2 New Zealand Shellfish Regulations and Management

Bivalve Molluscan Shellfish Regulated Control Scheme (BMSRCS)

The commercial shellfish industry operates under the Bivalve Molluscan Shellfish Regulated Control Scheme (BMSRCS), administered by the New Zealand Food Safety Authority, encompasses the Animal Products (Regulated Control Scheme – Bivalve Molluscan Shellfish) Regulations 2006 and the Animal Products (Specification for Bivalve Molluscan Shellfish) Notice 2006 (Refs: Greening and McCoubrey 2010, Busby 2009.). The BMSRCS assimilates the requirements for the US FDA NSSP (see Section 4.1.2) and has been enhanced to manage NoV with the aim of having no sewage contamination in any shellfish growing areas. Although New Zealand has closely followed the USA program, the NSSP has gradually evolved to the current BMSRCS through a series of additions initially to allow New Zealand to meet EU export requirements and then to the degree where it has enhanced the original NSSP requirements. Initially in 1980, New Zealand directly followed the NSSP requirements via a MOU between New Zealand and US FDA in order to allow export shellfish to USA. In 1995 the New Zealand Fishing Industry Agreed Implementation Standards: IAIS 005.1 Shellfish Quality Assurance Circular 1995 which was issued pursuant to Regulation 19 of the Fish Export Processing Regulations 1995. Then the IAIS005.1 was rewritten/modernised to the current BMS RC in 2006.

The BMSRCS sets out control measures for classification of production areas around continuous discharges as well as emergency measures following outbreak or sewage event. As an overview during NoV illness and sewage events the BMSRCS requires:

- Closure and/or reclassification of the production area
- Identification of the NoV catchment contamination sources
- Elimination of the sources
- Closure of the production area for 28 days after elimination of sources
- Taking 5 spatially separated representative samples from production area for examination of NoV
- Absence of NoV in all samples before re-opening of area

Prohibition Zones Around Wastewater Discharges

As with the NSSP the BMSRCS makes provision for prohibition zones around continuous discharges following a sanitary survey to identify sources of contamination likely to impact on growing areas. This allows appropriate classification of shellfish production areas and provides the necessary separation from discharge point sources.

Clause 29 of the Animal Products Notice 2006 specifies the criteria for the setting of prohibition zones as described below:

- Sub-clause (1) makes provision for extraction of undersize shellfish spat from prohibition zones with a requirement to relay for a minimum of 6 months (Sub-clause (6)) within an area of appropriate classification status.
- Sub-clause (3) links prohibition status to Risk Assessment (Part 13) criteria which are considered further in the following sub-section.
- Sub-clause (4) sets out that the prohibition zone should allow:
    - Area immediately surrounding point source discharge is prohibited
    - Prohibited area must be large enough to allow sufficient time for emergency closure of an adjacent shellfish production zone in a event of an emergency failure
    - A minimum 500m zone set for sewage discharges
    - Location of shellfish resources and production area boundaries are clearly defined.

- Zone sizing to encompass: discharge characteristics, decay rate of pathogen, receiving water characteristics, dilution/dispersion characteristics and use of an approved model to determine sizing of zone.

Current and dilution studies are undertaken to support sewage plume dispersal modelling using US EPA supported models (see Visual Plumes Modelling in Section 5.2) to make sure that sewage does not impact growing areas by the setting of appropriate Prohibition Zones.

Vessel discharge prohibition zones are also considered in the Animal Products Notice 2006 in common with NSSP requirements. Part 14 (Clauses 81-83) set out the classification status and zoning requirements which are similar to that of sewage discharges in their need to model bacteriological loadings and dilution in order to attain water quality faecal coliform standards. In the absence of marina occupancy data set loading criteria defaults are provided. No mention of viral considerations is made.

Emergency Conditions - Outbreaks / Spill Events

Overall the threat from significant continuous discharges is well managed through wastewater treatment and careful proximity controls between discharge locations designated shellfish production areas. This has largely been achieved by high levels of wastewater treatment, with disinfection to inactivate pathogens, coupled with good separation between the main urban areas and commercial growing areas. However, smaller localised intermittent spill events which are difficult to manage are more problematic and present the principal NoV threat in New Zealand (Ref: Greening and Lewis, 2007b).

The Animal Products Notice 2006 makes provision for 'sewage events' within the Risk Assessment section (Part 13). These measures recognise that bacterial indicators may not always provide a robust management tool and make provision to encompass pathogens such as NoV. Part 13 sets out the roles and responsibilities of the Animal Product Officer which are comparable to that of Environmental Health Officers (EHOs) in the UK. This is centred on investigation of outbreaks in which Bivalve Molluscan Shellfish have been implicated and sets out a framework for investigation, area closure and reassessment of classification status. As an overview:

- Clause 77 relating to the presence of human pathogens in shellfish indicates that once the health officer has investigated the outbreak if the growing area is implicated as the source the area will remain closed until the correct classification status can be determined.
- Clause 78 states that the growing area can be closed for 28 days if the animal product officer reasonably believes impact from a sewage event.
- Clause 79 sets out risk management and tolerance levels for when a pathogen is present in shellfish samples regardless of whether illness has occurred and is considered further in the following sub-section.

Busby (Ref: 2009) reports that the New Zealand approach to NoV outbreaks and ‘sewage events’ was successfully applied 4 times between implementation in 2006 and the time of reporting in 2008. It is understood that 28 day closures are instigated around 5-6 times a year although a site specific evidence based response is adopted case by case. Wastewater spill conditions and frequency are considered further in Sub-Section 4.3.4.

Viral Testing of Shellfish and Future Management Tools

Although regulatory tools incorporate viral testing to help support risk management decisions there is no drive to embed routine testing into regulatory programmes.

Clause 79 Sub-clause (3) states that when a tolerance level for a pathogen is not known then the Animal Product Officer must seek guidance from the regional shellfish specialist to help assess the public health significance of the levels of pathogen found in the shellfish. Then depending on whether levels are acceptable or not there is scope to either open or close the growing area. The threshold for NoV is not specified within the Animal Products Notice 2006, although the assessment of the legislation (Refs: Greening and Lewis, 2007b; Greening and McCoubrey 2010 indicates that even the presence of enteric viruses will prevent a re-opening of the growing area.

The Institute of Environmental Science and Research recommended post-spill viral sampling at 2, 4 and 6 weeks following an event to ensure absence of viruses before the re-opening of shellfish sites (Ref: Greening, 2007b). However, it is understood that routine NoV shellfish testing is not considered an integral part of future regulatory controls, particularly whilst viability remains an uncertainty with current RT-PCR tests. This perspective and need to develop appropriate management tools for a range of shellfish related threats (including Vibrios and biotoxins) has led to the development of a range of regulatory control measures in New Zealand without reliance on the use of a NoV standard (Ref: Busby, 2009). It is understood that ordinarily so long as no related illness has occurred the area is reopened after the 28 day period without the need for further sample analysis.

New Zealand researchers have progressed with the development of new molecular tools to assess NoV viability (Ref: Wolf *et al.,* 2009) and development continues in this field. In addition to viability uncertainty the CEN quantitative NoV method is thought unlikely to provide a consistent risk management tool in New Zealand as main commercial shellfish growing areas are away from major centres of population and are only subject to sporadic NoV loading.

Post-Harvest Decontamination

Most New Zealand shellfisheries are equivalent of EU Class A status and as such are largely market ready. Depuration is not considered an effective viral risk management option and only one plant is in operation, although even this is primarily for conditioning stock. Although most shellfish growing areas are fit for direct human consumption there is scope for post-harvest treatment through cooking or relaying.

4.3.3 New Zealand Illustration of NoV Impact and Industry Engagement

NoV Incidence in New Zealand Shellfish Stocks

Despite the good bacterial quality of commercial shellfisheries in New Zealand the consumption of shellfish has historically been associated with gastroenteritis outbreaks caused by NoV following faecal contamination of growing waters with human wastewaters. Over 50 NoV outbreaks have been reported since 1994 in New Zealand have been linked to consumption of either New Zealand commercially grown oysters or imported oysters (Ref: Greening, 2007). In some cases foreign imports have been implicated such as in 2006 where Korean stocks were traced as a source, whilst in others such as in 2008 commercial growing areas have been identified as the source.

In order to characterise New Zealand shellfish quality an extensive survey was conducted from 2004-2006 obtaining 360 samples from 28 sites (Ref: Greening and Lewis, 2007b). Forty-eight percent of samples were positive for one or more human enteric viruses with all but 2 sample sites exhibiting some presence of viruses on at least one occasion. In addition, apart from sites in a close proximity to wastewater discharges there was a poor correlation between viral incidence and FNRA phage levels. The inclusion of non-commercial sample sites in the survey was reflected in the high incidence (>83%) of samples which exhibited *E. coli* levels >230 counts/100g flesh and ~33% of samples with >4,600 counts/100g flesh.

Impact of NoV Outbreaks

Historically, prior to the introduction of the BMSRCS some high profile NoV outbreaks provided a strong impetus to introduce control measures. A number of cases of gastroenteritis involving NoV were linked to oyster consumption from leases in the Lower and Mid Waikare during the period from 1994 until 2001. Following the final outbreak in 2001, the Lower and Mid Waikare Inlet in the Bay of Islands area were reclassified as "restricted", leaving only the leases in the Upper Waikare as "conditionally approved". Following these changes, a number of improvements were put into operation within the catchment by the territorial authorities and others to try to improve the seawater quality of the area. A second 12 year report was filed in 2005 however, the writer of this report found that there was insufficient evidence to reclassify the area at that time i.e. remained restricted – although relay conditions were for at least 60 days to another growing area were allowed. From 2001 for a period of seven years, 14 oyster farms in the Bay of Islands area were unable to operate their farms and businesses to directly place product on the market as a result of contamination. The closed area accounted for 40% of New Zealand oyster exports and employed over forty people. The shellfish farmers contented that the source was sewage contamination from the Kawakawa WWTP into the Waikare Inlet. A long and expensive legal battle ensued in which the marine farmers tried unsuccessfully to sue the Far North District Council who operated the WWTP on the basis that they did not provide a pumping station of sufficient capacity leading to the release of 23,000m^3 of sewage. The Council highlighted that improper stormwater connections had been made to the system and ultimately the case failed when the claimants were unable to prove the source of the contamination with recreational boats also implicated as a potential source. Ultimately the Council had to invest significant sums in removing infiltration problems and increasing system capacity and provide substantial upgrading of the Kawakawa WWTP, improvements at Opua marina, new bylaw for onsite sewage systems, better policing of the marine environment and further study of the tidal impacts affecting the Inlet.

At the request of farmers and with the full co-operation, contribution and involvement of the territorial authorities and Ministry of Fisheries, a Reclassification Sanitary Survey was filed in April 2009. This report recommended the upward classification of most of the leases – from restricted to conditionally approved. As a result, New Zealand Food Safety Authority (now MPI) approved the area to be classified as "conditionally approved" subject to a rigorous Management Plan, leaving only one lease as "restricted." The case had significant financial impacts to both sides in the prolonged legal battle and the implications were felt at a national level leading to a heightened awareness of viral issues and ultimately to the implementation of the BMSRCS.

Although the BMSRCS control measures are comprehensive they cannot fully prevent foodborne illness as even minor spills from the wastewater system can have a profound impact upon commercial shellfish production. In 2008, two New Zealand outbreaks of NoV were traced to commercially produced oysters from the Coromandel region (Ref: Wall *et al.*, 2011). GI NoV was found in food samples and from the growing area and matched with epidemiology and faecal stool samples. Ultimately the source of the contamination was traced to leaking of partially treated wastewater into a pipe which discharged into a stream adjacent to the growing area. BMSRCS measures were effectively put into place leading to closure of the growing area and catchment investigations leading to location and termination from the contamination source. As with many other NoV outbreaks *E. coli* levels in shellfish samples were below the legal 230 counts/100g limit.

Industry Role in Regulatory Process

It is understood that the New Zealand regulatory programme has been fully cost recovered since 1980s. Furthermore, as indicated previously New Zealand operates a comprehensive system which incorporates both US and EU standards. Whilst this places a high level of cost burden it also provides a degree of ownership and engagement in surveillance monitoring and dynamic management of shellfish beds. Various components of shellfish regulation and management are considered below:

- *Sanitary Surveys*

  It is understood that New Zealand Sanitary Surveys are more comprehensive than UK equivalents and may take 1-2 yrs for completion before opening an area for production. It should also be noted that as the shellfish operators are paying for the Sanitary Survey they will have already undertaken their own preliminary work to assess the suitability of the area before undertaking the further investment of New Zealand $30-100K per Sanitary Survey. New Zealand Sanitary Surveys may include dye and drogue studies to assess water dispersion and dilution rates and also encompass a greater focus upon sampling and characterising worst case conditions. This is primarily so that growing areas with a 'conditional' status can be opened and closed on a predictable basis. This enhanced knowledge of the shellfishery is required to set catchment specific rainfall trigger thresholds which are vital as regulatory samples are obtained immediately following re-opening of an area following storm closure. Whilst regulatory sampling regime and classification is based upon bacteriological (Water and flesh) standards a viral oversight is always paramount and human wastewater faecal sources are always the principal contaminant aspect of interest.

- *Environmental Monitoring*

  New Zealand operates Active Management of beds using environmental data input from salinity data buoys, rain-gauges and in some cases river gauges. In all cases water quality management following storm events is linked back to the intensive studies undertaken during the original Sanitary Survey when associations between deterioration in microbial water quality is managed using surrogate parameters. Figure 4.3 provides an illustration of the Golden Bay salinity monitoring system in response to riverine loading.

- *Regulatory Sampling and Analysis*

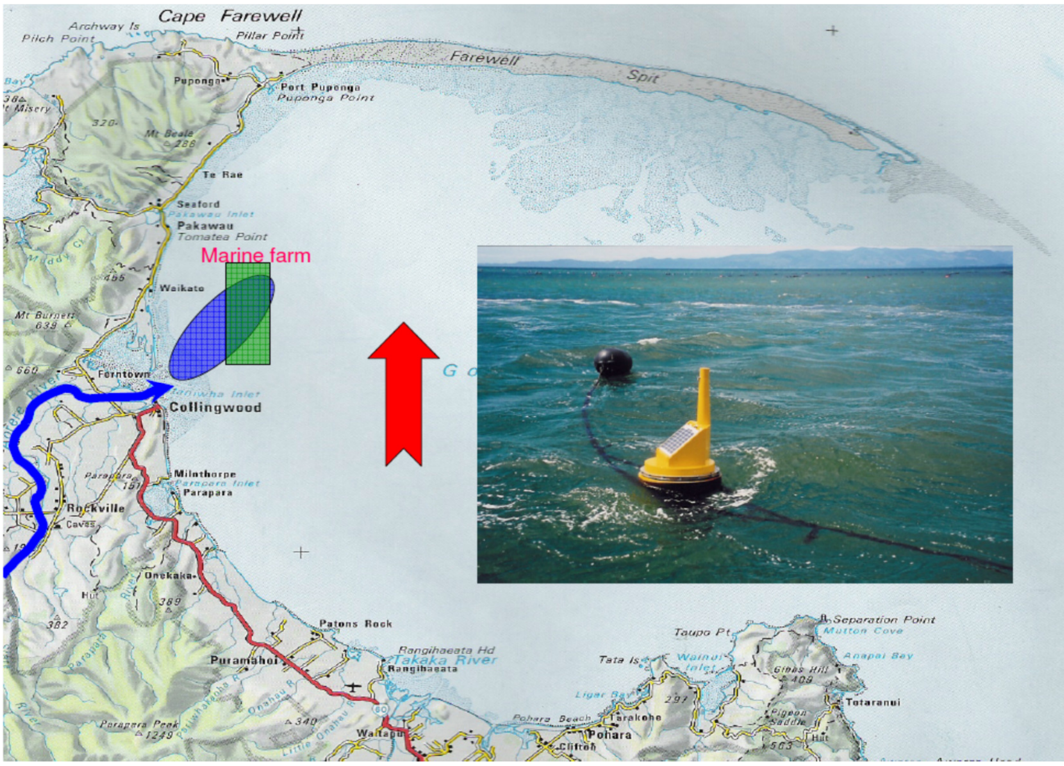
  Industry form 'regional pools' to group together to pay for accredited third party testing and sampling which is periodically let to contract and audited by regulator.

  In addition to regulatory samples some New Zealand operators retain defensive 'library' samples stored on a rolling programme for each batch to allow rapid and easy testing in the event of an outbreak.

The comprehensive engagement of New Zealand shellfish farmers in the regulatory process and access to monitoring data gives industry power to provide significant support to regulators allowing prompt action in the event of problems. Furthermore, industry awareness of local issues provides a high level of scrutiny upon other marine users potentially aiding with the identification of other catchment contamination sources.

Figure 4.3: Active management data buoy and telemetry for adverse river impact monitoring

(Source: Courtesy of Jim Sim)



4.3.4 New Zealand Wastewater Discharge Controls and Requirements

This sub-section is provided to give context to the New Zealand wastewater NoV risk profile. This will give a comparison to the UK and a means to consider where New Zealand regulatory viral risk management methods and zoning are appropriate.

Wastewater Treatment Process (WWTP)

As outlined in Section 4.3.1 above, the majority of New Zealand population is situated within urban areas which are serviced by mains sewerage systems which have seen significant investment in wastewater treatment over recent years. Many New Zealand wastewater systems were treated by relatively low technology waste stabilisation ponds which if appropriately designed and sized can provide effective treatment but lack the process control and degree of log reductions to indicator organisms and pathogens offered by modern disinfection processes. Auckland, the principal urban area on North Island has recently had its waste stabilisation ponds replaced by a state of the art WWTP at Mangere WWTP with full biological treatment and UV disinfection. This plant and associated measurements of viral loads and treatment efficacy has provided a model for much of subsequent New Zealand viral risk assessments (see Section 5.1). Hewitt *et al.* (Ref: 2011) showed that the levels of reduction through the treatment process were somewhat variable with appreciable loads in the final effluent although it was acknowledged that viability status could not be ascertained.

Although Dunedin has recently seen an upgrade to its Tahuna WWTP Section 4.3.1 provides an illustration of the potential NoV impact of a continuous discharge which at the time of study only received primary treatment with chlorination.

Wastewater Spills

As indicated in Section 4.1.1 much of the New Zealand population is urban based and largely serviced by sewerage networks. Unlike the UK the sewerage system is not generally combined and therefore not subject to the deliberate introduction of surfacewater / rainwater with the resultant high frequency of CSO spills. However, New Zealand is geologically active and the sewerage network is subject to a high level of pipework disruption giving rise to infiltration problems in some areas which can lead to spills following increased groundwater ingress resulting from higher rainfall levels.

Wastewater spill impact should differentiate non-commercial and commercial shellfish areas. Recreational shellfish collected from areas such as estuarine sites are known to present an increased risk (Ref: Greening, 2009). A QMRA report for Avon-Heathcote was conducted in 2009 (Ref: Palliser *et al.*, 2009) which highlighted the issue of wastewater spill frequency impact to estuarine systems. The QMRA was used to compare two potential consent spill frequency scenarios with Annual Reoccurrence Intervals of either 2 years or 6 months. As may be expected the study highlighted that wastewater spills significantly increased the risk of infection, especially for shellfish consumption relative to that of recreational contact. Although the QMRA study modelled shellfish exposure it was acknowledged that the Avon-Heathcote estuary itself was prohibited to commercial shellfish production due to the presence of a direct WWTP discharge. The absence of commercial shellfishiries designated in this area was also confirmed by MPI. However, the potential impact of wastewater spills upon non-commercial shellfisheries was illustrated in 2011 when sewage spills arose due to sewerage network damage caused in the New Zealand earthquake resulting in high NoV levels being found in shellfish from the Avon-Heathcote estuary. The Canterbury Medical Health Officer posted health warnings on the internet and recreational shellfish collectors were advised not to gather shellfish from the mouth of Christchurch estuaries with the internet notification supported by appropriate signage.

A study of estuarine shellfish quality (Ref: Greening *et al.*, 2009) referenced a number of mainly small sewage spills at different sites relating to leaking pump stations or broken pipes. It is understood that study areas were mainly rural with an upriver WWTP which serviced a small (< 10,000 PE) population.

Despite the high level of treatment periodic wastewater spills still occur into the Otago Harbour ~2/yr impacting upon cockle beds. After each spill the Animal Products Officer with their technical expert uses their judgement based upon specific variables such as discharge volumes, currents dilution, etc. to help determine impact, although if in doubt the growing area is closed. On one occasion Microbial Source Tracking was applied to Otago harbour stock to help decision making and allow early open of closed growing area.

NoV in Wastewater

The NoV pathogen concentration in wastewater greatly varies according to catchment health and will have a direct bearing on the magnitude of impact for any wastewater discharge or sewage event spill. Hewitt *et al.* (Ref: 2011) undertook a New Zealand wide survey of viral loads and incidence over a 6 month summer period from the end of 2003 with samples obtained from 10 WWTPs of varying size and process type. Output from this survey generally showed lower NoV counts than those experienced in some EU studies. Whilst enterovirus and adenovirus incidence in crude wastewater was related to the size of the catchment population, NoV was found to be sporadic for all WWTP. Unlike many Northern European countries, where NoV is known as 'winter vomiting bug', New Zealand NoV incidence is not thought to be seasonal and has similar reported incidence of outbreaks in the general population throughout the year (Ref: Greening, 2007).

NoV load *in crude wastewater and the subsequent treatment efficacy is a principal requirement of discharge characterisation* used in the QMRAs (see Section 5.1). The prototype QMRAs for Mangere WWTP (Ref: Ball *et al.*, 2008 was supported by intensive data gathered on a site specific basis. Subsequent QMRAs have obtained limited additional NoV measurements and drawn on previous New Zealand experience.

The impact of wet weather wastewater spills are likely to be strongly influenced by the concentration of potential pathogens which can vary by many orders of magnitude. Recognition of this important factor from a risk management perspective led to recommendations for ongoing viral monitoring of wastewater (Ref: Greening, 2007). It is understood that there is no current viral wastewater monitoring programme as a viral management tool and existing BMSRCS measures are considered adequate.

Quantitative Microbial Risk Assessments (QMRAs) for WWTP Discharges

In addition, to the NSSP requirements for the setting of Prohibition Zones under national BMSRCS requirements (see Section 4.3.2) there are also often local requirements implemented by the Regional Councils as part of the wastewater discharge consenting process but also in relation to public health notifications. Quantitative Microbial Risk Assessments (QMRAs) are a tool often used to assess potential health impacts of wastewater discharges. Many discharge consents are now supported by a QMRA although as this is not a national regulatory requirement this is not universal. It should be noted that this wastewater regulatory discharge consent requirement provides protection to all shellfish sites including recreational areas which may be closer to increased risk areas. Furthermore, QMRAs allow thresholds of acceptable risk and the recognition that an overly precautionary approach cannot prevent illness in all of the people all of the time.

Microbial water quality guidelines for recreational waters have been produced by the Ministry for the Environment (MfE, 2003). These guidelines provide microbial 'standards,' which are largely similar to shellfish water quality standards and outline the use of QMRAs. These guidelines although not legislative standards do link to other related statutory instruments. For example, Section 57 of the Resource Management Act 1991 places a requirement on Regional Councils to produce 'Coastal Policy Statements'. These include a policy statement to provide a level of care to the public and ensure adequate warning is provided to the public if water quality is degraded or rendered unsafe for shellfish gathering. The requirement to warn public is devolved from the Regional Councils to the territorial authorities with advice provided by the Medical Officer of Health.

QMRA modelling work was developed on Mangere WWTP in 2002 (reported in Ref: Ball *et al.,* 2008). Since then a number of Regional Councils have utilised this approach to help assess both water contact and shellfish foodborne related public health risks.

Although the Mangere viral model has provides a QMRA template many studies maybe adapted for specific wastewater discharges with differing study objectives.

Canterbury District Council and Hastings District Council commissioned QMRAs for the Christchurch and Hastings discharges respectively (Refs: Miller *et al.,* 2004; Norquay and Loughran, 2013). In both cases secondary biologically treated wastewater is discharged via long sea outfalls over 2km offshore with multiple port diffuser sections. Hydrometric model output is used to support the probabilistic Monte Carlo analysis which assesses impact against the MfE 2003 public health impact thresholds upon shellfish areas many kilometres away from the discharge point. It is understood that this approach has been used in recent years to assess the impact of wastewater discharges for a number of other areas (Hamilton, Tauranga and Moa Point WWTPs).

Details of the computer modelling aspects of the QMRAs are considered further in Section 5.1.

4.3.5 New Zealand Future Developments

Impact of Storm Events on Catchment Bacterial Loading

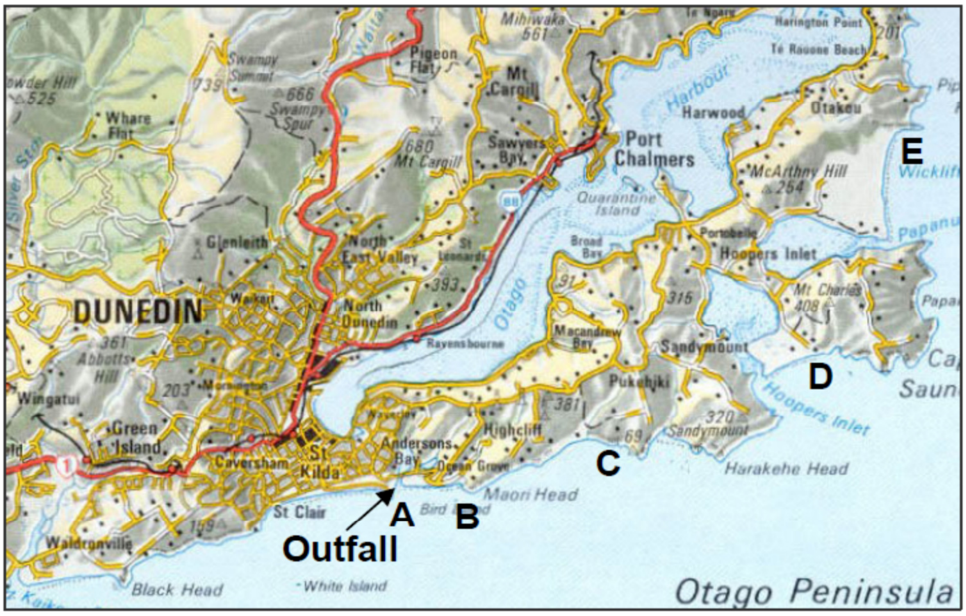
Now that appropriate management controls are in place to control NoV, principal efforts in New Zealand are now focussed upon faecal coliform indicator status. As indicated previously New Zealand is subject to elevated bacterial loading from diffuse catchment agricultural sources. Whilst high intensity rainfall events trigger temporary shellfishery closure under NSSP criteria increased storm loading to riverine systems can give rise to short term ‘spikes’ in *E. coli* water and flesh quality potentially compromising classification status in some areas. It is understood that there are plans to develop appropriate Microbial Source Tracking tools to help differentiate risk. This area has been identified as an aquaculture research area under the seafood safety programme with Cawthron planning to undertake work to assess pre- and post-harvesting risk from microbes (Ref: MPI 2013c).

4.3.6 Coastal Discharge from Large Town (Dunedin, New Zealand)

This section provides an illustration of how a continuous wastewater discharge can potentially impact NoV shellfish quality relative to the distance from the outfall. It should be noted that this illustration highlights NoV concentration profile in non-commercial shellfish and is not typical of managed New Zealand shellfish stocks.

In 2004-2006 survey was conducted to ascertain background incidence of viral contamination in shellfish throughout New Zealand as outlined in Section 4.3.3 above (Ref: Greening and Lewis, 2007). This study included a profile of NoV along the coastline from the outfall of the City of Dunedin extending down the coast for over 10km with mussel samples obtained for viral analysis at sites A-E along the Otago Peninsula (see Figure 4.4).

Figure 4.4: Shellfish viral monitoring sites for Dunedin study
(Source: Greening and Lewis, 2007)



The city of Dunedin with a population of just under 120,000 is the South Islands second largest urban area and at the time of the survey, wastewater from Dunedin received primary treatment with limited disinfection through chlorination at Tahuna WWTP. Primary treatment will have provided minimal NoV removal and whilst chlorination may have provided some inactivation the NoV RNA content is unlikely to have been reduced. In consequence, the Dunedin study illustrates the potential impact of a continuous crude discharge into a deep water setting.

Table 4.2 provides the incidence of positive virus shellfish samples which demonstrate a clear profile with 100% presence in the vicinity of the outfall but decreasing incidence with distance from the discharge point. Table 4.3 gives a breakdown of virus type between NoV, enterovirus and adenovirus and whilst there was no direct correlation on a sample by sample basis the aggregated samples again demonstrated a similar profile with decreasing incidence with distance from the outfall.

Table 4.2: Number and percentage of positive enteric virus samples for Dunedin study

(Source: Ref: Greening and Lewis, 2007)

NB. Akatore is a background sampling point from far to the SW as shown in Figure 4.6

Map Code	Site	Human waste impact [1]	No. of samples	Virus negative	Virus positive	% Virus positive
A	Lawyers Head	VH	25	0	25	100.0
B	Smaills Beach	H	21	0	21	100.0
C	Boulder Beach	H	25	3	22	88.0
D	Sandfly Bay	H	21	6	15	71.4
E	Victory Beach	L	13	4	9	69.2
	Akatore	0	3	2	1	33.3

Table 4.3: Number of positive adenovirus, enterovirus and/or norovirus samples for Dunedin study

(Source: Ref: Greening and Lewis, 2007)

Map Code	Site	No. of samples	Total viruses detected	Norovirus	Adenovirus	Enterovirus
A	Lawyers Head A	25	55	22	19	14
B	Smaills Beach	21	46	20	13	13
C	Boulder Beach	25	41	14	18	9
D	Sandfly Bay	21	30	13	9	8
E	Victory Beach	13	10	8	2	0
	Akatore	3	1	1	0	0

Quantitative NoV data for GI and GII types are presented in Table 4.4 from which it can be seen that aggregated mean NoV levels were ~1,000 genome copies/g at the outfall location and ~500 genome copies/g at 2.5km from the outfall (Smaills Beach, Site B). Although NoV was detected at other beach sites the mean NoV levels reduced with distance and were ~50 genome copies/g at 10km from the outfall location. Figure 4.5 suggests a ~1 log reduction in NoV levels over ~9km distance from the outfall. Although NoV was found in the majority of shellfish, samples 10km from the discharge quantitative levels were below the Cefas proposed standard of 200 genome copies/g Dt.

Table 4.4: Relationship between shellfish NoV levels and distance from Dunedin outfall

(Source: Ref: Greening and Lewis, 2007)

	Distance in km from outfall	GI positive /total	GII positive /total	Mean GI Units/g	Mean GII Units/g
Lawyers Head	0 km	18/25	17/25	226	707
Smaills Beach	2.5 km	13/21	15/21	269	228
Boulder Beach	7.5 km	7/25	10/25	28	41
Sandfly Bay	10 km	6/21	8/21	15	34
Victory Beach	Control	0/13	8/13	<1	61

Figure 4.5: Relationship between mean virus detection and shellfish distance from outfall

(Source: Ref: Greening and Lewis, 2007)

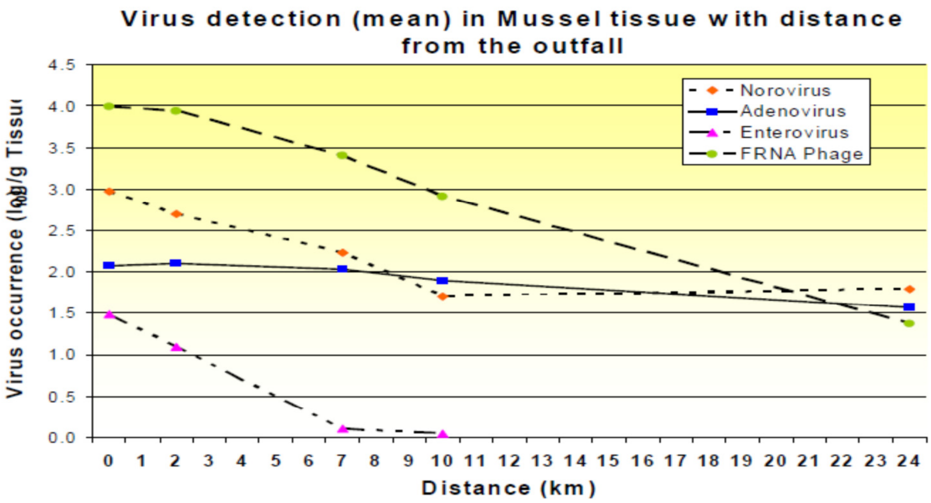
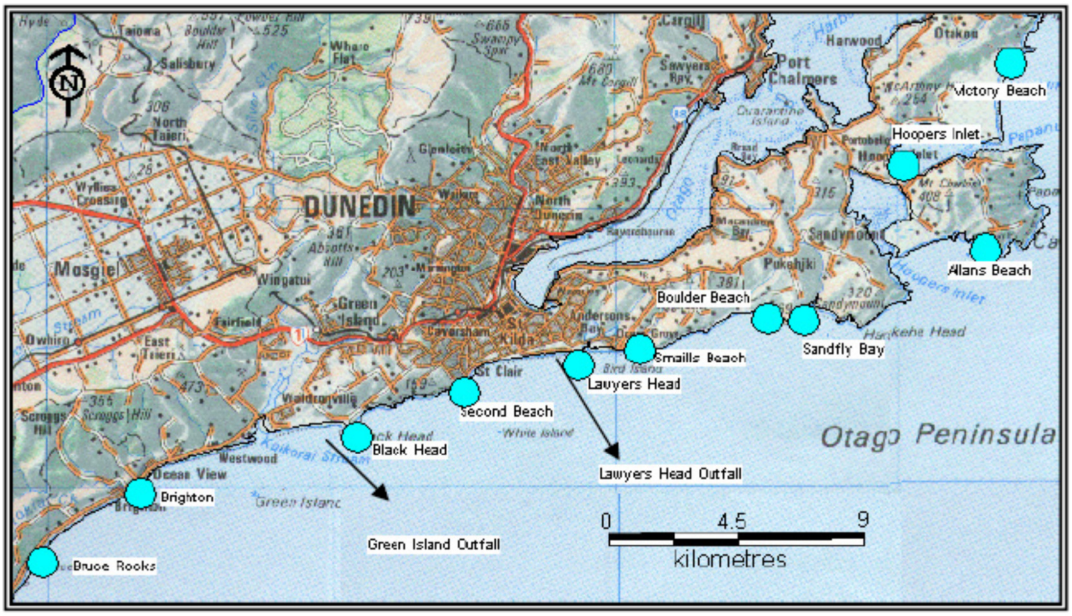
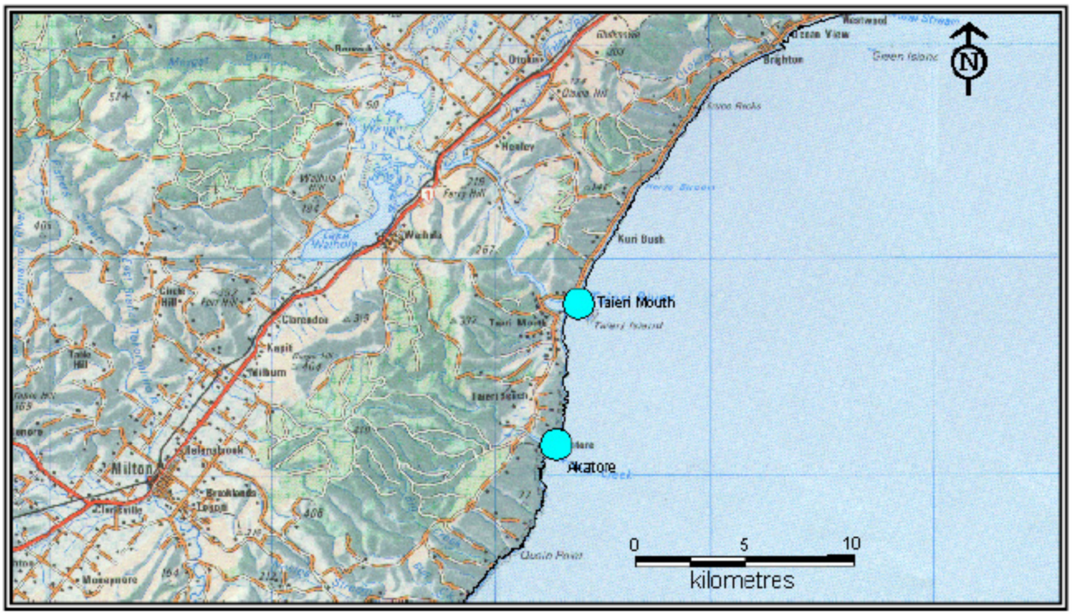


Figure 4.6 shows the seawater and shellfish flesh sampling positions monitored by the Dunedin City Council for routine faecal coliforms levels which were the same positions as those listed in Table 4.3 and 4.4. Historical bacteriological data under a similar discharge regime to that of the Greening and Lewis, (Ref: 2007) viral study provides an interesting comparison between indicators and pathogens. In 2001 Dunedin City Council commenced a consent application process to upgrade the discharge from the Tahuna WWTP (Ref: Dunedin City Council, 2001). This report reviewed seawater and shellfish flesh quality (1998-2000) along the coastline which indicated a similar pattern of reduced quality in the vicinity of the outfall. Another later (2004-2006) review of Coastal Water Quality of the Otago region coastline (Ref: Otago District Council, 2006) again showed a similar pattern of faecal coliform levels in waters and shellfish flesh with elevated bacterial levels >230

counts/100ml in the vicinity of the outfall. Although data points are not paired and numerous studies have highlighted a poor direct correlation between faecal coliforms and NoV, the data sets do exhibit similar proximity based patterns of contamination.

Figure 4.6: Shellfish and seawater bacterial monitoring sites for Dunedin city council (Note: Second map located to SW of First map) ***(Source: Ref: Otago Regional Council, 2006)***





As indicated previously this illustration is intended to highlight the potentially large scale impact of NoV wastewater discharges and is not an example of routine New Zealand management with respect to commercial shellfish areas. The wastewater from Tahuna

WWTP does not impact upon commercial shellfish aquaculture production areas with Stewart Island ~250km to the SW and the Banks area ~300km to the NE, although nearby fishery cockle beds are present within Otago Bay and are periodically impacted by intermitted spill events. A QMRA was not required for the Tahuna WWTP discharge consenting process (Ref: Dunedin City Council, 2001).

Over recent years significant wastewater scheme improvements have been provided at the Tahuna WWTP with addition of a 1,100m long sea outfall in 2009, followed by full secondary biological treatment with UV disinfection from 2013 (Ref: Crosbie *et al*., 2013). It would be interesting to ascertain what the current NoV and faecal coliform profiles are in shellfish from this region under the improved treatment regime.

4.4 European Studies - Shellfish NoV Levels and Wastewater Discharge Proximity

A limited number of studies have been conducted which relate NoV concentration (by RT-PCR) in shellfish in relation to wastewater discharge proximity. These studies are useful as they provide a 'real world' illustration of potential uptake profiles.

Studies include:

- US – Mobile Bay (Ref: Goblick *et al*., 2013) – Section 4.1.3
- New Zealand (Ref: Greening *et al.,* 2009) – Section 4.3.6
- Ireland (Ref: Dore *et al.,* 2007) – Section 4.4.1
- UK – Scotland (Ref: Cook *et al.,* 2009) – Section 4.4.2
- UK – Wales (Ref: Winterbourn *et al.* 2013) – Section 4.4.3
- UK – England (Ref: Campos *et al.,* 2013) – Section 4.4.4

In most cases the shellfish NoV results have been analysed by a method similar to that of the standardised CEN approach and are expressed in terms of genome copies/g Dt. In contrast, the US Case Study from Mobile Bay Alabama and the Irish study use different methodology or reporting approaches. Whilst their stand-alone quantitative value is useful to assess discharge proximity profile they are not inter-comparable against the proposed NoV standards under consideration.

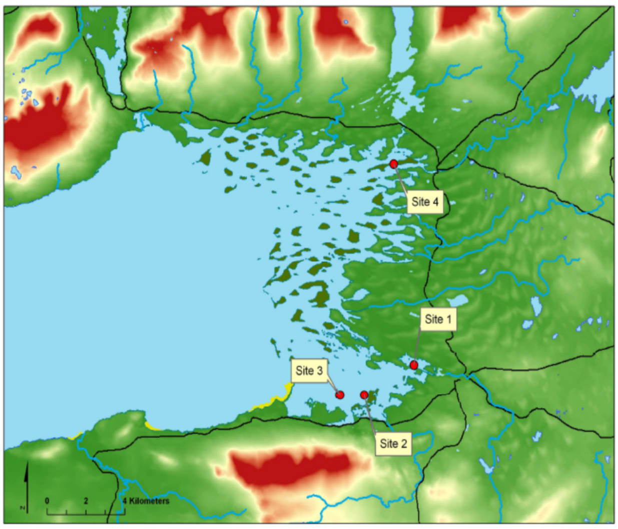
4.4.1 Coastal Discharge from Small Town (West Coast, Republic of Ireland)

Researchers from the Marine Institute in Ireland have undertaken key work in a number of NoV related research areas over a number of years. The EU Framework 6 SEAFOOD Plus-REDRISK project (Ref: Dore *et al.*, 2007) with the principal objective to identify the key environmental factors responsible for viral contamination in shellfish harvesting areas. The project further aimed to investigate the potential to develop a risk based management strategy for controlling the risk associated with viral contamination.

Figure 4.7a shows the oyster monitoring sites off the west coast of Ireland with varying proximity (300m to 4,500m) to a secondary treated discharge serving a 6,600 PE settlement. A summary of the microbial indicator and quantitative NoV data is reproduced in Figure 4.7b from which a clear concentration profile related to proximity can be seen. Figure 4.8 presents the time series of NoV concentration for Sites 1-3 from which it can be seen that increasing distance from the outfall not only reduced concentration but also incidence of detection varying from NoV detection on most occasions at Site 1 (60%) to only periodic detection at Site 3 (10%).

Figure 4.7: SEAFOODplus – REDRISK wastewater NoV impact study sites and data summary *(Source: Ref: Dore et al., 2007)*

a) Site Locations (Distance from outfall, 1=300m, 2=3500m and 3=4500m)



b) Microbiological Data in Oysters at Sites 1/2/3.

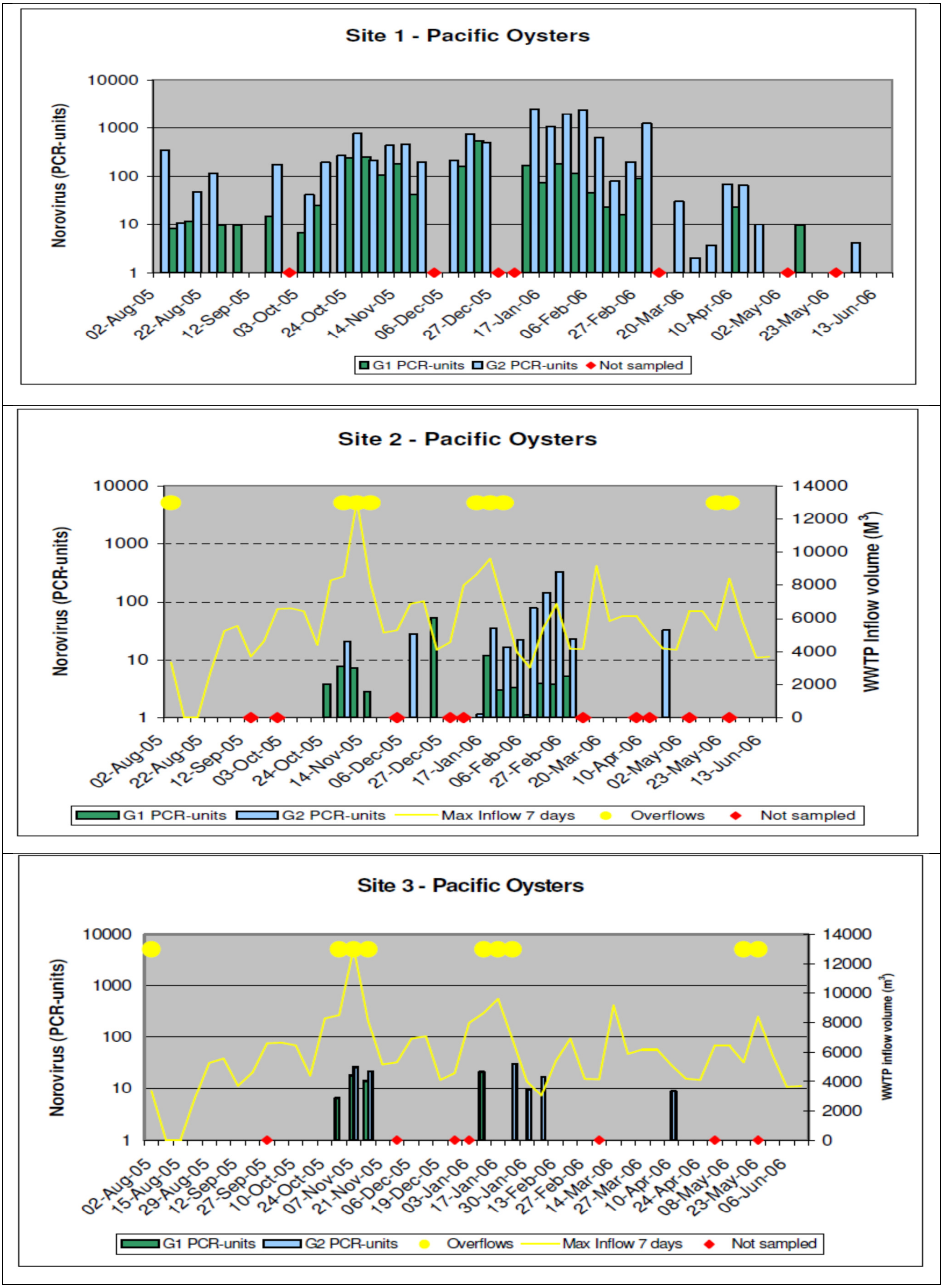
	Site 1	Site 2	Site 3
E coli (MPN $100g^{-1}$)	n=41	n=36	n=41
Maximum level	3500	5400	2400
Geomean	406	30	28
FRNA phage (pfu $100g^{-1}$)	n=37	n=33	n=38
Max	40,050	6,043	825
Geomean	406	40	14
NoV GI	n=40	n=36	n=42
Max (PCR units)	544	54	21
% positive	60	36	10
NoV GII	n=40	n=38	n=42
Max (PCR units)	2471	325	31
% positive	78	31	14

Although this study demonstrates a clear relationship between proximity and NoV concentration it also highlights how periods of increased risk can have a profound effect upon shellfish quality for an extended distance from the discharge point. All three sites experienced a similar pattern of contamination with peak levels corresponding to a winter maxima and potential storm spill impact. Overflows of untreated sewage caused by high rainfall events occurred on four occasions two of which were over the winter when increased NoV loading is expected. The winter overflow events were associated with virus contamination in oysters whilst the summer events were not.

In terms of measuring potential impact against the proposed 200 genome copies/g DT NoV standard it should be noted that only Site 1 in close proximity to the discharge (300m) had mean NoV levels which frequently exceeded the 200 genome copies/g level. However, even Site 2 (3500m) from the outfall experience a peak NoV level above this threshold illustrating the extensive impact of a relatively small wastewater population discharge. Although NoV positive levels were found at Sites 2 and 3, it is clear that on most occasions the 200 genome copies/g threshold was rarely exceeded – and could potentially be managed if seasonality and spill event risk factors were adequately understood.

The report highlights that such overflow events corresponding to seasonal increased NoV wastewater catchment loading present an increased risk of shellfish contamination. Notification of these spill events could act as triggers to initiate increased public health control measures such as temporary suspension of harvesting or increased post harvest treatments. It is noted that this source highlights the need to develop close links between waste water treatment plant managers, shellfish producers and risk managers to adopt this approach. Near real-time interventions triggered by environmental monitoring in shellfisheries to improve consumer protection against the risk of viral illness is considered further in Section 6.1.4.

Figure 4.8: SEAFOODplus – REDRISK time series of wastewater flow/ regime and NoV in shellfish ***(Source: Ref: Dore et al., 2007)***



4.4.2 Coastal Discharge from Small Settlement (West Coast, Scotland)

Small septic tank discharges can still have a significant impact upon shellfish NoV quality which is not necessarily reflected by routine bacterial indicator monitoring. Following an outbreak of viral gastroenteritis in the summer 2007 attributed to suspected NoV contaminated Pacific oysters, a year long investigation was undertaken by Cefas to ascertain patterns of contamination in the area (Ref: Cook *et al.*, 2009).

Sample sites relative to discharge positions from a Western Scottish production area are shown in Figure 4.9. The study measured GI and GII levels in oysters in relation to their proximity to two septic tank discharges as shown in Table 4.5. Monitoring over a 12 month period showed a high prevalence of GI and GII with a concentration gradient consistent with proximity to the suspected source of contamination. Although contamination levels did not reach that from the original suspected oyster batch a marked seasonal pattern in GII contamination levels was obtained although it should be noted there was no significant correlation with *E. coli* levels. Four shoreline oyster beds were monitored for NoV and *E. coli* adjacent to a couple of septic tank discharges ~0.5km from nearest bed. The NoV data showed both seasonal variation and a clear spatial pattern with a concentration gradient in the shellfish related to proximity to the discharges. Peak NoV levels were experienced in February 2008 and exceeded the proposed 200 genome copies/g standard at three of the four stations upto ~1.2km distance from the closest discharge. A maxima NoV GII of 470 geome copies/g Dt was found ~0.5km from the nearest discharge. The population served by the septic tanks were relatively small at PE 35 and 50 people in the Argyll and Bute area showing that even relatively small discharges can have a significant impact.

Figure 4.9: West Coast Scotland NoV impact wastewater sources and sample stations *(Source: Ref: Cook et al., 2009)*

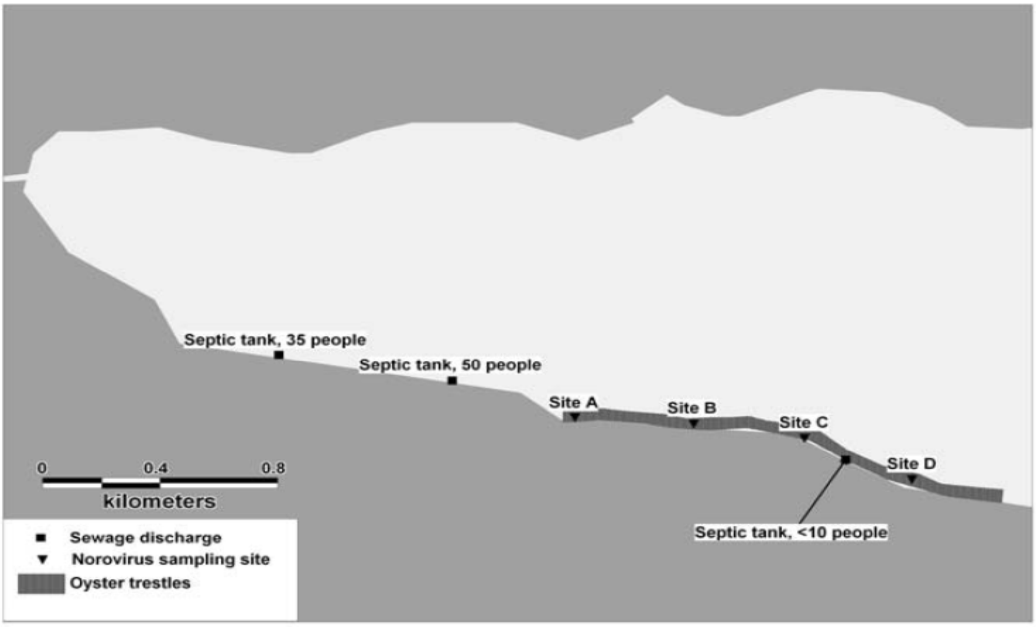


Table 4.5 West Coast Scotland NoV 2007-2008 data

((Source: Ref: Cook et al., 2009)

	Norovirus Genogroup I				Norovirus Genogroup II			
	Site A	Site B	Site C	Site D	Site A	Site B	Site C	Site D
No. of samples	11	12	12	11	11	12	12	11
Geometric mean result	2.4	1.9	3.2	3.3	28.0	17.5	8.2	4.7
Prevalence [1]	64%	42%	58%	64%	82%	83%	75%	55%
October 2007	ND	ND	ND	ND	19	9.1	25	7.3
November 2007	3.9	16	21	4.8	ND	8.8	ND	ND
December 2007	NS	1.9	8.7	NS	NS	ND	3.3	NS
January 2008	7.6	15	5.3	15	240	180	140	42
February 2008	15	15	21	8.2	470	310	250	100
March 2008	14	ND	4.4	9.7	180	27	25	100
April 2008	ND	ND	18	13	16	33	39	38
May 2008	ND	ND	ND	18	120	29	ND	ND
June 2008	ND	ND	ND	ND	ND	75	7.2	ND
July 2008	1.5	ND	ND	ND	70	46	15	7.6
August 2008	0.96	ND	ND	ND	37	ND	ND	ND
September 2008	28	39	26	7.6	20	9.5	2.8	ND

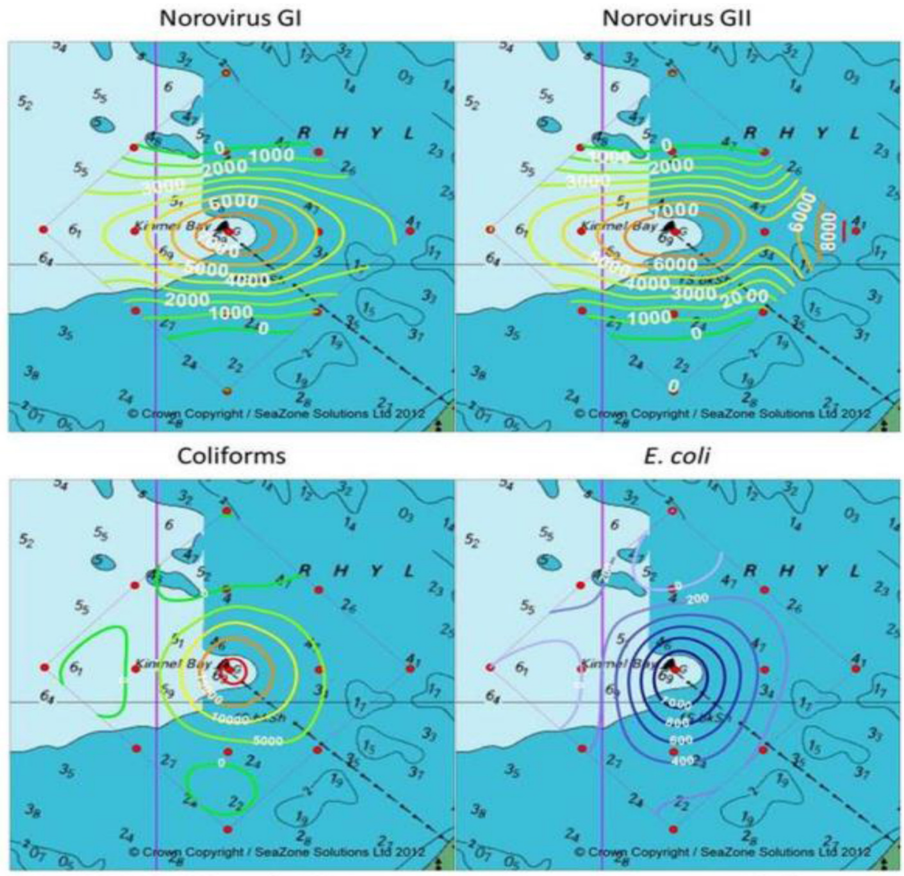
ND = not detected (assigned a nominal value of 0.48 PCR units)
NS = no sample

4.4.3 Coastal Discharge from Moderate Town (North Coast, Wales)

Researchers from Bangor University have been assessing microbial interactions in the marine environment and associated impact upon shellfish (see Figure 4.10). Part of this work included NoV monitoring within an offshore setting in the vicinity of a long sea outfall discharging treated wastewater (Ref: Winterbourn *et al*. 2014). Secondary treated wastewater from a municipal WWTP serving ~78,000 population was discharged in 6.9m depth (below Lowest Astronomical Tide) 4km offshore which should provide a good mixing environment and compliance with regulatory requirements.

Within this study moorings holding mussel bio-sentinel shellfish were deployed at 13 offshore stations in a 1km grid density around the outfall location for a 30 day period at the end of a NoV winter season when wastewater could be expected to contain NoV loading. The resultant pattern of contamination along an E-W axis was considered to be consistent with the current regime influence upon wastewater plume movement. Figure 4.10 reproduces the pattern of bacterial indicators and NoV results obtained at the end of the deployment period.

Figure 4.10 North Coast Wales NoV and indicator levels in mussels after 30day offshore exposure *(Source: Winterbourn et al., 2014)*



Peak NoV GII concentration of ~10,000 genome copies/g was found at 2km to the east of the outfall demonstrating considerable plume impact despite the good mixing environment.

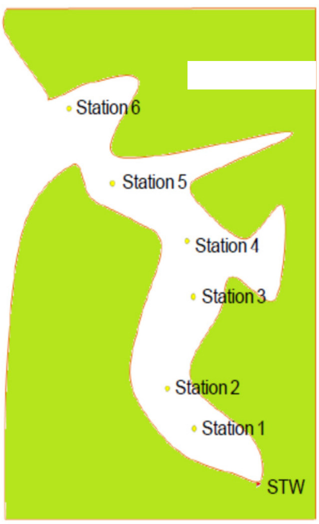
Comparison between bacterial and NoV concentration patterns did not show a good correlation which was considered to reflect a number of potential variables. Factors were thought to include differing faecal contamination sources and differing association with particles and microbial clearance rates. This dataset, as with other studies, highlights the limitations of standard bacterial indicators in assessing the health risk presented by NoV within shellfish.

4.4.4 Coastal Discharge from Large Town (South Coast, England)

Cefas recently completed Phase 1 of a project funded by Defra (Ref: Campos, 2013) to assess the impact of NoV in wastewater upon shellfish at various proximities to a wastewater discharge as illustrated in Figure 4.11a. NoV results presented in Figure 4.11b show the trend in reducing NoV level with increasing distance from the wastewater discharge from Stations 1-6 over 12km. The overall NoV concentration gradient was ~0.6 log_{10} /10km in contrast to the *E. coli* contamination gradient across the harbour was more pronounced with geometric mean levels decreasing >1 log_{10} /10km,

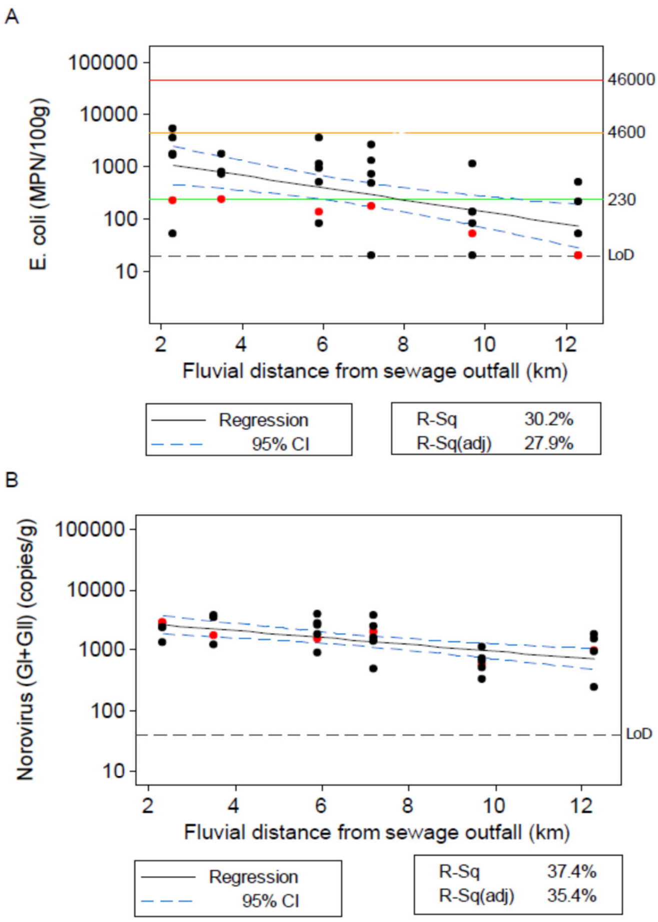
Figure 4.11: Environmental dispersion of NoV and *E. coli* and impact on shellfish quality *(Source: Ref: Campos, 2013)*

a) Schematic of Sample Stations Relative to WWTP (or STW) Discharge



Linear models: (A) log_{10} *E. coli* (MPN/100g)=3.296-0.1166*fluvial distance (Km). (B) log_{10} norovirus (GI+GII) (copies/g)=3.542-0.05694*fluvial distance (km); R^2=37.4%. Reference lines indicate thresholds for classification of shellfish production areas: class A–230 *E. coli*/100g; class B–4600 *E. coli*/100g; class C–46000 *E. coli*/100g. Red dots represent dry weather samples. LoD - limit of detection.

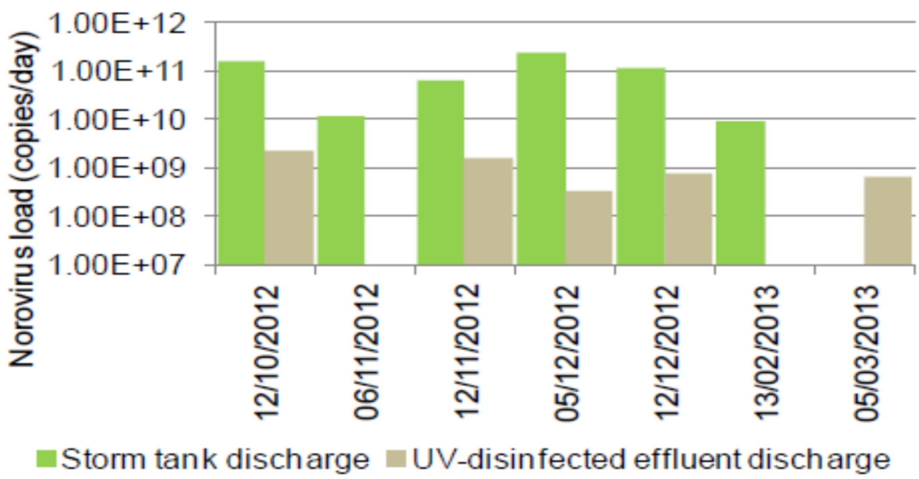
b) Relationship between E.coli and NoV in Oysters and Distance from WWTP Outfall



It is notable that 8km of dilution and dispersion was required to reduce the mean NoV contamination level down to the proposed 200 genome copies/g Dt End Product standard threshold. Of even greater significance is that even beyond this point maximum NoV levels were still occasionally greater than the proposed 1,000 genome copies/g harvest NoV threshold.

This Cefas study for Defra also highlighted the potential contribution of NoV loading from CSO discharges by determining 97.6-99.9% of NoV load from settled storm tank. Figure 4.12 reproduces the NoV loading data from the storm tank which ranged from $9x10^{9}$ to $2x10^{11}$ copies/day. Under equivalent discharge conditions, these were 1–3 orders of magnitude higher than those from the UV-disinfected effluent.

Figure 4.12: Illustration of relative untreated and treated NoV load
(Source: Ref: Campos, 2013)



This survey work is the first phase of a series of studies commissioned by Defra/FSA which will have a key bearing on the future assessment of potential proximity zones.

4.5 Case Studies - Summary

Section 4 considers Case Studies from the US/Canada, Australia and NZ who employ NSSP zoning to separate wastewater and shellfish areas on bacteriological water quality criteria. Developments to encompass viral considerations in NSSP area are currently underway and are highly significant in the determination of proximity zones around wastewater discharges from WWTPs.

- *National Shellfish Sanitation Programme (NSSP) Proximity Zones.* Proximity zones have been established elsewhere in the world with respect to the separation of wastewater discharges and shellfish harvest zones, although it should be recognised that these are with respect to bacterial indicators rather than NoV. This is particularly in the case of the US and its shellfish suppliers who participate in the NSSP. Countries include: Canada, Australia, NZ, South Korea and Chile.

  The countries participating within the NSSP are currently reviewing their control measures with respect to NoV and the applicability of current zoning and associated control measures. Although not specifically designated as ‘NoV Exclusion Zones’ these approaches provide a valuable series of case studies which are considered in Section 3.

- *Case Study Risk Assessment Output.* NoV is currently a key topic of regulatory consideration in both EU and NSSP related countries. The US/Canada case study highlighted three current NoV related programmes underway which should report in the coming year or so the NoroCORE project, the NACMCF project and the joint US/Canada NoV in Shellfish Risk Assessment. In particular the later project is currently working on a whole system model which would be very relevant to the UK considering the similar risk profile for US wastewater system types and population density. Initial indications are that a ‘whole system’ approach to risk management is currently under consideration. Australia is also understood to be currently undertaking their own Risk Profile for NoV in shellfish, whilst New Zealand has already undertaken its Risk Profile.

- *Zone Dilution and Associated Requirements.* Table 4.6 below summarises the various zone requirements for wastewater and shellfish harvesting areas.

Table 4.6: Summary of case study zone definition and associated requirements

Case Study	*Zone Definition*	*Alert System*	*Wastewater Discharge Risk Assessment*
US	1000:1 dilution zone *(Note 1)* Conditional Zone	Rapid alert system in event of system failure.	N/R
Australia	1000:1 dilution zone *(Note 1)* Conditional Zone	Rapid alert system in event of system failure.	N/R
New Zealand	1000:1 dilution zone *(Note 1)* Conditional Zone	Rapid alert system in event of system failure.	Site Specific QMRA *(Note 2)*

Note 1: Continuous treated discharge dilution zone defined through dye tracking

Note 2: Quantitative Microbial Risk Assessment to determine viral infection potential between discharge point and shellfish harvesting point (see Section 5.1)

- *Wastewater Spill Closure/Re-opening Viral testing Requirements* Despite a common NSSP framework different participating countries have varying levels of national implementation. A summary of viral related testing criteria is provided in Table 4.7 In addition, the case studies examples the French Winter Norovirus Protocol (Section 7.1.5) has a 28 day closure period but with an option for testing based early opening.

Table 4.7: Summary of case study wastewater spill event shellfish closure/re-opening viral criteria

Case Study	*Closure Period*	*Early Re-opening Option*	*All Clear Viral Testing*
US	21 days *(Note 1)*	>7 days coliphage testing *(Note 1)*	N/R
Australia	21 days	No	N/R
New Zealand	28 days	No	5 samples with no viruses detected

Note 1: Early opening testing requirements <50/100ml Male Specific Coliphage

- *Case Study Development for Impact Studies.* There is a need to obtain industry feedback from case study countries to ascertain perceived impact of currently developed viral and exclusion zone measures in NSSP countries. For example, the New Zealand Case Study illustrated the trauma of expensive high impact conflict between the oyster industry and WWTPs operators following the Bay of Islands NoV outbreak and farm closures. It would also be useful to discuss with water utility providers their discharge regulatory framework to see how it compares with the UK and in particular how wastewater management data is utilised to inform Active Management of Conditional shellfish waters.

- NoV coastal profile case studies provide quantitative RT-PCR NoV data to illustrate the impact of wastewater proximity impact on shellfish:
    - Coastal deep water discharge from large town in New Zealand (Section 4.3.6). NoV profile in mussel obtained at 2.5km exceeded the 200genome copies/g Dt threshold (>500 genome copies/g Dt) dropping to ~50 genome copies/g Dt after 10km.
    - Coastal inshore discharge from a small town on West Coast Ireland (Section 4.4.1). Direct comparison of this data against the proposed 200 genome copies/g Dt standard is not possible as the analytical method was not the CEN accepted method. Although NoV levels in oysters were frequently elevated at 0.3km from the discharge 'event' wastewater spill occasions increased contamination to the degree that equivalent NoV levels were occasionally exceed at a site 3.5km from the discharge.
    - Coastal inshore discharge from a small settlement on West Coast Scotland (Section 4.4.2). NoV profile in oysters a shoreline intertidal site had >200 genome copies/g Dt at 3 of 4 sites upto ~1.2km from two septic tank discharges serving a combined PE of 85. NoV contamination in shellfish presented a clear seasonal profile with peak levels in the winter (February). *E. coli* was not found to be a good indicator.
    - Coastal offshore discharge from a large town on North Coast Wales (Section 4.4.3). Secondary treated wastewater was discharged from a 4km long sea outfall at a depth of nearly 7m. Despite this a peak NoV GII concentration of ~10,000 genome copies/g Dt was found at 2km from the outfall

demonstrating considerable plume impact despite the good mixing environment.

- Coastal inshore discharge from a large town on South Coast England (Section 4.4.4). NoV profile in oyster obtained at 12km with 200genome copies/g Dt threshold reached after 8km. This study also demonstrated the impact of storm wastewater discharge which had a NoV loading which was 1-3 orders of magnitude greater than the UV disinfected discharge.

5 COMPUTER MODELLING

The objective of this section is primarily to assess whether existing computer modelling tools previously developed to meet *E. coli* environmental water quality regulatory requirements can be utilised to determine or help manage NoV 'exclusion zones'.

Computer models have been used for some time to understand potential impacts on shellfish waters. This has included point and areal assessment, and assessment of impacts over multiple, and varying, weather, tidal and flow conditions. These assessments have principally been undertaken for Faecal Indicator Organisms (FIOs), the bacterial indicators currently used to quantify quality. In terms of predictive model application, the use of bacteria or virus to establish impact is irrelevant; input characteristics will change, but the operation of the models, and the established techniques of assessment, remain effective and of demonstrable practical value.

A key element of current model use is the provision of detailed information on impacts across multiple scenarios, an essential element to consider in the consideration of the use of these models as management tools for Norovirus. The application of models over the last 15 years, to both shellfish water and bathing water considerations, has provided a methodology and experience which can be applied with little change to the management of Norovirus issues.

The potential setting of NoV exclusion zones based on the grounds of distance, time or dilution factors can therefore be effectively assessed using computer modelling techniques. Comprehensive coastal hydrodynamic and water quality models have been developed for a number of shellfisheries in the UK and are commonly commissoned by most Water Utilities who manage wastewater schemes discharging into Shellfish Waters and Bathing Waters. In consequence, computer models can effectively encompass the consideration of hydrological and meteorological factors that could impact on the dispersion of wastewater discharges (Section 3.5).

Hydrodynamic computer model output can also be interfaced with additional modelling components for a variety of related functions which may help understand NoV impact and behaviour:

- *Population health impact.* Computer modelling applications (such as QMRA) use Monte-Carlo models to assess shellfish impact upon population health (Section 5.1).
- *Sewerage modelling components.* Wastewater loading is particularly variable from CSOs in response to various storm events (Section 5.2.2).
- *Hydrological and microbial washoff components:* Modelling of catchment microbial loading under changing rainfall and river flow conditions.

- *Source apportionment components*. Modelling of catchment microbial loading has increasingly aimed to assess human and animal sources under baseload and storm conditions. Although these models are primarily based on assessing *E. coli* there could be benefits for NoV modelling (Section 5.2.3)

- *Sediment coupled components.* There is evidence to suggest that microbial *in-situ* processes occur which are strongly linked to sediment behaviour (Section 5.2.4).

- *Natural UV Dose Modelling.* Environmental degradation of NoV is likely to be strongly influenced by UV decay. Models exist to assess penetration through the water column as a function of meteorological and water quality features (Section 5.2.5). Current modelling practise for bacterial modelling requires a good understanding and representation of decay, which is generally driven by UV light, and this experience and approach would be readily transferrable to the modelling of NoV.

Some early NoV modelling work has already been attempted in other countries alongside standard bacterial models (Section 5.3.1). Future modelling may include the development of more predictive Artificial Neural Network (ANN) models which may link into wider environmental monitoring (Section 5.3.2). However, adaptation of existing models for NoV applications could provide a valuable risk management tool for shellfish operators and managers and could be readily available to use in some areas (Section 5.3.3).

5.1 Quantitative Microbial Risk Management (QMRA)

QMRA is a whole system modelling approach used to determine potential wastewater discharge impact on shellfish quality. The use of QMRA Monte Carlo package models such as "@RISK" for shellfish water wastewater discharge impact studies is outlined in the New Zealand case study (Section 4.3.4). QMRAs have been adapted for predicting human health effects for a range of wastewater schemes having different emphasis such as the impact of wet weather (Ref: McBride and Reeve, 2011) or of changing wastewater spill frequency (Ref: Palliser *et al.*, 2008). QMRAs can also be used to model multiple scenarios with differing discharge, riverine, seasonal and future growth/treatment efficiency input variables (e.g for Warkworth WWTP in Ref: Stott and McBride, 2008).

Use of QMRAs is becoming internationally recognised within scientific literature for a variety of uses such as recreational waters (Ref: McBride *et al.*, 2013) and even NoV impact when using wastewaters for crop irrigation (Ref: Mok *et al.,* 2014). However, shellfish water QMRA applications are strongly dependent on having a good understanding of the underlying distribution curves for each variable. In consequence, QMRAs would benefit from improved understanding in a number of areas such as Bioaccumulation Factors and human illness dose-relationship.

5.1.1 Risk Assessment Principals

As indicated previously in Section 4.3.4 in New Zealand MfE 2003 guidelines provide thresholds against which to assess QMRA output. Thresholds of illness for contact and shellfish consumption are:

- No Observable Adverse Effects Level (NOAEL), <1% illness
- Lowest Observable Adverse Effects Level (LOAEL), 1-5% illness
- Substantial elevation, 5-10% illness
- Significant risk of high levels of illness, >10% illness

Using a precautionary approach dose-response threshold for NOAEL is actually set at the risk of infection rather than risk of illness.

Ball *et al.* (Ref: 2008) makes it clear that although NoV is the principal public health enteric virus of concern, the input variables used in calculations have been drawn from a variety of viral model sources owing to the incomplete understanding of NoV behaviour. Rotavirus has been used as a representative pathogen and model virus for dose-response although bioaccumulation factors and T_{90} decay rates have been taken from other viral models (e.g. bacteriophage). A breakdown of risk assessment stages and assumptions are provided below:

- *Hazard Identification* Viruses were identified as the principal group. Rotavirus identified as 'representative' model viral pathogen particularly for NoV as:
  - moderate numbers in sewage
  - quite infectious
  - can survive for several days in environmental waters
  - detected both in environmental waters and frequently in oysters
  - waterborne and shellfish borne outbreaks have occurred
  - good clinical trial and associated dose-response data (which is limited in NoV)

- *Exposure Assessment* (what kind and level of exposure to populations) A number of parameters considered including:
  - Range of pathogen concentration in raw sewage (min, max and most likely) – generally for New Zealand assumed to be random!
  - Removal efficacy of pathogens from WWTP
  - Dilution and inactivation of pathogens in estuary (often conservative with no flocculation removal)
  - Inactivation in harbour – hourly seasonal variation in inactivation rates (based on coliphage data for New Zealand) Ref: Sinton *et al.,* 1999) and somatic coliphage

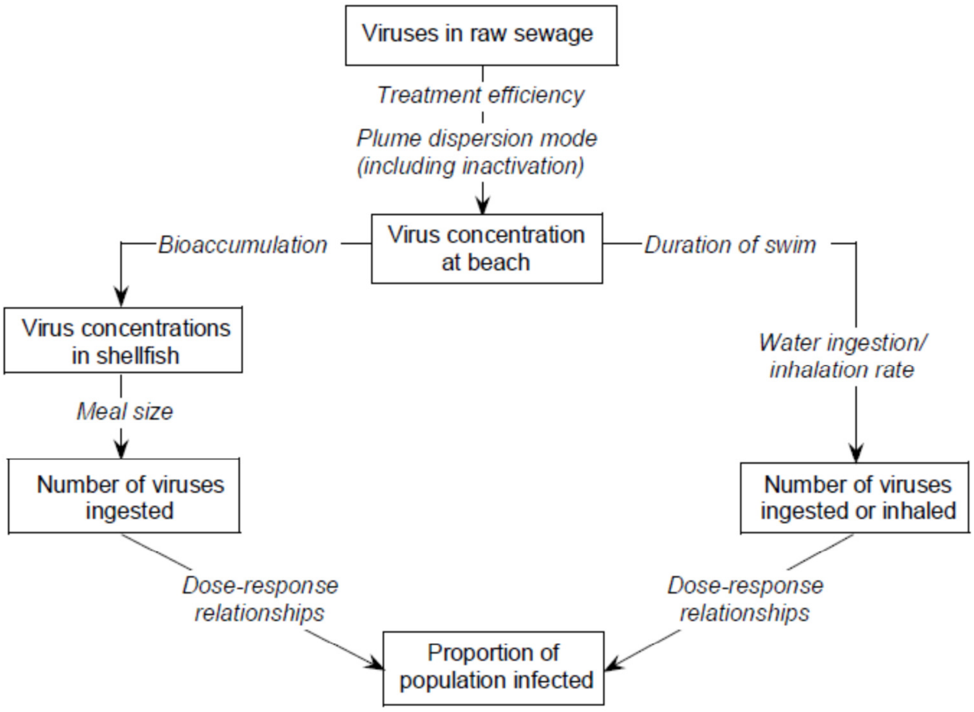
- *Dose-response assessment* (how much infection or illness would arise in population exposed to potential distribution of pathogens in shellfish). Dose – response curve from literature ID50 = 6.17 – with probability that population has variable susceptibility. Curve described by Alpha and Beta parameters for focus forming units (FFU - literature suggests 100 rotavirus particles per FFU)

– similar dose response curve to NoV – NB Infection rather than illness is threshold selected for risk assessment – precautionary response

- *Risk Characterisation* (integrates previous steps to indicate the extent of public health concern) Consumption of shellfish
  - meal size – based on national survey data (distribution curve)
  - bioaccumulation factors (BAF) from literature for FRNA phage (Ref: Burkhardt and Calci, 2000)

Input variables are modelled by Monte Carlo risk analysis even using package generic software such as '@RISK' where distribution relationships to describe variables can be selected along with number of iterations (normally 10,000). The output produces a risk profile in the form of percentage of time when people can be infected at defined points from defined at risk activities (i.e. bathing or shellfish consumption). This approach takes account of random variation and uncertainty by running a number of simulations over a large number of iterations by modelling against a defined statistical distribution. Figure 5.1 provides an outline of the QMRA sequence and variable components.

Figure 5.1: Conceptual Flow Diagram for QMRA Calculation Sequence for Swimmers and Consumers of Shellfish. *Italicised items are subject to variability and uncertainty* ***(Source: Ref: Stott and McBride. 2008)***



5.1.2 QMRA 'Water Quality NoV Standard'

A potential NoV water quality standard of 0.04 NoV/L was back-calculated by Ball *et al.* (Ref: 2008) as a threshold for potential infection through consumption of raw shellfish. This encompassed assumptions of Bioaccumulation Factor and meal size to provide an infective dose from a single exposure. A similar exercise was performed by Washington State Department of Health (Ref: 2007) as described in Section 5.3.1 which generated an even lower viral water quality standard over shellfish of 1 virus/10,000L (0.0001NoV/L).

It should be noted that the authors of the report stress that this NoV level should not be considered as a practical water quality 'standard' as it could not effectively be detected this low concentration. This 'standard' is however a composite of multiple assumptions for assessment purposes. Further work is required to provide a better understanding of underlying science such as Bioaccumulation Factors (Section

3.6.4). This critical data gap is highlighted in recommendations for future work (Section 8.1).

5.2 Existing Water Quality Models

This section considers based hydrodynamic models and additional coupled components which have been developed to provide an increasingly comprehensive tool for the assessment and management of the marine environment.

5.2.1 Standard Hydrodynamic Water Quality Models

Conventional water quality models have routinely been used to assess regulatory performance for many years. For Bathing Waters compliance over a summer season can be modelled against Guideline and Mandatory *E. coli* and Enterococci standards. In the case of Shellfish Waters in England and Wales compliance on an annual basis is considered against a 100 counts/100ml faecal coliform geometric mean as a 'standard' to achieve the Class B Shellfish Hygiene flesh quality standard. In both cases similar impact assessment approaches are used to ensure that wastewater schemes are suitably designed to meet bacteriological water quality standards.

Computer modelling is common in NSSP affiliated countries (e.g. New Zealand and US – See Section 4) and used to define the extent of 'prohibition zones' around wastewater discharges against bacterial standards. New Zealand the regulations defining prohibition zone requirements specify the use of the US EPA 'PLUMES' model. The US EPA website describes the later generation of windows based VISUAL PLUMES (VP) model which updates the previous PLUME DOS based model. VP allows use of a multi-stressor pathogen decay which predicts coliform mortality based on temperature, solar insolation and water column light adsorption (see Sub-Sections 3.5.2 and 5.2.5). VP also allows input from other 3D models to provide system flexibility. A range of simple and complex 3D MIKE hydrometric models are employed by Canadian authorities to help assess WWTP discharge impact upon shellfish waters (Ref: Roberts *et al*. 2014). It is suggested that future wastewater regulatory proximity computer modelling should review NSSP modelling practice and compatibility with existing UK 3D computer models. The Canadian authority's use of MIKE models which are also commonly used in UK (as demonstrated in Appendix B) would suggest good inter-comparability.

Hydrodynamic modelling of microbial water quality can be enhanced with additional model components to vary wastewater loading conditions (Section 5.2.2), catchment land loading conditions (Section 5.2.3) and *in-situ* processes (Sections 5.2.4 and 5.2.5) as considered further in the following sub-sections.

Researchers at the Irish Marine Institute have developed an ecosystem services approach to shellfish modelling (Dabrowski *et al.,* 2013). This Dynamic Energy Budget type of model has the potential to incorporate microbial bioaccumulation (Section 3.6.4) and when supported by appropriate *in-situ* monitoring systems (Section 7.1.4) may have scope to provide near-real time classification output. This level of sophistication is currently unlikely in the near-term for NoV applications due to the variability and uncertainty of appropriate viral input parameters. However, similar types of shellfish growth based models are established for a number of Northern Ireland shellfish waters and may have scope for future development particularly in this region.

5.2.2 Sewerage Models Components

Continuous wastewater loading from treated WWTPs (as considered in Section 5.2.1) provides a relatively constant microbial input flux for model calculations. CSO intermittent discharges provide a much more complex microbial loading situation as the volume and pattern of discharge (for catchments with multiple CSOs) will vary according to the wastewater flow regime which in turn is determined by the timing and intensity of the rainfall profile. To enable this complex loading scenario to be hydro-dynamically modelled sewerage flow modelling can be used to provide loading inputs.

With appropriate sewerage flow surveys linked to local catchment rainfall data it is possible to understand how a sewerage system will respond and spill intermittent discharges in response to rainfall events. These in turn can allow linkage of historical rainfall events of varying intensity, duration and antecedent conditions. Sewerage model output can be used both directly and indirectly for shellfish water requirements:

- Direct use of spill output can be applied to assess whether agglomerated CSO outputs discharge <10 'significant' (>50m^3) spills a year. The Water Utilities hold sewerage models for many of the UKs larger urban areas as it is one of the principal tools to assess flooding risks and the impact of CSO discharges.
- In current integrated impact assessment methodology for shellfish and bathing waters, spill output from sewerage models is used to provide pollutant loading inputs for a coupled hydrodynamic coastal model (where available). This can then be used to assess the microbial water quality at the margin of the designated shellfish area as a result of a spill event. This approach enables both the temporal and spatial variation of intermittent discharges to be assessed, as well as providing source apportionment information for particular receivers.

This study has included an adaption of an existing CSO model for NoV related parameters to provide an interactive EXCEL based 'tool' for CSO spill model output. This 'tool' can be input of CSO spill scenarios of differing profile which can be run over a range of hydrodynamic conditions (Section 5.3.3). This model is based on output from an integrated impact study using both coastal models and network models.

5.2.3 Source Apportionment Model Components

Bathing water quality (in common with the shellfish water quality under the WFD) is assessed against the faecal bacterial indicator *E. coli*. As *E. coli* and other faecal coliform sources can be derived from the guts of multiple warm bodied species, humans are not the only source of potential non-compliance. It has long been recognised that the balance of sources is site specific and that at certain seasons and conditions ruminant or avian sources may outweigh human bacterial loads.

Significant progress has been made over recent years in particular by David Kay and co-workers from Centre for Research into Environment and Health (CREH), University of Aberystwyth in understanding catchment based source apportionment and how specific basin systems may response under baseload and storm conditions. Hampson *et al*. (Ref: 2010) and Crowther *et al.* (Ref: 2011) describe apportionment modelling from a number of catchments around UK to assess land use influence on faecal bacterial water quality with potential to impact both bathing water and shellfish water compliance. This supported by Microbial Source Tracking techniques and has highlighted how diffuse agricultural loads may dominate the contribution of faecal coliforms under storm conditions with significant implications for both bathing water and shellfish water compliance (in contrast to NoV contamination). Models have been developed to determine potential loads based on landuse, agriculture type and catchment characteristics to better reflect compliance when human wastewater sources may not be the dominant controlling variable.

Buogeard *et al.,* (Ref: 2009) coupled catchment watershed and marine models for the river Daoulas in the Bay of Brest. This work encompassed a Soil and Water Assessment Tool (SWAT), which is a catchment process-based model to address microbial contamination of water caused by point and non-point sources integrating land use, soil, topography, rainfall and other climatic data. Simulation of the dispersion and dilution of the contaminated riverine plume in the estuary was conducted using a MARS-2D hydrodynamic model created by Ifremer incorporating realistic wind and tide values and a die-off rate for bacteria. Plume status for HW and LW spring and neap tides post-rainfall event were modelled and using a range of bioaccumulation factors used to provide comparison against shellfish flesh microbial data.

A clear understanding of source apportionment on a catchment basis would be beneficial to differentiate regulatory compliance issues (using *E. coli*) from viral risk management. The concept of source apportionment should be clearly understood, as it can be used in a number of ways;

- The field based approach described above provides an apportionment of various sources as they enter the environment. It does NOT provide quantified information regarding the actual breakdown of significance of impact at the receiver, as generally the field survey only identifies a small number of environmental scenarios within which load and impact is measured.
- Source apportionment derived from modelling assessments provides a detailed breakdown of the significance of individual sources to impact, as the models calculate the importance of each source. As many thousands of

impact scenarios can be modelled, across a wide variety of source profiles, a detailed understanding of the importance of particular sources is gained.

This is potentially significant, as dependent on the proximity of the source to the shellfish water or harvesting area, and the spill profile of the source, can have a dramatic effect on performance. This may not be identified during the relatively short period of a field survey.

5.2.4 Sediment Coupled Models

Water quality models can be effective at assessing hydrodynamic movement and physical dispersion characteristics to determine water quality as a result of microbial loading. However, existing generic models struggle to effectively reflect *in situ* processes which may control decay rates and resuspension. These complex processes are influenced by a number of variables (see Sections 3.5.2 and 3.5.3) which are not fully understood and often very site specific. Most existing models use first order decay rates for microbes and will accommodate a degree of ground truthed data to reflect different conditions (e.g. light or dark). In practice, within the water column the interaction between microbes and suspended solids is a complex dynamic process which differs over a diurnal and tidal basis, in response to storm events and proximity to the shoreline (Thupaki *et al.*, 2013).

The association between bacterial indicators and solids and its impact on microbial water quality is particularly notable when modelled for environments with high levels of suspended solids such as the Bristol Channel (Ref: Stapleton, 2007). Harris *et al.* (Ref: 2004) highlighted potential limitations of computer modelling for complex hydro-environmental catchments. This study showed how modelled faecal coliform concentrations were dependent on microbial decay rates which were functionally controlled by sunlight intensity and the need for site specific day-light / night-time decay rates. One case study of CSO spill impact based on the Cardiff Barrage was modelled using field measurements of turbidity in order to determine selection of appropriate decay rates. Light and dark bacterial T_{90}s of 10hr and 100hr respectively where used which when modelled showed a completely different pattern of water quality depending on whether a spill occurred during morning or evening. These considerations are equally likely to apply to NoV environmental degradation and modelling.

In addition to the impact on UV deactivation, the adsorption of microbes upon suspended solids (Section 3.5.3) will slow environmental degradation and compound issues regarding NoV viability. Sediment coupled components might enhance existing models to provide better site specific modelling of microbial water quality. In particular, this approach might be important in areas with a high level of suspended load and to understand the aberrations in quality which occur under storm or extreme events when compliance is most under threat.

5.2.5 Natural UV Dose Models

Section 3.5.2 highlights the importance of natural UV in the inactivation of NoV in the environment with viral T_{90} decay rates cited ranging from 14 minutes to 30 days according to conditions and the parameter measured. In view of the extreme variation in literature viral decay rates it is apparent that an understanding of UV dose received in the water column is likely to be an important consideration if future models are to apply site specific decay rates. For this reason the shellfish model 'tool' developed in Appendix B has capacity for variable T_{90} inputs to allow for sensitivity analysis.

UV dose is a function of attenuation through both atmosphere and water column both of which have been successfully modelled (Ref: Vantrepotte and Melin 2006). Considerable progress has been made in recent years to use surface UV monitors to ground truth large mapping models based on satellite reflective measurements (Ref; Verdebout, 2006). Similarly progress in modelling UV penetration through the water column has become more comprehensive. HYDROLIGHT ™ provides a package tool available over the internet to calculate light field for different wavelengths for a variety of water bodies where Inherent Optical Properties (IOPs) can be input to calculate UV dose at depth. Workers in the UK at Plymouth Marine Laboratory (Ref: Smyth, 2011) have successfully managed to combine both satellite and water column models to provide comprehensive monthly averaged UV maps.

Although this approach provides some exciting possibilities it is acknowledged that modelling is much more difficult in the optically complex coastal shelf seas.

Therefore nearshore modelling in dynamic shellfish waters is likely to require considerable work. However, a systematic collection of appropriate water quality parameters to help characterise IOPs (perhaps for selected target regional catchments) should be able to provide a crude seasonal and condition related attenuation factors which could drive more appropriate selection of viral T_{90} decay rates.

5.3 Adaption of *E. coli* Computer Models for NoV

5.3.1 Early NoV Modelling Experience

There are limited examples of computer models which have also included NoV components in addition to standard bacterial parameters.

Irish Modelling

Researchers at Cork University developed a model for Cork Harbour which has a sensitive oyster fishery in close proximity to the port and a major public treated wastewater discharge (Ref: O'Kane and Barry, 2007). The post-scheme model output (Case 3) indicated that mean faecal coliform levels would range from 2-40 counts/100ml and be compliant with regulatory requirements. This study indicated that scheme improvements would reduce NoV load by >90% with peak modelled NoV levels (outside of the immediate mixing zone) likely to improve from 18/L to 2/L. Although the model work indicated likely regulatory compliance and an overall scheme improvement there was no NoV water quality 'standard' against which to assess scheme viral performance.

French Modelling

France and Ifremer have championed the use of hydrodynamic models for protection of shellfish waters from wastewater inputs with key developments in a number of related areas:

- Bacterial and NoV comparative modelling was considered by Pommepuy *et al.*, (Ref: 2004)
- Integration of computer modelling with active management systems (Ref: Gourmelon *et al,* 2010).

- Coupling hydrodynamic models with catchment loading models (see Section 5.2.3).
- Modelling work in Bretagne has been used to delimit Class D prohibited areas with respect to riverine and wastewater contamination sources. This example was raised by the French NRL within EURL buffer zone discussions (Ref: EURL, 2014a)

US Modelling of Vessel Impact

Vessel and marina wastewater microbial loading can also be modelled against attainment of bacterial water quality standards with some slight modifications as discharges to not occur from fixed point continuous wastewater discharges. Section 6.3.2 highlights how even small discharge volumes from vessels can have a significant adverse impact on shellfish waters due to the potentially close proximity discharge with high microbial levels. Marinas and cruise ship discharges have been modelled:

- For marinas the US FDA provide model input guidance with assumed levels of faecal coliform vessel loading and levels of contributing vessel occupancy. Although individual vessel discharges may be considered diffuse within the marina common prohibition zoning is applied to the whole area under US FDA guidelines. Modelling of zone boundaries can use hydrodynamic models, or simple volumetric spreading radial disc models.
- For cruise ships computer modelling has been attempted for a ‘moving outfall’ which in effect provides initial dilution as a result of ship movement and vessel propulsion. A comprehensive modelling of potential NoV impact on shellfish waters in Puget Sound was developed to help manage potential viral impact from wastewater discharged from the large number of cruise ships visiting Seattle. Modelling of ‘ship to shore’ impact is described in Saranson *et al*, (Ref: 2006) and presented as an Appendix within a report by Washington State Department of Health (Ref: 2007). Modelling work used a 3D Princeton Oceanographic Model backed up by dye release studies to ascertain dilution rates. This lead to the recommendation of a 0.5 nautical miles prohibition zone between cruise ship passage and shoreline shellfish areas to preclude vessel discharges. In addition, to dilution levels time-of-travel was also modelled to assess potential authority notification times in the event of a system failure. In consequence, management measures were recommended

to contain wastewater flows if upset conditions occurred leading to loss of $4\log_{10}$ disinfection from the ships advanced on-board WWTP (see Section 3.3.3). A 'whole system' approach to health impact calculations (similar to the QMRA modelling) were also made within this study using literature values leading to the setting of a NoV water quality standard over the shellfish of 1 virus/10,000L.

5.3.2 Self Learning and Artificial Neural Network (ANN) Models

Under the Revised Bathing Water Directive 'discounting' of potential compliance failures is allowed if the regulatory agency is able to predict and appropriately notify the public at affected beaches. In Scotland, SEPA are already operating this approach whilst in England and Wales comprehensive work by Environment Agency South West Region has renewed interest in a similar national scheme. This principal could also be applied to shellfish waters especially if coupled with Harvesters Own Sample Protocol.

In essence, past historical bathing water compliance is assessed against other contributing variables which could be a predictor of performance such as catchment rainfall. The difficulty of this approach is that generally bathing water compliance is good at most sites and therefore provides little data from ~20 samples/year with which to assess potential failure modes. Initial work by the EA yielded insufficient prediction accuracy until additional high resolution sampling (and some wet weather periods) provided a larger failure data set which allowed the models to 'self learn' and provide a more robust performance.

Current work by EA modellers uses rainfall intensity in previous 24hours as the principal predictor for qualitative pass:fail status. However, parallel work being undertaken in association with the University of Exeter is focussed upon the use of Artificial Neutral Networks (ANN) aim to take a more comprehensive array of data inputs and provide a more quantitative assessment of potential output quality. This approach may in the future provide an even more powerful tool for Bathing Water discounting predictions.

At least two water companies are currently operating predictive systems based on use of hydrodynamic models and weather forecasting, to provide information on poorer water quality at bathing waters, in order to provide warnings to beach users. At least one of these systems is operating with an accuracy of 95% (compared to EA bathing water sampling data).

The use of self learning and ANN models for predicting shellfish water compliance status could have many parallels to that of bathing waters in that the principal water quality influences (e.g. rainfall events) are largely similar. Indeed, as highlighted in Section 4, NSSP counties have rainfall intensity criteria to dictate shellfish closure which could be an appropriate precautionary measure to areas subject to such inputs. However, care will need to be exercised to differentiate between rainfall events which may impact on catchment bacterial quality using intensity on a 24hr basis and CSO spill viral quality on a shorter (e.g. mm/3hr) intensity basis.

US researchers have recently proposed a range of modelling tool components to forecast and predict oyster related NoV outbreaks to assist management options (Ref: Wang and Deng, 2012). The potential close proximity of CSOs to shellfish waters would ideally mean that early warning alert systems (see Section 7.2.2) could help develop predictive modelling. Gourmelon *et al.*, (Ref: 2010) describe how computer modelling and early warning system outputs (Section 7.1.4) can be integrated to help guide shellfish management decisions. Clearly, any monitoring system which links environmental measurement with resultant microbial quality could lend itself for informing ANN model development.

However, as many CSO spills have only qualitative Event Duration Monitors (EDMs) there is currently only a poor understanding of the relationship between actual spill volumes and compliance status. The lack of both quantitative flow and wastewater concentration data makes NoV impact assessment even more problematic. Any future self-learning models would therefore need to be coupled to appropriate monitoring and data assessment tools in order to ensure meaningful output.

5.3.3 Suitability of Existing Models for NoV Applications - Intertek report

It should be noted that this study component is intended to provide a preliminary review of whether existing models can be used for NoV exclusion zoning in their current form. It is beyond the scope of this report to undertake new specific development work.

The full Intertek Ltd report provided in Appendix B demonstrates that existing models used for the impact assessment of both CSO and continuous treated wastewater discharges has the potential to be upgraded for NoV applications. Some of the Appendix B output is reviewed in this discussion section to provide zoning and risk management context.

Modelling of Continuous WWTP Discharges

Two different types of computer models operating for shellfish waters were considered:

- The North Coast model which incorporated continuous discharges from coastal and catchment sources (see Appendix B, Figure 5-1 and 5-2). Model output can be provided on a concentration basis if a target water quality standard is available. Alternatively, output can be provided as dilution rates (see Appendix B, Figure 6-1 and 6-2). This might be appropriate if US NSSP 1000:1 dilution zoning were to be employed (see Section 2.3).
- The South Coast model analysed a couple of offshore discharges within a nearshore embayment (see Appendix B, Figure 5-3 and 5-4). These examples have been modelled under ‘dry’ and ‘wet’ conditions to assess the impact of storm events on both continuous and riverine microbial loads.

Assessment Tool for CSO Spills

A full description of the Intertek assessment tool is provided in Appendix B with model input parameters shown in Appendix Table B1. Example output (Appendix B, Figure 3-1) shows how the assessment tool incorporates timeseries (see Appendix B, Figure 4-1 and 4-2) and spatial data (see Appendix B, Figure 4-3 and 4-4) for both viable and non-viable concentrations. It should be noted that any threshold (e.g. <10 genome copies/100ml) are for illustration purposes only and not an assessment against a definitive water quality standard.

Table 5.1 provides a listing of run parameters and example output from the Intertek spill tool. This collated listing highlights how the tool can be used to re-run analysis for the same $49m^3$ spill scenario under a range of different tidal and wind conditions.

- *Tidal state.* A HW or LW spill might be expected to have a bearing as to whether the spill would move seaward or landward on the first tide. For a single spill at a HW release there might be an expectation of lower peak NoV levels within the estuary as more wastewater would be expected to be flushed from the estuary on the first ebb tide. The data indicates a more complex picture with a difference in pattern between spring and neap tidal ranges.

- *Tidal range.* A spring or neap tide might be expected to govern the overall rate of flushing from the estuary as well as the current speeds which would influence mixing rates. Again the table output provides a complex picture not meeting classical expectations. Ordinarily for a single spill release to an estuary following a HW spring discharge might be expected to be advected to the nearshore coastal waters on the first ebb tide with an expectation of low NoV on the subsequent flood – surprisingly results indicate NoV levels higher than that of an equivalent LW release. More detailed examination showed that this pattern was as a result of simultaneous CSO spill release further up the catchment which was better able to impact the lower estuary under these tidal conditions. This complex analysis was possible because the model allows you to disaggregate the x4 component spill volumes which made up the total cumulative 49m^3 spill.
- *Wind.* A limited number of NW wind runs were compared to calm conditions to show that for this location wind was not a major factor in controlling plume behaviour and the magnitude of NoV. This was probably because the estuarine setting did not provide a sufficiently long fetch for the formation of wind induced currents and wave mixing. A more exposed coastal outfall setting would be likely to provide more stark differences.
- *NoV Viability*. It should be noted that the model inputs of initial viability and T_{90} are for demonstration purposes with examples drawn from the literature (see Sections 3.3.3 and 3.5.2). Model output has been illustrated for two levels of 'Viable' and 'Genomic' decay to show how the slower degradation of genomic NoV could give rise to a more rapid decrease in viable NoV with a resultant increase in non-viable NoV levels. Peak concentrations shown in Table 5.1 were obtained in the first couple of tides before mixing and decay reduce concentrations of both viable and non-viable NoV. However, the slower genomic decay rate provided a longer relative persistence of the non-viable NoV. Table 5.1 output provides a powerful illustration of how important T_{90} decay rates are in determining NoV infectious threat. Viability has been one of the key problems facing implementation of the RT-PCR analytical

results. The spill 'tool' capacity to easily alter T_{90} values could be useful for sensitivity analysis and might highlight the importance of different seasonal UV light conditions in shellfish derived outbreaks.

Table 5.1: Listing of example model output from Intertek CSO spill 'tool'

Tide State *(Notes 1, 2*	Tide Range	Wind *(Note 3)*	T_{90} Decay Rates *(Note 4)*		NoV Peak NoV/100ml *(Note 5)*	
			Viable	Genomic	Viable	Non viable
HW	Neap	Calm *	40	80	4.8	12.7
HW	Neap	Calm	60	120	8.9	17.3
HW+6	Neap	Calm	40	80	2.4	8.9
HW+6	Neap	Calm	60	120	6.2	12.3
HW	Spring	Calm	40	80	11.0	18.3
HW	Spring	Calm +	60	120	19.6	20.0
HW+6	Spring	Calm	40	80	8.8	13.3
HW+6	Spring	Calm	60	120	15.1	14.1
HW	Neap	NW *	40	80	4.2	12.4
HW	Spring	NW +	60	120	14.9	15.2

Note 1: Spill Volume = 49m³ (over x2 days)
Note 2: Spills from x4 discharges (10m³, 20m³, 1m³ and 18m³) lower estuary & catchment
*Note 3: Re-runs with differing wind conditions marked with symbols * and +*
Note 4: Initial spill volume modelled with wastewater NoV as 90% viable:10% genomic at onset of spill
Note 5: Output results from position '39' in SW

Model Advantages of Zoning and Risk Management

Current models have a number of advantages as outlined below:

- *Determination of dilution factors.* As indicated in the previous sub-section US FDA guidelines define prohibited : conditional zone boundary at a 1000:1 dilution threshold. In the case of the Appendix B North Coast model example it could be seen that the offshore coastal discharge does achieve a 1000:1 dilution before reaching the shellfish water area whilst the up-catchment riverine sources received a 10,000:1 dilution factor before reaching the shellfish water.
- *Wastewater plume time of travel.* US FDA guidelines require the prohibited : conditional zone boundary to provide sufficient time for responsive action in the event of system failure (see Section 2.3 and 4.1.3). The Appendix B model showed that the riverine catchment discharge took 3 ebb tides (~36hours) to reach the shellfish water whereas the coastal discharge took 1 flood tide

(~6hr) to reach the shellfish water. In addition to reactive management the time-of-travel is also important in terms of microbial decay (T_{90}s).

- *NoV Viability.* As demonstrated in the Appendix B illustration 'tool' and discussed in the previous sub-section (see also Table 5.1) NoV viability can be effectively modelled by employing different decay rates (T_{90} = time for 90% to decay) for NoV inactivation and NoV genomic degradation. This could be a powerful tool to help interpret impact assessments for areas subject to UV disinfected wastewater discharges (which may be only 1% viable) and CSO crude discharges (which maybe ~100% viable).
- *CSO Spill Scenarios.* As described in Appendix B and the previous sub-section a preliminary tool was developed which allowed emulation of different spill volumes under different conditions of tidal state, tidal range and wind conditions. This could potentially be a useful tool for both regulators and industry in terms of quantifying potential levels of contamination and differentiating a potential harvest area into levels of relative risk. Furthermore, models can provide an assessment tool in complex environments where multiple wastewater discharges and contaminant sources occur. This is also demonstrated in the Appendix B illustration 'tool'. The potential spill impact modelled by the assessment tool is markedly influenced by the NoV concentration of the wastewater and as such is strongly seasonal. As initial NoV wastewater concentration can be adjusted in the model there is scope to model seasonal impacts based on appropriate use of NoV in wastewater input values.
- *Extensive UK coverage*. There is a long history of computer modelling microbial water quality with a wide level of coverage for UK coastal waters. The Section 6 database lists model types and availability for areas supporting oyster fisheries with ~90% coverage within England and Wales. The scope of model coverage is illustrated by Intertek UK experience shown in Appendix B Figure 1-1. Although previously developed models within oyster production areas may be available, an assessment will be required to determine the level of work required on a case by case basis to make them fit for purpose. Coarse scale models also exist for much of the UK coastal zone having been developed for other applications. These models present some scope for nesting further higher resolution model components for future local shellfish applications.

The marine environment is complex with multiple variables impacting NoV concentration and viability threat. The capacity to readily alter a number of environmental parameters and wastewater discharge conditions has the potential to provide a valuable risk assessment tool. Input of catchment specific rainfall, CSO spill conditions, wind regime and tidal conditions would allow the compilation of model output of frequency occurrence tables to assess risk against threshold conditions.

The ability to superimpose the impact of various contributory sources could be very powerful in the prioritisation of any future wastewater zoning or management measures. Models can differentiate between a UV treated discharge with high levels of non-viable NoV from a smaller volume CSO spill with high viability. Model output could be a useful tool in guiding judgement calls when high shellfish RT-PCR results are produced with no means to assess potential viability risk.

It is concluded that this sort of computer model output could provide valuable tools to both regulators and shellfish operators when evaluating risk. Future optimisation of the spill 'tool' user interface (e.g. the EXCEL variable input fields) might need to be adjusted to the target audience. For example a less interactive 'traffic light' type of risk management tool might be suitable for a shellfish operator, whereas a regulator user may prefer access to a wider range of inputs and more complex output.

5.4 Computer Modelling - Summary

A number of computer modelling tools currently used for marine microbial assessment could be utilised to help manage NoV shellfish issues including zone parameters for dilution, time and proximity.

- *Quantitative Microbial Risk Assessment* (QMRA) modelling (Section 5.1) has been used to assess separation zones between wastewater discharge and shellfish collection areas on the basis of public health impact. QMRA has been developed as a stochastic tool using Monte Carlo techniques to evaluate potential illness arising from shellfish consumption in relation to wastewater discharge variables. QMRAs are commonly used in New Zealand as part of wastewater discharge consenting to assess viral risk of shellfish contamination. They can be a powerful tool to provide an unambiguous output which the general public can understand despite being based on a range of complex test variables. With improved scientific understanding of the underlying variables this type of approach may be more commonly used around the world for wastewater discharge impact assessments.

- *Existing Computer Models.* Hydrodynamic computer models have been used to model the potential impact from both continuous treated wastewater discharges and intermittent (e.g. CSO) discharges for many years. Models outputs indicate potential microbiological water quality against Bathing Water and Shellfish Water design standards. Models have become increasingly complex with coupled modules to enable additional modelling capabilities:
    - *Sewerage Models* These models provide output to help model storm derived CSO spill events (Section 5.2.2).
    - *Source Apportionment Models*. Catchment microbial fluxes under baseload and storm conditions are increasing important in assessing shellfish regulatory priorities (Section 5.2.3).
    - *Sedimentology Linked Models* These computer models have greater capacity to encompass *in situ* changes as a result of sedimentological processes which influence microbial decay(Section 5.2.4).
    - *Natural UV Dose Models*. Microbial decay through the action of UV is one of the principal environmental inactivation processes. Modelling of UV dose in the water column has become increasing sophisticated and could help inform site specific decay rates (Section 5.2.5).
- Computer modelling of NoV and future developments.
    - A few early NoV models have been used in other countries alongside standard bacterial parameters for public WWTP wastewater and vessel discharges (Section 6.3.1)
    - Self-Learning and Artificial Neural Network (ANN) modelling approaches could potentially offer powerful tools (Section 6.3.2). However, these approaches rely on collection of appropriate high quality and resolution data to continuously develop understanding of relationship between variables. Although this approach may be promising the current monitoring systems are unlikely to support this approach in the UK in the short term.

- *Adaptation of existing models with NoV input variables –*. This modelling approach adopted by Intertek (Section 6.3.3) offers a number of key advantages from a potential NoV management perspective:
  - *NoV Water Quality or Dilution Rates* can be adapted from modelling faecal coliforms to output NoV water quality or dilution rates. Computer models often exist for both baseline conditions (e.g. performance of continuous treated discharges) and for storm spills of untreated wastewater. However, in the absence of NoV water quality standard there is no definitive guide to the setting of an appropriate dilution factor which will ensure safe shellfish production. Current US FDA proposals are to maintain the 1000:1 dilution requirement (Section 4.1).
  - *Plume Behaviour.* Modelling tools can be used potentially both by regulators and shellfish operators to output a range of scenarios with differing loading, tide and wind conditions. This can be useful to determine potential wastewater plume trajectories which may help risk management decisions (e.g potential time-of-travel for a spill to impact a shellfish area or to differentiate patterns of resultant contamination as considered for catchment characterisation in Section 7.2.3).
  - *Modelling NoV Viability.* As models can superimpose multiple inputs they have the capacity to differentiate NoV load from continuous treated disinfected discharges (potentially with low viability) and intermittent CSOs (potential with high viability). As NoV decay can also be modelled it is possible to determine potential viable and non-viable patterns of contamination (Section 5.3.3).
  - *Applicability to UK*. Many regions of the UK already have developed computer models which can be adapted relatively easily to present NoV output. Adaption and updating of these models will be more appropriate and cost effective in some regions than others according to what models have already been developed.
  - *Model Flexibility and Sensitivity Analysis.* Models allow comprehensive analysis of complex environments which are subject to the influences of multiple variables. In consequence, models are powerful tools because they can allow great flexibility in input variables. This allows cost effective testing in ways which cannot be effectively surveyed. Furthermore, the ability to extrapolate input variables and systematically

re-run models provides scope for sensitivity analysis. This is important as it allows determination of the relative importance of the controlling input variables (e.g. microbial decay). This can help prioritise research needs and assess the efficacy of potential management strategies.

6 ASSESSMENT OF APPLICATION TO UK

This section aims to assess the practical applicability of the various exclusion zone approaches identified to the UK's shellfish waters. It will:

- Utilise a UK oyster database developed using regulatory and industry data listings and reports to obtain a preliminary profile of oyster industry proximity to wastewater discharges within relevant shellfish production areas (Appendix A). This component will help highlight next steps which can be assessed by a subsequent impact study.
- Consider how the various types of wastewater discharges may impact UK oyster production. Discharge types include:
  - Continuous treated wastewater discharges (Section 6.1)
  - Intermittent CSO discharges (Section 6.2)
  - Diffuse catchment wastewater sources (Section 6.3)
- Explore the various ways in which exclusion zoning might be implemented in order to highlight potential adverse impacts. It is however beyond the scope of this study to undertake an impact assessment (Section 6.4)
- Illustrate site specific implementation issues by developing a couple of Scenarios for real shellfish waters (Section 6.5).
- Consider potential multi-component approaches to make exclusion zones more workable by combining with other management measures (Section 6.6)

Cefas, on behalf of FSA, obtained a comprehensive prevalence database of NoV data from oyster beds around the UK over an 18 month period on behalf of the FSA (Ref: Lowther, 2011). This dataset would provide a useful source of data to assess shellfish NoV content in relation to the wastewater loading potential for a number of different types of catchment. Unfortunately, this database has not been available for analysis in the current study, although Defra has recently commissioned Cefas to undertake statistical analysis of 'contamination risk factors' (Ref: Campos, Unpublished). This study undertook further analysis of the NoV database assessing measured microbial contamination levels against catchment hydrometric, climatic, physical and demographic factors. The draft report concludes that significant risk factors for NoV contamination in shellfish waters include:

- water temperature,
- volume of sewage discharges,
- catchment area,
- number of continuous and intermittent discharges,
- resident population in the catchment and,
- river flows (to a lesser extent)

Wastewater discharge impacts are key ‘risk factors’ although other environmental factors may influence shellfish NoV levels.

This Section has been prepared with input from Aquafish Solutions Ltd.

6.1 UK Oyster NoV Levels with Respect to Continuous Wastewater Discharges

Microbial loading from continuous WWTP wastewater discharges have long been recognised as one of the principal factors in determining receiving water quality and resultant shellfish quality. In consequence, they have formed the focus for regulatory controls which have been the cornerstone of pollution reduction strategies in coastal waters. Considerable improvements in wastewater treatment have been made in the UK over the last 20-30 years through a series of regulatory drivers such as the Bathing Waters Directive, The Urban Wastewater Treatment Directive and then the Shellfish Waters Directive.

This section focuses on considering the significance of continuous treated discharges to shellfish quality in the UK today and how this may be impacted by any exclusion zoning applied. In addition, the significance of continuous discharge impact upon shellfisheries is also considered elsewhere within this report:

- Section 3.3 – detailed review of NoV removal through wastewater treatment
- Sections 5.2 and 5.3 - computer modelling of continuous WWTP discharge impact
- Section 7.2.1 – improved wastewater treatment requirements
- Section 6.5.2 – considers site specific illustration of potential WWTP discharge zoning impact on shellfish waters

6.1.1 Continuous Discharges as a NoV Risk Factor

Section 3.2 reviewed the levels of NoV within wastewater from which it is reasonable to expect larger wastewater WWTPs to contribute more NoV to the environment. The Cefas 'risk factors' study (Ref: Campos, Unpublished) showed that overall catchment urbanisation, as a proxy for population loading alone, did not show a relationship with shellfish NoV content. This may have been a result of co-variation with other factors as larger settlements adjacent to estuarine shellfish waters are often located on major rivers which correspond to large frequently rural catchments. This would result in a low overall population density for the whole catchment. It is suggested that use of catchment urbanisation as a risk factor may be improved if weighted by a proximity factor. In this way the degree of urbanisation immediately adjacent in the lower catchment could have a greater weighting than urbanisation (or lack of urbanisation) in the wider catchment.

The 'risk factors' study highlighted that the highest risk of NoV contamination is associated with areas with more than 80,000 people and with more than two large continuous sources of sewage pollution. This factor has been developed to provide proximity output using the oyster database as described in Appendix A.

6.1.2 Continuous Discharges in Proximity of Oyster Areas

As indicated in the previous sub-section, proximity to discharges with Population Equivalent (PE) of >80,000 is considered a significant risk feature in the magnitude of NoV contamination. In consequence, the oyster database (see Appendix A) has been used to map oyster shellfisheries against discharge magnitude. Figure 6.1 presents a UK map of PE based discharge load with a log based colour scheme and differentiation between native oyster, Pacific oyster and combined shellfish areas.

Figure 6.1 would seem to provide stark regional variation in the magnitude of population based discharges between Scotland and the other regions. As may be expected for the largely rural coastal fringe of Scotland most shellfish waters are not adjacent to continuous discharges from large population centres. This geographical pattern would concur with the FSA NoV prevalence findings presented in Figure 1.4 which show a much lower NoV level in Scottish shellfish.

Discharge Proximity

Section 6.1.1 highlights the difficulty in applying catchment population density as a risk factor. This illustrates how important it is to consider the basis for a proximity assessment when determining potential discharge impact upon a shellfish water. The methodology caveats provided in Appendix A regarding the oyster database need to be understood before further analysis is conducted on the output. Figure 6.1 is based on data obtained from the Pollution Reduction Plans (PRP) which consider discharge impact upon the shellfish waters. This data source was driven by the Shellfish Waters Directive (SWD) which focused upon potential *bacterial* impact upon the relevant shellfish water. As it is believed that NoV persistence in the environment exceeds that of *E. coli* indicator (Section 3.7) then the potential proximity zone maybe much wider. This means that some shellfish waters indicated to have a low risk might in fact exhibit higher NoV risk than apparent from the PRP. Appropriate recommendations in relation to NoV environmental degradation have been made in Section 3.5.

Level of WWTP Treatment

In terms of human health risk, it should be noted that Figure 6.1 is purely based on the size of the consented discharge and does not take into account treatment levels (see Section 3.3). This means that:

- 80,000 PE from a <u>secondary</u> WWTP providing 2 log_{10} reductions in NoV would provide the same NoV load as an 800 PE crude discharge, or primary treated WWTP.
- 80,000 PE from a <u>tertiary</u> WWTP providing 2 log_{10} reductions in NoV load and an assumed further 2 log_{10} reductions in NoV viability would provide equivalent viable risk as an 8 PE crude discharge or primary treated WWTP.

Figure 6.1: UK Oyster Production Areas – Population Wastewater Loading

Figure 6.2: UK Oyster Production Areas – Number of CSO Intermittent Discharges to Shellfish Area

Scottish shellfish areas may still be threatened by NoV wastewater load from small volume non-connected small crude discharges or poorly functioning septic tanks. These types of discharges have been proven to provide a significant NoV threat (see Australian case study Section 4.2.3, and Scottish example in Section 4.4.2) despite their apparent small PE size – especially where a potential ‘crude’ NoV load is discharged in close proximity to a shellfish production area.

If population size for continuous WWTP discharges were the principal risk screening criteria for the imposition of exclusion zones then there would be a markedly regional variation in impact. The Scottish industry would remain largely unaffected whilst the oyster industry for much of the rest of the UK could face potential closure. The inability of the RT-PCR test results to take NoV viability into account and the degree of treatment afforded by WWTP discharges would undermine the strength of the observed relationship as a risk factor. In consequence, discharge population size alone may not be a direct ‘risk factor’ but rather an indicator of potential risk. It is suggested discharge population magnitude alone as a criteria for exclusion zoning would not be founded in firm science and would be open to legal challenge by affected industry operators. It is recommended that further work on presenting treatment level (i.e. UV disinfection) should also be included in future impact assessments (see Section 8.1).

Illustrations of potential site specific concerns using of using exclusion zone criteria around continuous discharges within real shellfish water examples are provided in Section 6.5.

6.2 UK Oyster NoV Levels with Respect to CSO Intermittent Discharges

CSO discharges are one of the wastewater sources with a potential to impact shellfisheries (Ref: Campos, Unpublished). Other Emergancy Overflows (EOs) can give rise to intermittent discharges as a result to wastewater system failure. Whilst EOs can impact shellfish quality they are relatively rare and generally receive urgent attention. In contrast, CSOs are legal and part of the storm system design.

This sub-section will consider UK CSO discharge numbers with respect to exclusion zoning. In addition, the significance of CSO spill impact to shellfisheries is highlighted elsewhere within this report:

- Section 4.1.3 – where US regulatory experience with CSOs are considered
- Section 5.3.3 - computer modelling of CSO impact
- Section 7.2.2 - CSO monitoring systems using Event Duration Monitors (EDMs)
- Section 6.5 – considers site specific illustration of CSO impact on shellfish waters

CSO wastewater spills can originate from sewerage network overflows, pumping station overflows and stormwater storage overflows at WWTPs. These overflows are release points designed to spill when the combined system is overwhelmed by surface-water infiltration following intense rainfall events. In all cases the wastewater is essentially untreated from a microbiological contaminant perspective.

It should be noted that CSOs deemed to impact shellfish waters are designed to spill whilst not unduly impacting Class B shellfish hygiene compliance status (i.e. only impacting within 10%ile compliance margin). In some Water Utility areas this has been achieved through providing a combination of network improvements and storm water storage to limit the number of 'significant' (deemed to be $50m^3$) spills to <10 spills per year. Other Water Utilities have undertaken comprehensive modelling to demonstrate that wastewater spills do not adversely impact shellfish waters for >10 spills per year. In both cases the critical parameter considered is compliance with bacterial indicator standards. Unfortunately, viral threats undermine the foundations of both the modelling criteria and the significance of wastewater spills. Furthermore, the increased potential survival of viral pathogens relative to bacterial indicators means that many additional up-catchment wastewater sources can be possible contaminant contributors.

The need for storm management of CSO wastewater impact has been recognised within the CODEX guidelines with implications to both regulators and shellfish operators.

- CSO Storm-water storage within WWTPs: *"Treatment plants should be designed to minimize storm overflows that may affect the fishery."* (CODEX, 2012)
- Harvest implications to shellfish operator: *"After heavy rainfall, during risk periods (e.g., untreated or partially treated sewage that has or is suspected to have entered a growing area) and/or after overflow from sewage treatment plants, harvesting of bivalve molluscs should cease for a period, until the water and/or bivalve molluscs quality of the harvesting area has been assessed and has been returned to normal background levels for the area."* (CODEX, 2012)

6.2.1 CSO Discharges as a Risk Factor

A number of studies have demonstrated how untreated CSO wastewater discharges of even relatively small volume can have a significant impact upon shellfish due to their high viable NoV content (Refs: Dore *et al*., 2007, Campos and Lees, 2014).

The impact of CSO spills was also recognised by the recent Cefas 'risk factors' study (Ref: Campos, Unpublished). The FSA NoV prevalence data was further analysed for a 10 site data sub-set where spill data was available. Results indicated higher levels of NoV in oysters from sites impacted by a high (>10 per year) number of spills, although NoV was still detected in sites with <10 spills per year. As quantitative EDM data is not generally available the basis for this assessment is not known and >10 qualitative spills may be considerably less than the water industry 50m^3 definition of a 'significant' spill.

Detailed analysis of site specific quantitative spill data and corresponding rainfall data may also be necessary. The Cefas report went on to highlight a seeming contradiction with the lack of relationship between NoV contamination and rainfall which was reported to potentially 'dilute' NoV contamination. It is probable that a site specific relationship might need to be developed in order to determine a potential rainfall intensity (and antecedent conditions) likely to produce a CSO spill so that variables above and below this threshold can be differentiated.

Although the Cefas work is promising it raises issues which will need further exploration before this risk factor can be fully developed and utilised for potential management measures. Recommendations are considered further in Section 8.4.

6.2.2 CSO Intermittent Discharges in Proximity of Oyster Areas

Catchments with a large number of CSOs in close proximity to shellfish waters are likely to present an increased risk of NoV contamination. This will have implications for shellfish management and have a bearing on the potential applicability of exclusion zone controls.

Figure 6.2 provides a clear contrast between Scotland, with no CSO influence, and the rest of the UK, where CSO discharge numbers are significant. Generally, the pattern of potential impact is similar to that of the continuous discharges shown in Figure 6.1. This feature is not surprising as higher population density areas are likely to be served by both larger magnitude WWTPs and more extensive and complex sewerage systems which tend to need more CSOs.

As highlighted previously in Section 6.1.2 with respect to continuous discharges it should be remembered that CSO numbers in Figure 6.2 will be an underestimate of risk. This is because the environmental persistence of NoV is thought to greater than that of *E. coli* suggesting that that the sphere of influence will be more widespread. For example, DOENI use <2km proximity threshold to the shellfish water as inclusion criteria; as a ‘direct’ discharge likely to influence shellfish quality (DOENI official, personal communication). However, if the CSO numbers from the wider catchment, as obtained from the Sanitary Survey, are applied the number potential CSOs impacting the shellfishery is vastly greater as demonstrated in Table 6.1 below.

Table 6.1: Illustration of proximity zone threshold on CSO numbers likely to influence shellfish water
(Source: DOENI Data and Sanitary Survey Documents)

SHD Production Area	SWD Area	DOENI Data			Sanitary Survey
		<1km	<2km	<3km	
Strangford Lough	Reagh Bay	5	7	11	124 (whole catchment)
	Marlfield Bay			7	
	Skate Road	2	4	10	
Lough Foyle	Longfield Bank			9	37 (<5km) 110 (<20km)
	Balls Point		1	1	

Appendix A methodology description highlights how different devolved administrations use different proximity thresholds for discharge inclusion. Further catchment based GIS work is needed to better assess CSO NoV risk on a catchment by catchment basis perhaps using riverine time-of-travel against various NoV decay rates to ascertain potential sphere of influence. Any CSO scheme improvements will need to pass a disproportionate cost test (Section 6.7.2). Any calls to improve a specific CSO discharge (e.g. consideration of UV treatment) are likely to have to take into account whether up-catchment loading will undermine significant environment improvement from the proposed scheme improvement.

The number of CSO discharges will not directly relate to risk as it takes no account of the volume of wastewater released (i.e. multiple small CSOs might contribute less wastewater than one large CSO). However, the number of CSO discharges does highlight the potential complexity which any active management plan would require. An enhanced Sanitary Survey would be needed to characterise individual CSO impact (Section 7.2.3) and subsequent spill monitoring (Section 7.2.2).

CSO impact is likely to be a critical threat to NoV contamination in some UK shellfish waters. There are however a number of problems in developing criteria for establishing potential exclusion zoning around CSO discharge points:

- *Lack of quantitative CSO data.* CSO spill data is qualitative as discussed in Section 7.3.4. In consequence EDM data may have limited relationship to risk.

- *CSO spill significance.* The water industry considers a 'significant' spill of $50m^3$ regardless of the actual impact it may have on a shellfish area or NoV content. If CSO zoning were to only apply following a spill then it would be necessary to have an understanding potentially how each contributing CSO might impact on the various points within shellfish waters. For example, a $5m^3$ CSO discharge (i.e. not 'significant' spill) near a shellfish bed might have a *significant* NoV impact, whereas a $50m^3$ CSO discharge (i.e. 'significant' spill) a distance away from the shellfish bed on a favourable tide might have *no significant* NoV impact! Computer modelling can be an effective tool to assess relative contributions for amalgamated CSO spills following a storm event (Section 5.3.3) and could potentially help in those catchments suitably modelled.
- *Variable nature of CSO operation.* As CSO operation is largely linked to rainfall intensity the level of operation (and potential impact) varies on a seasonal and inter-annual basis. Design criteria for CSO considers a 10 year average with no effective means currently in place to ensure operation even meeting engineering expectations. This is problematic as it is difficult to establish a meaningful spill inclusion threshold criterion. Inclusion of every CSO would be a massive undertaking and disproportionate to risk as many such intermittent consented discharges may rarely even discharge to the environment.
- *Widespread geographical coverage of CSOs.* The number and extent of CSOs far exceed that of continuous WWTP discharges. This could result in a significant loss of shellfish production area if exclusion zones were imposed around all potential CSO discharge points. As such zoning could be disproportionate to risk and could be open to challenge.

These issues raise concerns as to how CSO discharges could be used to implement potential exclusion zone controls. There may however, be potential to use EDM data within a wider 'enhanced management' approach as considered further in Section 7.3.3.

In view of these limitations no further analysis has been performed on this output until the database can be further developed ideally with a clearer inclusion rational and a means to scale the degree of potential pollutant loading. Recommendations are provided in Section 8.4.

Illustrations of potential concerns about using exclusion zone criteria around CSO discharges are illustrated with real shellfish water examples are provided in Section 6.5.

6.3 Impact of Diffuse Wastewater Sources

In addition to measures to improve WWTP NoV removal performance, environmental hygiene guidance (CODEX, 2012) states:

"With regard to risks for virus contamination some of the specific areas to be addressed are as follows:

- *Growing areas that are contaminated by sewage discharge or disposal of faecal matter from ships, recreational boats and bivalve molluscs harvesting vessels.*
- *Overflow from sewage treatment plants that may contaminate the growing waters after heavy rainfall.*
- *Quality of sewage collecting network and private septic tanks.*

Despite a generally urbanised population the UK is still subject to a high level of diffuse wastewater sources input. This can provide a significant potential for NoV contamination in some catchments which may impact adjacent shellfish waters. Generic diffuse wastewater issues are considered in this sub-section whilst site specific examples are discussed in Section 6.5.

6.3.1 Septic Tanks & Soakaways

Section 6.5 highlights how small private discharges and septic tanks can contribute to microbial load within a specific catchment Scenario A. This sub-section considers septic tanks from a more generic perspective.

Point source discharges from septic tanks serving small populations have been shown to impact upon local shellfish quality. The use of soakaways from septic tanks is a low-tech approach to limit contamination to surface waters used around the world. Poorly designed or maintained systems can however, present a problematic diffuse source of contamination which can be difficult to track and resolve. In the UK septic tanks with soakaways are still widely used in largely rural catchments where sewerage systems and communal WWTPs are not affordable or practicable. For oyster production areas away from major population centres (e.g. in Scotland - see Section 4.4.2) catchment populations maybe generally low and rural and serviced by septic tank soakaways. Unfortunately, as individual NoV load from a symptomatic infected person is so high and the infective dose is so small, contamination from even small diffuse sources can be problematic. In consequence, even a small poorly maintained system can have a profound adverse impact on contamination of an oyster production area as demonstrated by the Australian Case Study (Section 4.2.3).

Viral inactivations within soils and from clays have long been a topic of research with a view to designing suitable wastewater disposal systems (George et al. 1968). Shields 1986 assessed soil adsorption of viruses and considered the dynamic equilibrium which helps to describe how viruses maybe removed from diffuse soakaway sources. Soil type (content of clay, loam, oxic/anoxic, pH and ionic concentration) has a profound effect of viral retention. For example, 20% adsorption of poliovirus in a high organic content soil increased to 98% adsorption with the addition of cations (e.g. calcium chloride). Similarly low ionic strength waters (such as rainwater) can lead to desorption of previously adsorbed viruses. Other chemical influences within the wastewater or soil such as the presence of sodium metaphosphate (such as from detergents) and high protein solutions block virus adsorption process potential reducing removal efficacy in a soakaway. Indeed understanding of these adsorption: desorption kinetics form the central basis for NoV extraction and concentration laboratory procedures during assay techniques.

The potential for diffuse leaching of NoV from soakway sources is a complex problem. It is probable that diffuse source risk from soakaways may vary on a season and regional basis with soil saturation and soil type. This could help identify potential enhancement measures for regions and time of increased risk. For example, soakaways over winter periods with low ionic strength rainwater permeating acidic humic peaty soils may benefit from a lime-rich aggregate buffer zone to maintain a high pH and aerobic environment to encourage viral adsorption to soils. It is recommended that management and assessment of catchment soakaways is built into future catchment risk profiles.

Consideration of septic tank discharges and small scale private discharges from unconnected population is considered further in the site specific shellfish Scenarios in Section 6.3.

6.3.2 Vessel Discharges

Vessel discharges from shellfish harvest vessels, commercial vessels (such as cruise liners) and pleasure craft have the potential release to release NoV contaminated wastewater in close proximity to shellfish waters and adversely impact flesh quality. As indicated in Section 2.1 and 2.2 a number of EU and NSSP countries respectively operate exclusion zones around marinas and ports to preclude shellfish harvesting.

The FSA's Advisory Committee on the Microbiological Safety of Food (ACMSF, 2014) have recently recommended:

*"Prohibition of overboard disposal of sewage from boats should be mandatory under local byelaws in all water bodies and coastal areas with designated shellfish waters. Inshore Fisheries and Conservation Authorities (IFCAs) and the Marine Management Organisation (MMO) should take the lead on this." (*Recommendation R6.8)

Vessel impact of wastewater discharges in the UK is likely to be very catchment specific but potentially significant as illustrated in Scenario A and B (Section 6.5). This sub-section considers generically how vessel impacts can impact shellfish quality.

Harvest Vessels

The high titre of viruses shed by an infected symptomatic person and the low infective dose mean that a large body of seawater around a vessel can be contaminated by even a small quantity of wastewater discharged from a vessel. The potential for a little vomit or faecal matter to go a long way was highlighted in the US when multiple state outbreaks of gastroenteritis in Louisiana were tracked back to a batch of stock originating from a single production area thought to be contaminated by a sick shellfish worker who had been sick from the harvesting vessel (Berg *et al.* 2000).

CODEX 2012 highlights a number of issues with respect to harvest vessel wastewater disposal: *"Suitable precautions should be taken to protect bivalve molluscs from being contaminated by human faecal material, in particular:*

- *No overboard discharge of human faecal material should occur from harvest (or assisting) vessels around bivalve molluscs growing areas.*
- *All necessary measures should be taken to prevent contamination of bivalve molluscs by faecal materials on board of harvest vessels.*
- *Facilities and toilets should be such to ensure that an appropriate degree of personal hygiene can be maintained, especially on harvest vessels. "*

For many of UK oyster businesses operating within intertidal areas the working duration and proximity to shellfish beds is tidally constrained which may limit risk of inadvertent staff initiated contamination of stocks. Whilst most UK cultured oysters are intertidal some stocks are held in sub-tidal cages and serviced by harvesting vessels. However, some stocks (i.e. native oysters and wild settled Pacific oysters) are directly fished using harvesting vessels. Harvest vessel contamination potential may have training and facility implications to some shellfish business operators in some shellfish production areas (see oyster database).

Pleasure Craft

Pleasure craft have a significant potential to directly contaminate shellfish waters as a diffuse source which can be in very close proximity to shellfish production areas and have long been noted as potential sources of contamination (Milliken and Lee, 1990). A classic study undertaken in North Carolina measured bacterial water quality in the vicinity of a marina over an extended period and matched observed degradation in faecal coliform levels with levels of occupancy and holiday periods of increased use (Sobsey *et al.*, 2003). Two marina areas had faecal coliform levels 6-9 times higher than background levels. Similar results were obtained from a study in Saint Gervais harbour with a 650 pleasure vessel capacity (Guillon-Cottard *et al.*, 1998). Mussel samples were collected in the evening from 3 stations in the harbour on a 2 week basis for 1 year in 1995/1996. Highest faecal coliform values were obtained generally in the summer and on the holiday weekends of Whitsun and All Saints Day. These periods of peak microbial flesh concentration were indirectly associated to vessel uses which were on average only out of port for around 1 week/yr being used primarily within port despite limited wastewater facilities.

Recent EURL Cefas Good Practice Guidelines (EURL 2014b) propose a 300m exclusion zoning around marinas in recognition of the potential for vessel wastewater derived contamination to impact shellfisheries. In addition, to defined marinas it should also be noted that potential recreational vessel discharges are not confined to marinas and that mooring areas and visiting anchorages may also pose an increased potential for uncontrolled contamination. Recreation vessel impact is considered further in Scenarios 1 and 2 (Section 6.5.5).

UK pleasure craft discharge impact is likely to be a significant risk in many oyster fisheries within England, Wales and Northern Ireland with limited legislative measures in place other than for inshore waterways. The Royal Yacht Association (RYA) and British Maritime Federation support the ‘Green Blue’ website which provides a range of resources to inform recreational vessel users on waste disposal related issues. A ‘Green Boat checklist’ (*http://www.thegreenblue.org.uk/pdf/Green%20Boat%20Checklist%20TGB.pdf)* provides a scoring criteria which includes negative and positive score factors for a range of vessel operation and resourcing issues including wastewater disposal. This checklist does make mention of flushing in environmentally sensitive areas and the potential to impact on shellfish. However, the level of emphasis on these aspects is relatively low and the guidance relating to sensitive areas somewhat limited.

A private shellfish industry survey of Pump Out facilities for England and Wales listed in the RYA Green Blue directory showed generally very low levels of utilisation (FitzGerald, 2007a). Recent communications with RYA Green Blue representatives indicate that new initiatives such as the ‘Love where you sail’ have been developed which include measures to encourage use of pump ashore facilities. Preliminary work in the Solent negotiated free Pump ashore discharges over Cowes week and raised skipper awareness over discharge issues (RYA representative personal communication).

In Scotland pleasure craft pump ashore facilities would require a licence and should be controlled by SEPA. However, direct pleasure craft discharges lie outside SEPA sphere of control and are not thought to be controlled by anyone (SEPA official, personal communication.). NoV risk from this source is likely to be very catchment specific and could larglely be informed from the Sanitary Surveys.

Site specific scenarios including vessel impacts are considered in Section 6.5.5.

Cruise Liners

US regulation of vessels discharges and the recognition that discharges in the coastal zone can adversely impact shellfish from a NoV perspective was comprehensively considered for cruise ships transiting Puget Sound whilst visiting Seattle (Washington State Department of Health, 2007). Modelling of NoV exclusion zone and wastewater management requirements is described further in Section 5.3.1.

UK consideration of this potential threat is likely to be relevent in a limited number of oyster fisheries. Some operators (e.g. Cunard liners) visit major ports such as Southampton, Oban, Belfast and Kirkwall. Other operators (e.g. Fred Olsen cruises) visit multiple UK locations supporting oyster production including remote largely pristine Scottish island settings. Preliminary risk profiling of this potential source is recommended to ensure Port Health authorities are effectively monitoring wastewater treatment discharge performance from a NoV perspective.

Vessel Discharge Regulation

Shellfish water quality regulation in the US as with the EU is based on bacterial indicator levels rather than viral pathogens. The US and countries operating the NSSP system (e.g. Canada, New Zealand and Australia) all make provision for buffer zones between marinas and shellfish waters in recognition of the potential for adverse wastewater related contamination. Case Studies in Section 4 expand upon the extent of the zoning requirements for each country, although the NSSP provides guidance for the calculation of a mixing zone based upon assumed levels of faecal coliform loading and vessel occupancy (Section 2.2).

The US Clean Vessel Act came into force in 1992 to help reduce the impact of vessel discharges and provided 10 years of grant aided improvement schemes to marinas without adequate treatment facilities. In addition all states now have designated 'No Discharge Zones' (NDZ's) for both inland waterways and sensitive marina environments of which shellfish are a primary designation criteria. It has been recognised that the effectiveness of the NDZ's is dependent on compliance. Critically the NDZ's have three components: marina pump-out facilities should be "reasonably" available and adequate to the number of users, vigorously enforced which includes vessel inspections to ensure that vessel "Y-valves" have been disabled from discharging to the marina environment and includes an education programme for vessel users.

In the UK there is relatively little regulation of vessel discharges although marina pump ashore facilities will require appropriate connection to mains sewerage or access to a consented facility. Newer pleasure craft are equipped with wastewater holding tanks, although many older vessels still in use have capacity to retrofit such facilities. The Royal Yachting Association (RYA) Environmental Code of Practice advises vessel users not to discharge their wastewater in close proximity to the shore but measures are voluntary unless port or harbour areas have generated local by-laws. In addition, the Environment Agency Pollution Prevention Guideline PPG14 document (2004) states "*discharges from sea toilets are not prohibited, but the use of toilets with storage tanks is recommended in preference*" and request operators to "*check for the proximity of potable water abstraction points and shellfish beds for human consumption*."

Local signage and notification guidance via marina websites (or appropriate phone APPS) might be useful low cost measures to help inform vessel users of responsible vessel operation in the vicinity of sensitive shellfish waters. Examples might include the US public information approaches such as the boating posters developed within the NoroCORE programme (courtesy of Lee-Ann Jaykus) shown in Appendix D.

Future zone impact studies will need to assess how many UK oyster shellfisheries may be vulnerable to vessel derived wastewater contamination.

It is recommended that a task force of responsible agencies and industry representatives assess the ACMSF proposals and consider a range of practical measures which can be available for implementation at a local level. Efforts should be made to work with vessel stakeholders to improve resources and voluntary guidance available to operators.

6.3.3 Sludge Disposal

Human sewage sludge encompasses primary sludge (containing faecal solids with unknown NoV contribution) and secondary sludge (inferred to contain high NoV levels). Within the UK most primary and secondary sludges receive treatment at a regional sludge centre which often consists of anaerobic digestion, dewatering and potential lime stabilisation. Different regions have various disposal routes for these ‘biosolids’ one of which includes land application as a fertiliser. In consequence, sewage sludge disposal poses a potential risk as a diffuse NoV source for recontamination of surface waters.

This is a complex subject and requires a staged assessment of removal and viability at each step in the potential pathway:

- Removal and viability of NoV concentration from wastewater to sludge within WWTP
- Efficacy of ADAS Safe Sludge Matrix (designed to provide a 6 $\log_{10}$ reduction in faecal coliform count) to reduce NoV viability
- Degradation and adsorption of NoV into soil matrix
- Potential for desorption and remobilisation to groundwaters or surface waters

Most assessments of sewage sludge risk are related to faecal coliform indicator concentrations, although Gale. (2004) assessed the risk of infection as <1 case/year from bacterial pathogens and enterovirus. Unfortunately, the assessment did not include NoV or additional enteric viruses.

Shields 1986 reviewed the adsorption process between viruses and sewage flocs and the subsequent bonding to different sludge types along with the various dynamic processes by which viruses can become desorbed from bound solids as considered previously in Section 3.5.3. With increasing attention upon viral removal efficacy within wastewater treatment a number of researchers have pointed to the potential contamination link from sewage sludge biosolids and the need to appropriately controlled disposal. Cheng *et al.* (2012) also highlighted that whilst WWTPs may be effective at pathogen removal the concentration of pathogens within the sludge (including NoV) may also have implications to sewage sludge disposal as Biosolids for land spread.

There is no current consensus as to the significance of biosolids as a potential NoV contamination source to environmental waters – partially due to the difficulty in developing assay techniques which effectively desorb viruses back from sludge solids. Sima *et al.* (2011) attempted to quantify NoV levels in sludge giving counts of ~10^8 genome copies/kg, although low mengovirus control extraction efficiencies (<10%) were acknowledged and not factored within enumeration. New sewage sludge recovery methods and verification techniques are under development with Amdiouni *et al*. (2013) reporting enhanced virus extraction efficiencies from synthetic sludge samples. Further work is recommended on sewage sludge biosolids to assess NoV load and potential viability to inform risk assessments.

A comparative assessment of NoV risk against conventional faecal coliform performance criteria would seem a pragmatic way forward. Modelling of potential infection risk could also be an option as demonstrated by the Quantitative Microbial Risk Assessment (QMRA) recently undertaken to assess NoV risks due to wastewater irrigation of crops in Australia (Mok *et al.*, 2014). QMRAs, which are considered further in Section 5.1, can provide a broad population-wide assessment of risk. However, as with other components within the NoV environmental pathway, it is critical to develop an appropriate NoV viability assay before the potential health implications of a NoV concentration can be evaluated.

6.4 Consideration of Exclusion Zone Options

Sections 6.1, 6.2 and 6.3 highlight that wastewater sources from continuous discharges, intermittent CSOs, smaller diffuse catchment and vessel sources can all contribute to NoV contamination of shellfish. Some of these wastewater sources have been subject to zone controls in the EU and US affiliated NSSP countries as described in Section 2. However, there are no current zone controls implemented for all of these potential sources on a NoV loading evidence based approach (Section 3).

This sub-section considers various zoning options from a generic perspective to help ascertain their principal benefits and disadvantages.

Table 6.2 provides a summary of various potential positive and negative features which might characterise different types of zoning which could be implemented. No option provides a clear evidence based rational to scale zoning with risk in view of the science gaps identified in Section 8.1.1. Principal problems are:

- Uncertainty in quantifying NoV risk from shellfish food product to human health and wider population impact (see Section 3.1.2).
- Limited understanding of relationship between NoV water quality and uptake in shellfish flesh (Section 3.6)
- Limited understanding of NoV behaviour in the environment (Section 3.5).
- Inability of RT-PCR analytical testing to determine NoV viability (Section 3.1.3)
- Extreme variation in risk profile for NoV relative to that of *E. coli* indicator organism used for regulatory purposes (Section 3.7).

With potential NoV risk from even small wastewater discharges there is potential for many shellfish production areas to be impacted from multiple wastewater contaminated sources (Section 6.5). More detailed consideration of generic zone approaches is provided in Table 6.3 where potential issues and solutions are developed further.

In addition to the technical and commercial challenges the cost and ease of zoning implementation is also an important consideration. Section 7.3.4 (see Figure 7.10) outlines a potential mechanism to initially use default proximity zoning on the basis of the *E. coli* NoV proxy risk scoring system (Section 7.3.2). The initial linkage between risk scores and distance thresholds for zones could be based on assummed theoretical levels and basic volumetric dilution calculations. For example:

a) default NoV wastewater concentration and flows to determine NoV load,
b) a simple spreading disc model with depth and sector inputs,
c) a target NoV water quality threshold (e.g. 20 genome copies/100ml as considered in Section 3.6).

Default proximity zones could later be amended with a more flexible and a site specific whole system risk scoring approach (Section 7.3.1) adopted to support ‘enhanced management zones.’ This is a relatively cost effective approach as many shellfish areas would either not require zoning or be allocated an appropriate default zone. Those shellfish areas wishing to develop a more expensive whole system approach will need to make an individual judgement on whether to pursue this method based on availability of appropriate site specific data/information.

Table 6.2: Summary of pros and cons for generic types of zoning

Basis	**Positive**	**Negative** *(Note 1)*
Geographical (proximity)	-Easy, cheap and quick to implement -Other EU national/regional examples	-Arbitrary with little science based evidence to proportion zone to risk -Open to possible legal challenge by potentially disadvantaged FBOs (e.g. those potentially contaminated by non-viable UV disinfected WWTP discharges)
Dilution	-Some areas could utilise previous Water Utility tracer studies and computer models -Better scientific evidence basis than geographical zones	-No consensus on what level of dilution is required (i.e. difficulties in linking water quality to shellfish flesh quality) -Dilution data/tools not available for all areas and expensive to undertake afresh
Time	-Existing EDM and Water Utility reporting systems already in existence to warn of 'failure event' (for many areas) -Existing computer models and hydrographic studies to help estimate potential time-of-travel between WWTP discharge and shellfish water (for many areas)	-Water Industry studies, tools and internal systems not readily accessible to shellfish industry and/or regulators -Time in isolation has no direct general relationship with risk and so is normally coupled with dilution (i.e. within US Conditional areas) – see above
NoV contamination levels (in shellfish)	-Can broadly link contamination level to risk in accordance to EFSA view on dose-response -Prevalence NoV data may give an indication of potential UK implementation impact -Responsive investigation could initiated for a NoV outbreak with demonstrable human health impact -Future analytical components to assess viral viability could make RT-PCR 'standards' more valid -Biosentinal monitors could be established to target proximity zones	-Not source specific, potential for multiple sources -Does not address fundamental shellfish industry concern regarding RT-PCR over-estimation of risk due to inability to measure NoV viability. -Unclear who would fund and implement -Would have a regional variation of impact within UK and may disadvantage some regions -Would vary significantly by season and by year (as a response to 'catchment health') -CSO impact could vary impact for each storm 'event'

Note 1: All types of zoning are likely to struggle to balance proportionate scaling against risk for catchments with storm impact (i.e. CSOs).

Table 6.3: Profile of possible zoning proposals and adoption issues

<table>
<tr><td colspan="2">a) – Proximity zoning for ‘major’ WWTP continuous discharges (based on P.E).
Proposal: Exclusion zone based on tidal excursion from 80,000 P.E discharge (Note 1)</td></tr>
<tr><td>Background
Re-analysis of the FSA NoV prevalence data (Ref: Campos Unpublished) indicated that proximity to WWTP discharges with P.E of >80,000 as a potential contributory risk factor.
Some EU countries have adopted proximity based zoning but with no basis for zone size. A half tidal excursion (see Glossary) would limit plume impact within a single flood or ebb tide.</td><td>Issues
Differentiation between differing levels of treatment and their impact on viability. For example as UV discharged wastewater is assumed to be ~1% viable a PE 80,000 UV treated discharge could be considered equivalent to PE 800 discharge with secondary treatment
There is no evidence base to set scaling on proximity zone. An exclusion zone based on a half tidal excursion maybe overly extensive</td></tr>
<tr><td colspan="2">Potential way forward: Enhanced sanitary survey (tracer studies or computer modelling) to try and assess relative NoV load contributions.</td></tr>
<tr><td colspan="2">b) – Dilution / Time based zoning
Proposal: To adopt the US FDA Conditional Zoning style (e.g. 1,000:1 dilution and time-of-travel plume impact systems)</td></tr>
<tr><td>Background
The US FDA provides guidance to US affiliated countries operating the NSSP system (Section 4.1.3). Current US FDA dilution guidance is designed to provide target bacterial water quality standards.
1000:1 dilution is required for Conditional Zones (where contamination events are ‘predictable’).
100000:1 dilution is required to differentiate Restricted from Approved zones</td><td>Issues
1000:1 Conditional zones also require a time based warning/response system to allow remedial actions in the event of system failure. The UK has not developed these systems. Potential failure events are not fully understood to provide a predictable impact.
1000:1 Dilution may be in-sufficient to provide water quality of a level to provide safe shellfish</td></tr>
<tr><td colspan="2">Potential way forward: CSO EDM monitoring to flag periods of likely increased risk. Water Utility companies often have conducted outfall tracer studies to ascertain dilution rates or have modelled plume dilution. Computer modelling will also help with time-of-travel to reach sensitive waters. Political pressure and cross-sector agreements would be required in order to access this data in addition to resource implications.</td></tr>
</table>

<table>
<tr><td colspan="2">c) – NoV contamination level - Testing (e.g NoV Prevalence results) to indicate risk profile
Proposal: Prohibit shellfish water if NoV ‘harvest’ level exceeds threshold (e.g. 1,000 genome copies/g Dt)</td></tr>
<tr><td>Background
EFSA (Ref: 2011) highlights a dose dependant response to NoV exposure and recommends the establishment of a standard threshold where a significant risk of infection could be posed through ingestion.</td><td>Issues
Approach will not allow differentiation between treated and untreated (i.e CSOs) wastewater. Shellfish in vicinity of UV discharge could be judged differently to that in vicinity of CSO outfall e.g. 200 genome copies/g Dt in vicinity of CSO may present greater viable NoV risk than 1000 genome copies/g Dt in vicinity of UV disinfected discharge. There is no easy way to scale zoning.</td></tr>
<tr><td colspan="2">Potential way forward: CSO EDM monitoring to flag periods of likely increased risk (i.e. Conditional zoning in event of spill)</td></tr>
<tr><td colspan="2">d) – NoV contamination level - Reactive prohibition following shellfish implicated outbreak.
Proposal: Exclude shellfish water in proximity to wastewater discharge following linked NoV outbreak</td></tr>
<tr><td>Background
It is increasingly common to employ reactive RT-PCR tools to analyse stool and shellfish samples to assess presence and Genotyping of NoV. If sampling of shellfish triggered by outbreak and analysis shows presence of NoV in shellfish regulatory authorities might assume outbreak was a result of consumption of contaminated shellfish.</td><td>Issues
Community based infection will increase potential for food handler derived contamination, whilst correspondingly increasing discharge loading to environment which can contaminate shellfish. In the absence of NoV wastewater assessment and routine monitoring it could be hard to differentiate this ‘chicken and egg’ situation. Low level positive NoV shellfish may have been released unknown into the market prior to outbreak with no ill effects in customers. FSA NoV prevalence study showed wide incidence of NoV in UK shellfish – even though most might not cause illness.</td></tr>
<tr><td colspan="2">Potential way forward: Outbreak must ideally be unequivocally a result of implicated shellfish. Robust EHO traceability food provenance, food handler swabbing and patient stool sampling. Increased defensive monitoring by FBOs (e.g. retention of previous batch samples demonstrating no ill effect).</td></tr>
</table>

Note 1: P.E = Population Equivalent

6.5 Catchment Scenario Illustration of Exclusion Zone Impact

In order to further explore the possible impact of exclusion zone imposition upon industry and regulators two catchment Scenarios as site specific illustrations have been developed.

6.5.1 Scenario Descriptions

Both areas are based upon real UK Shellfish Waters (SW) which currently support Class B shellfisheries and historically have supported production of multiple bivalve species. A summary of the key features for these contrasting areas are provided in Table 6.4.

Table 6.4: Comparison of shellfish catchment scenarios

Feature	Scenario A	Scenario B
Physical Characteristics a)-Estuary form b)-Depth LW c)-Freshwater flow	a)-Ria (drowned valley) b) 2-4m depth at LW (although deeper in places) c)~1m^3/s (multiple small streams)	a)Shallow estuary b) extensive sand and mudflats 1-2m at LW in channels c)~13m^3/s (x3 main rivers)
Catchment Details	Small <200km^2 catchment Largely agricultural Small resident population	Large 1500km^2 catchment Largely agricultural Large resident population
Continuous discharges *(Note 1)*	Number = 3 Treatment: UV= Village A1 (150m^3/day) Secondary= Village A2(25m^3/day) and A3 (50m^3/day)	Number = Multiple (75) Treatment: (main within 10km) UV= City B1 (40,000m^3/day), Town B2 (9,000m^3/day, Village B1 600m^3/day),
Intermittent discharges *(Note 1)* (CSOs, EOs) a)-Number identified b)-Spill status	a) x8 (mostly into catchment streams) b) EDM on most discharge points. Some have not been known to operate	a) x210 (mostly ~20km from shellfish water around City B1. Many to estuary) b) EDM to most in estuary and to some within catchment
Other wastewater sources: Vessels	600 moorings, 50 visiting vessels/night	1800 moorings, 200 pontoons
Shellfish Production	Sub-tidal cages Inter-tidal trestles	Differing immersion profile and potential for exposure to contamination
Number of production areas	Multiple - within estuary complex (also access to sites beyond in nearshore coastal waters)	Multiple - All within estuary

(Note 1: Public utility consented discharges only)

The shellfish waters are illustrated diagrammatically within Figure 6.3a) and b). Details of the two areas have been anonymised with key metrics rounded for simplicity. Public Water Utility continuous and intermittent consented discharge locations (provided courtesy of Environment Agency) have been plotted and analysed in relation to the corresponding shellfish Representative Monitoring Points (RMPs). Although both estuary sections are of similar length at around 10km Scenario B has a much larger catchment and resident human population (Figure 6.4). The higher number and intensity of both continuous and intermittent wastewater discharges is likely to provide a correspondingly higher risk exposure.

Various aspects are considered with detailed examples provided in following sub-sections and summarised in Table 6.5.

6.5.2 WWTP Discharge Proximity and Impact upon RMP

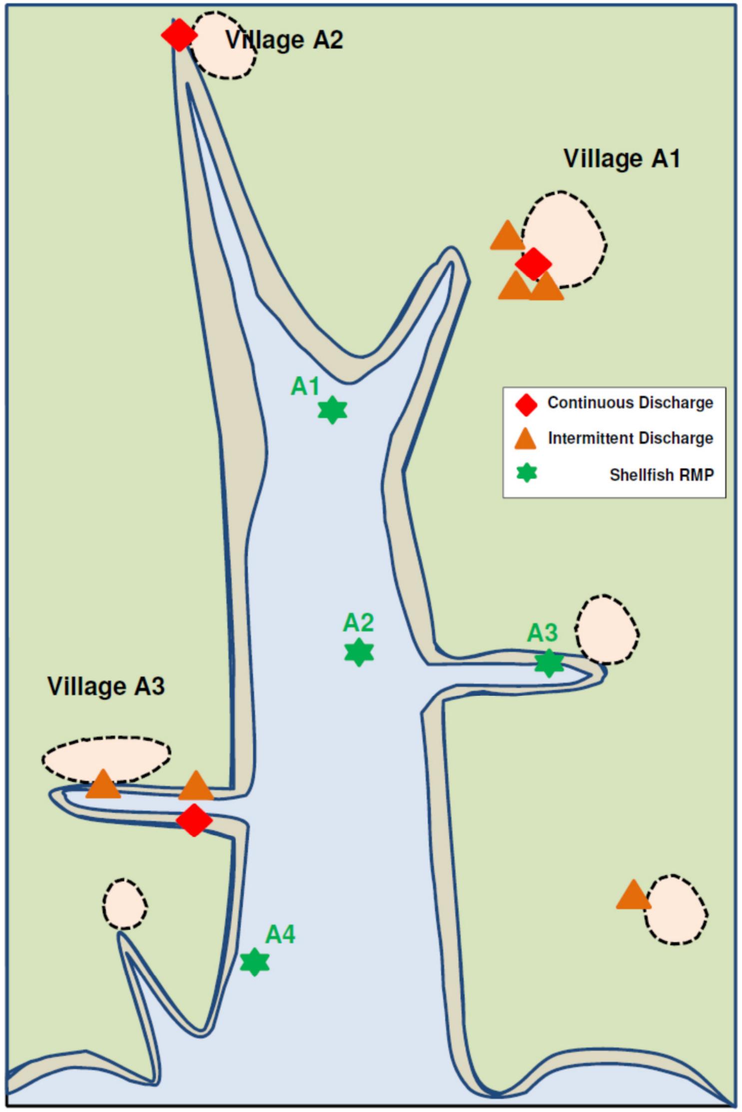
WWTP discharges to both SWs are treated to secondary or tertiary standards and discharge to estuarine waters with some positions close to shellfish RMPs which could conceivably be considered for exclusion zoning. The nature of the exclusion zoning (see Section 6.4) has a bearing on how it may impact upon the shellfish water particularly with regards to its relationship with the shellfish RMP.

The degree to which shellfish microbiological quality from a RMP reflects that of the actual shellfish stocks has long been a contentious issue with many shellfishermen arguing that RMPs tend to represent the ‘worst case’ condition of a shellfishery. From a public health perspective there is a rational that RMP data should not under-estimate risk. From an industry perspective there is a concern that the RMP can misrepresent the quality of the product harvested as there is rarely a comprehensive understanding of the spatial and temporal variation in microbial quality across a SW.

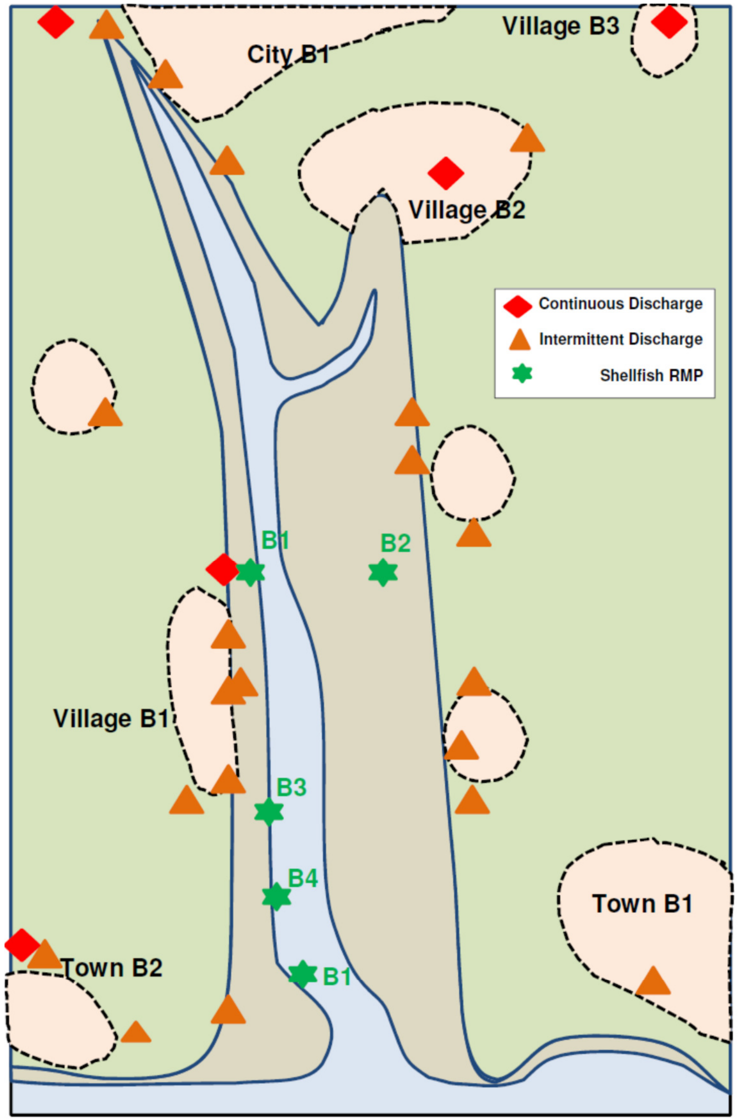
If exclusion zoning were considered for shellfish water some examples of possible impacts upon industry and regulators for Scenario A and B are considered in Example A1 and B1 below.

Figure 6.3: Illustrative Diagrams of Scenario Shellfish Water RMP and Wastewater Discharge Points

a) Scenario Shellfish Water A – Illustrative Diagram



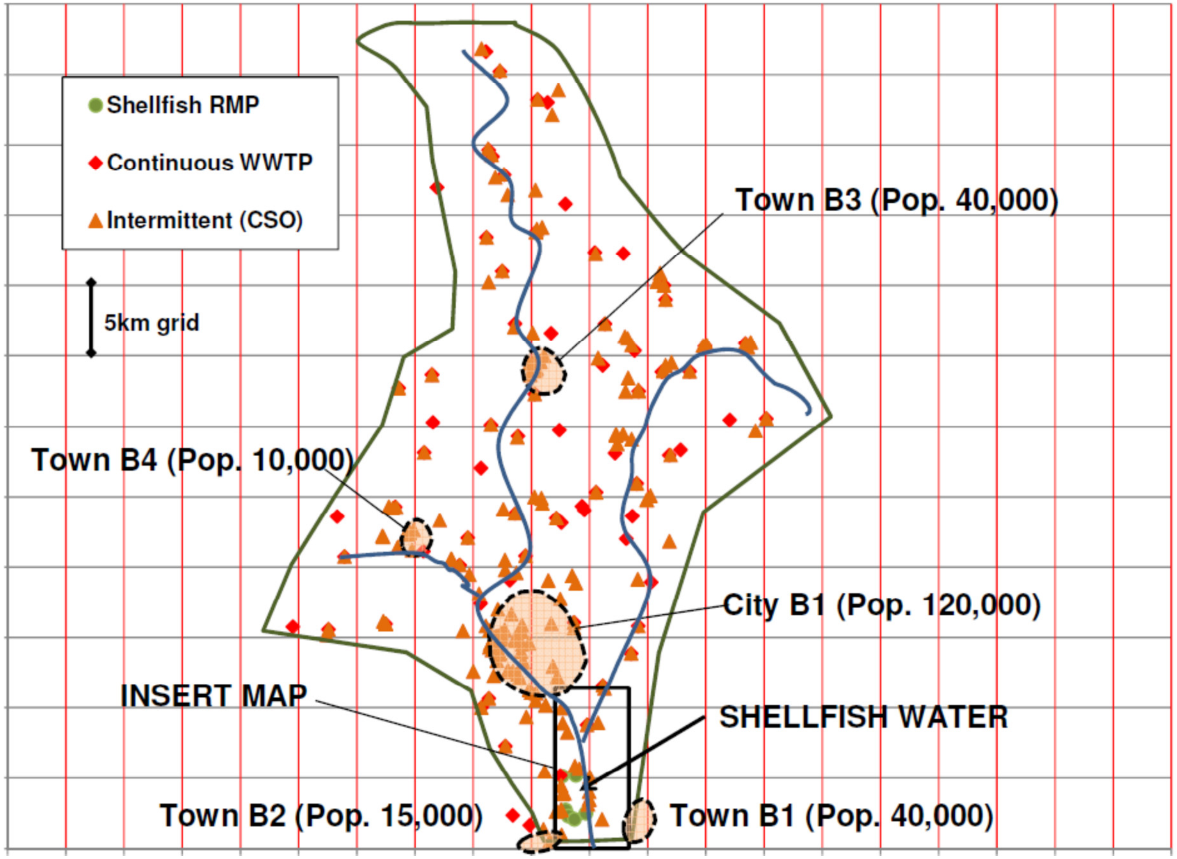
b) Scenario Shellfish Water B – Illustrative Diagram



Note: RMP = Representative Monitoring Point

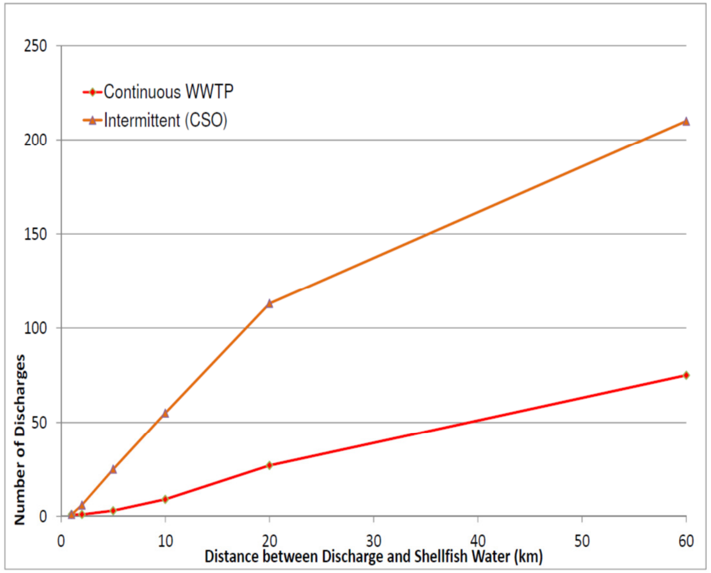
Figure 6.4: Proximity Relationship between Discharges and RMP Positions in Catchment Scenario B

Catchment B – Scatterplot of Discharge and Shellfish RMP Locations *(Note 1)*



(Note 1: Town, river positions and catchment boundary illustrative)

Catchment B – Discharge Proximity *(Note 2)*



Distance (km) Discharge to SW	Continuous Discharges	Intermittent Discharges
<1	0	1
<2	1	6
<5	3	25
<10	9	55
<20	27	113
<60	75	210

(Note 2: Distances relative to most inland Representative Monitoring Point (=RMP))

Example A1: - Exclusion based on use of 'representative' RMP NoV data

If exclusion zone criteria based upon NoV data were adopted for Scenario A then RMP A4 could be excluded as it may have historically produced NoV data exceeding potential harvest standard of >1,000 genome copies/g Dt. RMP A4 is at ~1,400m from the nearest discharge, whilst the edge of the shellfish water is just ~900m from the discharge so an exclusion zone to encompass the RMP (i.e. >1,400m) would in this case actually preclude a significant proportion of this sites production.

It should be noted that proximity also has a depth dimension which is not immediately apparent when viewing a map. In the case of Scenario A actual shellfish stocks adjacent to RMP A4 are located on the seabed sub-tidally, whilst the RMP stock for sampling is held below a floating mooring. The adjacent WWTP discharge is not actually continuous and only releases wastewater after High Water. Any ebb tide buoyant low density wastewater plume generated is likely to mix down from the surface within this deep water high salinity ria which will limit potential contamination at the seabed. It is therefore probable that near-bed commercial shellfish stocks will be cleaner than near-surface RMP samples.

Attempts to exclude this area would impact commercial viability for this operator at an otherwise generally good quality Class B site and with no indication that public health has been put at risk. The relevant operator might be inclined to challenge the exclusion on the grounds of weak discharge source association and aim to demonstrate that the RMP may not be 'representative.'

Example B1: - Exclusion based on geographical proximity data for RMP to discharge

If a proximity exclusion zone were adopted, say of an arbitrary 300m within Scenario B RMP B1 could be excluded as it has a separation of just ~200m from the nearest continuous discharge. In this case commercial stocks extend further away within the relevant shellfish water and the imposition of a 300m exclusion zone would have a limited impact upon commercial operations. Presumably a new RMP would need to be required in the shellfish water at the new exclusion zone boundary (i.e. at 300m away from the outfall). As no stock are affected in this case the exclusion zone would not improve public health, it would only undermine the regulatory continuity of the RMP long term data sets.

6.5.3 CSO Impacts and Zoning Implications

As highlighted in Section 6.1.2 the number of CSO intermittent discharges in shellfish water catchments varies considerably. This coupled with the uncertainties of spill volume and resultant impact make zone scaling considerations particularly difficult (Section 6.4).

Example A3: - Significant spill impact within a 'low risk' catchment

Figure 6.3 shows a relatively low number of CSO discharges for scenario A estuary with only 8 consented intermittent discharges in the whole catchment (6 of which within 5km of the SW). Many of these discharges are EOs from pumping stations which are known not to operate unless a failure occurs. A few discharges are CSOs which respond to storm events. All CSOs and most EOs discharge are equipped with Event Duration Monitors. Wider generic discussion of CSO operation is considered further in Sections 6.2 and 7.2.2.

For the purposes of illustration a single 'significant' $50m^3$ CSO spill is considered against the other continuous WWTP discharges within the catchment. This example uses bacterial indicator levels.

i.e.

- *'Significant CSO spill of $50m^3$:* Assume $1x10^7$/100ml with no microbial reduction = *E. coli* loading = $5.0x10^{12}$/day.
- *Public WWTP:* 2 secondary WWTP with combined flows of $75m^3$/day and 1 UV disinfected WWTP flow of $150m^3$/day (and an assumed reductions $3log_{10}$ and $5log_{10}$ respectively) from a crude *E. coli* concentration of $1x10^7$/100ml = *E. coli* loading = $7.6x10^9$/day

  N.B. CSO bacterial load maybe >650 times that of the combined WWTP load

The comparative load calculation above is based on *E. coli* related reduction factors through a WWTP which are unlikely to be so effective for NoV (see Section 3.7). In consequence, assuming ~$1log_{10}$ less treatment efficacy would result in a CSO viable NoV load of 65 times greater than the WWTP NoV load. However, the inability of UV disinfection to be able to demonstrate probable inactivation (Section 3.3.3) would further reduce apparent treatment efficacy by ~$2log_{10}$ indicating roughly comparable NoV loading levels (from a RT-PCR perspective. This means that although a 'significant' CSO spill would greatly increase the real viable NoV risk within Scenario A this would not be necessarily apparent through the shellfish analytical results.

Assessment of spill data from this catchment has in fact shown that 'significant' CSO spills are very rare. The simplicity and scale of Catchment A is such that there is a good potential to characterise how CSO spills respond and impact upon the shellfish water in response to rainfall events. This may lend the area to meet 'Conditional' zone requirement of being able to predict the impact of events (Section 4.1.3). As the number and intensity of CSO discharge positions within this area are relatively low there is scope for Active Management measures (Section 7.1.4) which might be more appropriate than blanket exclusion zoning.

It is concluded a 'significant' CSO spill could vastly increase NoV risk levels within this shellfish water at times of high NoV loading. it is suggested that alternative management measures may be more effective than exclusion zoning for this SW. There maybe scope to combine responsive zoning with complementary management measures (Section 6.6).

Example B4: - Significant spill impact within a 'high risk' catchment

Figure 6.4 shows that Scenario B shellfish water receives 25 CSO discharges within 5km and 210 for the wider riverine catchment with the majority of CSO associated with City B1 at the head of the estuary. Storm events will give rise to a number of associated spill events from various CSOs which from a scheme design perspective are amalgamated and assessed against 'significant' spill criteria.

For illustration a single 'significant' $50m^3$ CSO spill is considered against the other continuous WWTP discharges within the catchment. using bacterial indicator loading.
i.e.

- *'Significant CSO spill of $50m^3$:* Assume $1x10^7$/100ml with no microbial reduction = *E. coli* loading = $5.0x10^{12}$/day.
- *Public WWTP:* 3 UV WWTP with combined flow of $50{,}300m^3$/day providing an assumed $5log_{10}$ reduction in crude *E. coli* concentration of $1x10^7$/100ml = *E. coli* loading = $5.0x10^{10}$/day

  N.B. CSO bacterial load maybe >100 times that of the combined WWTP load

As with Scenario A from a NoV perspective WWTP treatment efficacy would be reduced and UV disinfection not so apparent – which would somewhat reduce the magnitude of the

NoV impact from a significant spill. However, unlike Scenario A this shellfish water example has a very large number of CSOs (see Figure 6.3 and 6.4) which are likely to provide a greater actual overall NoV impact in terms of frequency, volume and spatial coverage of significant CSO spills. The complexity of the system would make EDM monitoring and analysis difficult and expensive. At present for this catchment EDM data is qualitative so no-one actually knows what volume of wastewater is discharged by CSOs – limiting even theoretical calculations. Unlike the catchment in Scenario A, it may not be possible to relate quantitative CSO flows to rainfall intensity data. In consequence, attaining a 'Conditional' zone requirement for 'predictable' event response may be difficult.

A key difference between *E. coli* and NoV is the longer contaminant clearance rate which would extended NoV retention (Section 3.6.3) following a contamination event. This means even a short term CSO spill could impact shellfish flesh over a long period of a couple of weeks – CSO spill impact from a bacterial impact is considered to last for a day! If wastewater schemes are designed to allow 10 significant spills a year and the production area were to shut for 28 days following each CSO spill then harvesting could be prevented for much of the winter. In this situation shellfish commercial production could be compromised unless a reduced risk species were produced.

It is concluded that if exclusion zones were implemented the geographical extent and number of CSO discharges would be likely to limit production in much of this SW. Furthermore, as it is hard to assess the potential CSO impact within Scenario B shellfish water even Active Management within a 'Conditional' type of zoning might not be effective. The nature and type of shellfish operation may need to be appropriate to the risk profile.

6.5.4 Private Discharge Impact and Zoning Implications

Private wastewater discharges from unconnected population, private WWTPs and septic tanks can provide significant loading in certain catchments which may compromise water quality and exceed the level of load from public utility WWTPs (Section 6.3).

Public Water Utility WWTP consents are highly regulated and generally provide good wastewater quality with secondary or tertiary (UV disinfected) levels of treatment that often provide 3-5$\log_{10}$ reduction in *E. coli* concentrations. As indicated in Section 3.3.3 NoV removal and inactivation levels are not as effective providing assummed 2-4$\log_{10}$

removal/inactivation for secondary and tertiary WWTPs. The following scenarios consider the relative potential impact from these private wastewater sources.

Example A3: - Unconnected Population – Septic Tanks

Village A2 in Scenario A shellfish water is only partially served by a secondary WWTP treating an estimated 25m^3/day. Much of the village remains unconnected with private discharges and septic tanks. A comparative loading assessment has been performed assuming 50% of the village population remains unconnected and using bacterial indicator loadings.

i.e.

- *Unconnected village A2 private discharges of 25m^3:* Assume $2\log_{10}$ reduction in crude *E. coli* concentration of 1×10^7/100ml = *E. coli* loading = 2.5×10^{10}/day.
- *Public WWTP:* 2 secondary WWTP with a combined flow of 75m^3/day and 1 UV disinfected WWTP flow of 150m^3/day (and an assumed $3\log_{10}$ and $5\log_{10}$ reduction respectively) from a crude *E. coli* concentration of 1×10^7/100ml = *E. coli* loading = 7.6×10^9/day

  N.B. Private bacterial load from unconnected Village A2 private discharges maybe ~3 times greater than combined public WWTP load to estuary.

Any further reduced wastewater quality or crude discharges from the unconnected population would increase loading levels further. Freshwater loading microbiological investigations with source tracking technique reviewed in the Sanitary Survey would tend to reinforce the impact of diffuse human discharges to the catchment. This illustrates how potential septic tank loading which is symptomatic of diffuse rural catchments may compromise riverine water quality.

This scenario would suggest that exclusion zones would possibly need to be imposed upon freshwater courses in addition to WWTP discharges. Scenario A shellfish water receives minor stream input from around 10 sources which if all excluded could impact on area shellfish production. Loading determination and control from these sources is difficult to achieve. This highlights the danger that a relatively small proportion of rural population wastewater load could compromise microbial quality. It would be hard to establish evidence based risk proportionate zoning control for these sources.

Example B3: - Private WWTP Load

A private consented discharge of significant volume occurs to the estuary in close proximity to the SW. There are indications that the level of treatment may not have been optimal and as such comparative load calculations have been performed to demonstrate the impact of differing treatment efficacy using bacterial indicator loadings.

i.e.

- *Reduced treatment for 375m^3 /day:* Assume 2$\log_{10}$ reduction in crude *E. coli* concentration of 1x10^7/100ml = *E. coli* loading = 3.75x10^{11}/day.
- *Public WWTP:* 3 UV WWTP combined flow of 50,300m^3/day providing an assumed 5$\log_{10}$ reduction in crude *E. coli* concentration of 1x10^7/100ml = *E. coli* loading = 5.0x10^{10}/day

  N.B. Private bacterial load maybe ~600 times greater than the nearby UV WWTP load (and ~7 times greater than combined public WWTP load)

It is apparent that within Scenario B that this private discharge may be locally generating more microbial load than the adjacent public WWTP discharge and comparable loads to the total public WWTP contribution. In this particular case the private WWTP is not a seasonal holiday discharge and receives influent crude throughout the year and therefore capable of generating significant NoV load even during the winter high risk period.

Exclusion zoning around the public WWTP discharges in Scenario B shellfish water would have little tangible benefit to public health when poorly controlled private discharge loads can dominate local loading. Exclusion zoning around every consented discharge regardless of flow magnitude could prevent shellfish production along much of the estuarine and nearshore coastline.

Regulatory control of private consents is the responsibility of the Environment Agency within England. Generally, once a consent compliance issue is identified the EA can aim to enforce consent standards and require the site operator to rectify any problems. However, consented parameters are generally in terms of suspended solids, ammonia and BOD with little regard for microbiological performance. Older and smaller consents (<50m^3/day) may even be descriptive and difficult to drive private owners/operators to improve quality. The nature of NoV loading is such that even small quantities of poorly treated wastewater can contribute a significant load exceeding that of well performing

WWTPs serving much larger populations. In essence, poor wastewater performance for a minority of the population can exceed the impact from the majority of the population served by good WWTPs.

Most wastewater discharge 'easy hits' have already been addressed with diminishing returns relative to expenditure for future improvements. Societal choices for environmental quality and cost-benefits are likely to be key considerations in future WFD based spending for public utility discharges. Enforcement resources to chase 'minor' discharge compliance could also be an issue and may encourage shellfish operators to engage their own defensive monitoring.

6.5.5 Vessel Discharge Impacts and Zoning Implications

Section 6.3 considers the generic vessel impact issues whilst examples A4 and B4 assess the potential site specific vessel related impacts using data from the relevant sanitary surveys.

Example A4: - Scenario A Vessel Impact

Scenario A shellfish water is a popular destination for recreational craft. The Sanitary Survey for the area indicates the presence of around 600 vessel moorings and anchorage capacity for 50 visiting vessels. Vessel moorings are directly adjacent to shellfish production areas. Review of the RYA Green Blue directory indicates that there is no public Pump Ashore facility within the area for removal of wastewater from vessels.

NSSP (Ref: 2009) guidance on calculating potential vessel marina impact can be used to gauge potential impact from a bacterial indicator perspective.

i.e.

- *Vessels:* Assume 1% occupation of vessels and 2 people/boat contributing 2 $x10^9$/person/day = *E. coli* loading = $2.4x10^{10}$/day.
- *Public WWTP:* When compared to the largest secondary treated WWTP discharge (near RMP A4 which has a flow of $50m^3$/day and an assumed $3log_{10}$ reduction on a crude *E. coli* concentration of $1x10^7$/100ml = *E. coli* loading = $5.0x10^9$/day
- **N.B. Vessel bacterial load maybe ~5 times greater than main WWTP discharge (and ~3 times greater than combined public WWTP load)**

This theoretical comparison would suggest that even very low levels of vessel occupancy with associated wastewater discharges may contribute *E. coli* loads exceeding those from treated WWTP discharges. This may particularly be the case in the summer when observational data suggest peak vessel occupancy maybe closer to 10%. Fortunately vessel impact is likely to be reduced over the winter when the NoV risk is increased. However, vessel sources still might be problematic for spring and autumn holiday periods. In addition to pleasure craft in the lower reaches of the ria Village A2 at the upper tidal limit supports a number of live-aboard vessels which are likely to contribute to microbial load throughout the year.

It is concluded that vessel NoV loading direct into the shellfish water could at times result in risks to public health. Exclusion zoning around the wide-spread vessel mooring areas throughout the shellfish water area would exclude much of the production area. As seasonal vessel usage (with corresponding wastewater discharges) and NoV risk period are not likely to be synchronous exclusion zoning around vessel areas could be considered a disproportionate management tool. Increased shellfish NoV monitoring (and perhaps harvesting prior to holiday period) around Easter holidays might be a more appropriate management tool.

Example B4: - Scenario B Vessel Impact

The Sanitary Survey for the area indicates the presence of around 1880 moorings (of which 1620 in use) and 220 pontoon berths showing that this area too is subject to a high level of vessel use. Review of the RYA Green Blue directory indicates that there is no public Pump Ashore facility within the area for removal of wastewater from vessels. However, pontoon access to shoreside facilities might reduce the level of uncontrolled vessel discharges.

NSSP (Ref: 2009) guidance on calculating potential vessel marina impact can be used to gauge potential impact from a bacterial indicator perspective.
i.e.

- *Vessels*: Assume 1% occupation of vessels and 2 people/boat contributing 2 $x10^9$/person/day = *E. coli* loading = $7.2x10^{10}$/day.
- *Public WWTP:* When compared to the closest UV treated WWTP discharge (near

RMP B1) which has a flow of $615m^3$/day and a minimum $5log_{10}$ reduction on a crude *E. coli* concentration of $1x10^7$/100ml = *E. coli* loading = $6.1x10^8$/day

- **N.B. Vessel bacterial load maybe ~100 times greater than the nearest UV WWTP discharge (and ~comparable to the combined public WWTP load)**

As with Scenario A this theoretical comparison would suggest that even very low levels of vessel occupancy with associated wastewater discharges direct to shellfish water may contribute *E. coli* loads exceeding those from treated WWTP discharges. The corresponding theoretical relative NoV load would be less pronounced as removal rates through WWTP process are less effective.

It is concluded that vessel derived NoV risk has potential to be problematic to shellfish quality for this catchment. However, as with Scenario A the mis-match between NoV loading risk and likely vessel use would suggest imposition of an exclusion zone over the whole production area might be an excessive measure.

Guidance is provided to recreational vessel users to minimise sewage impact to the marine environment (see Section 6.3). In practice, visiting vessels to the highly utilised and busy waterways of the UK may have no idea when they enter an area at HW whether they are immediately adjacent to an oyster production area or not. Vessel users are unlikely to appreciate the potential adverse impact a single symptomatic NoV sufferer could have on a whole SW. Recommendations to address vessel related discharge impacts are provided in Section 8.

Table 6.5: Summary of wastewater discharge zone impacts on Scenario A and B catchment illustrations

Shellfish Water	Scenario A	Scenario B
Description *(Note 1)*	Catchment small with limited population and wastewater input. shellfish water deep with good dilution and degradation potential	Catchment large with significant city and towns providing multiple WWTP and CSO wastewater inputs. shellfish water shallow with limited dilution and reduced degradation potential
WWTP Impact on RMP (Section 6.5.2: Examples A1, B1)	Exclusion zoning based on NoV data at RMP. In this case an RMP 1,400m from a secondary treated discharge may have had elevated historical NoV shellfish levels exceeding the proposed 1,000 genome copies/g Dt. Exclusion of this RMP would have a significant impact on operator. Serious concerns about the representative nature of the RMP could lead to a legal challenge of exclusion.	Exclusion zoning based on proximity. In this case if an RMP 200m from a tertiary treated discharge were excluded on the grounds of proximity then there would be limited impact on operator for this bed. A new RMP would then need to be established at the new zone boundary which would break regulatory continuity for no tangible public health benefit.
CSO Impact (Section 6.5.3: Examples A2, B2)	A 'significant' 50m^3 CSO spill would greatly exceed WWTP microbial loads. Fortunately, CSO discharge number, frequency and magnitude of discharge is very limited within this catchment. There is good scope for Active Management to support a 'Conditional' zoning approach.	A 'significant' 50m^3 CSO spill would greatly exceed WWTP microbial loads. CSO number, frequency and magnitude would be problematic within this catchment. There is limited scope for Active Management. The spatial coverage of CSO discharge points would impact shellfish production areas in the event that exclusion zoning were imposed.
Private Discharge Impact (Section 6.5.4: Examples A3, B3)	Private discharges via septic tanks to the upper reaches of the estuary occur in rural areas with one village only partially served by a public secondary WWTP. Microbial load from the unconnected village with limited treatment efficacy was calculated to exceed that of the combined catchment public WWTP discharges. Exclusion zones applied to all minor freshwater stream inputs could prohibit production from a large proportion of the SW.	A private WWTP poorly performing discharge to the lower estuary could far exceed local public WWTP microbial loading and influence shellfish quality. Private discharge consent compliance and future quality improvements is likely to be a difficult topic and highlights how even small poor quality discharges may impact overall NoV quality. Exclusion zones to all consented discharges regardless of flow rate would prohibit production from a large proportion of the SW.
Vessel Impact (Section 6.5.5: Examples A4, B4)	High vessel use with potential for periodic reduced quality. Exclusion zoning would seriously compromise shellfish production viability but alternative management options might be appropriate	As with Scenario A

(Note 1: Impact compared relative to public utility WWTP loading)

6.6 Potential Application of Exclusion Zone Options

The Scenario illustrations considered in the previous section highlight that there are likely to be implementation problems with the application any singe exclusion zoning approach. There are two key issues:

- The zoning measure could poorly target the range of wastewater NoV sources and place an inappropriate zone around some sources whilst missing other more significant NoV sources
- A precautionary approach could place extensive zones on all potential wastewater sources and shellfish production would be commercially compromised in most shellfish waters with regulatory measures disproportionate to the level of risk

In an ideal world a scientifically robust evidence based approach would be adopted to set zoning requirements. This would be implemented with the use of appropriate analytical tools used to assess viable NoV risk in water quality and flesh quality. However, as extensively reviewed in Section 3 the current science base cannot yet provide evidence based zoning. In consequence, it is suggested that zoning cannot provide a complete viral risk management solution. It is however possible that some sort of zoning component could be combined with alternative management measures. A range of potential shellfish management and water industry management options are considered in Sections 7.1 and 7.2 respectively. It is suggested that no single measure will be completely effective and that every site or region might need to develop its own range of options which are most appropriate.

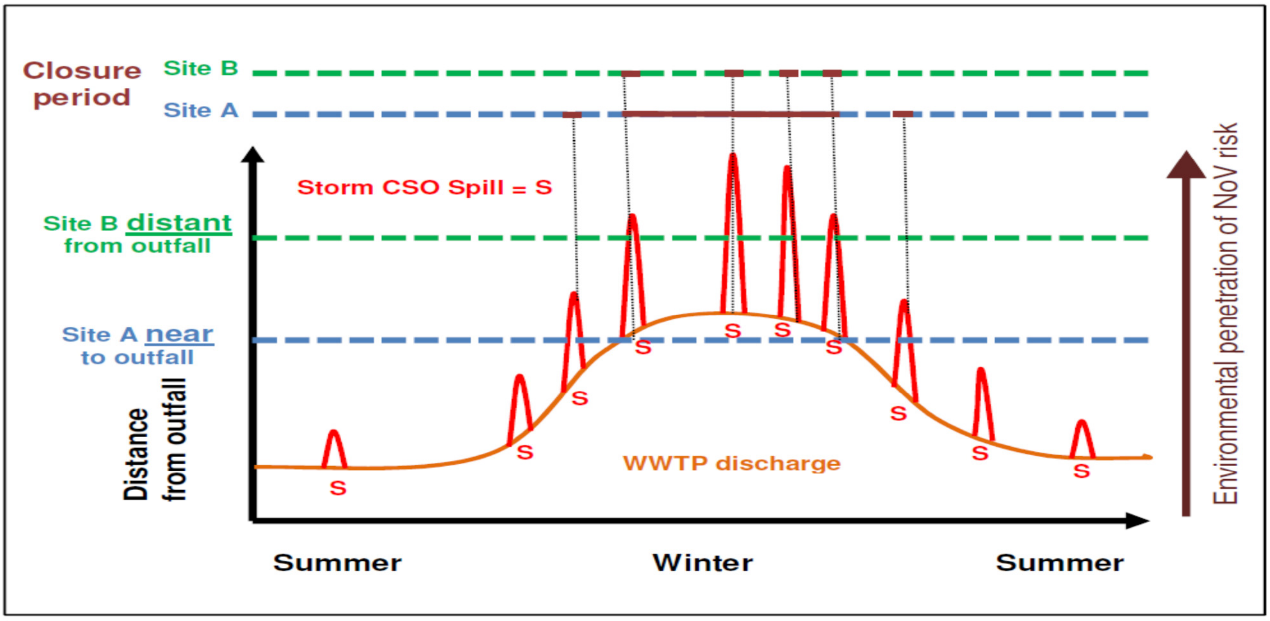
Exclusion zoning may be part of a combined viral risk management package for some areas at certain times. Examples of different zoning options and how they might be adapted are considered below:

- **Default Zoning** – Fixed zoning imposed on basis of proximity to wastewater discharge (e.g large continuous WWTP discharges). Range of zoning could possibly be scaled according to anticipated level of loading and modelled plume impact upon sensitive shellfish area. Alternatively, NoV impact could be assessed using US FDA style dilution/NoV uptake studies.
- **Responsive Zoning** – Emergency or event based zoning probably at precautionary fixed zone ranges without matching scaling to specific risk factors. (e.g. following outbreak or CSO spill conditions such as within the 'Winter Norovirus Protocol').

- **Composite Zoning** – It may be possible to combine 'default' and 'responsive' zoning criteria to a range of prescribed differing levels of control/management according to a combination of risk factors. The ability to scale zoning according to multiple factors could include:
    - **A) - Default minimum zoning**. Near outfall preventing production and harvesting
    - **B) - Seasonal zoning**. Production allowed but harvesting prevented at times of increased NoV
    - **C) - Spill zoning**. Production allowed but harvesting prevented following EDM notification of CSO spill

Figure 6.5 illustrates how the changing seasonal and spill event NoV risks may change over a year, This could result in extended winter periods of closure for Site A close to the outfall and periodic storm related closure for Site B more distant from the outfall.

Figure 6.5: Diagrammatic illustration of how NoV loading risk might influence shellfish harvest closure



The scaling and responsive nature of these zones would need to be evidence based and site specific in order to obtain buy-in from local shellfish operators. Ideally, if resources were available survey work could be undertaken to inform zone scaling, although for multiple wastewater sources this would be cost prohibitive. If resources were limited it is suggested that an initially precautionary zone is adopted and then adjusted through a self-learning process:

- Stage 1: Using NoV testing
  (whilst gathering sea temperature/rainfall /salinity data e.t.c)
- Stage 2: Using surrogate data e.g. sea temperature data and NoV data
  (backed up with periodic NoV data)
- Stage 3: Using surrogate data
  (with periodic NoV data)

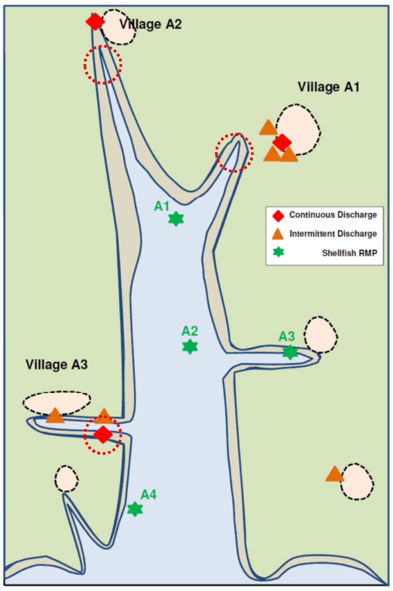
The use of site specific monitoring to develop understanding of local risk factors could be encompassed within a risk matrix scoring scheme (see Section 7.3). This could be pegged to action thresholds to move zoning from A→B→C as conditions develop. This adaptive management approach is based on ‘learning through doing’ as a practical approach to balance protection of public health with allowing continued shellfish operations. The objective of this approach would be to gain sufficient understanding of risk factors to develop a degree of impact ‘prediction’ in common with the US style ‘Conditional’ zones. Enhanced management such as this is considered further in Section 7.3.3.

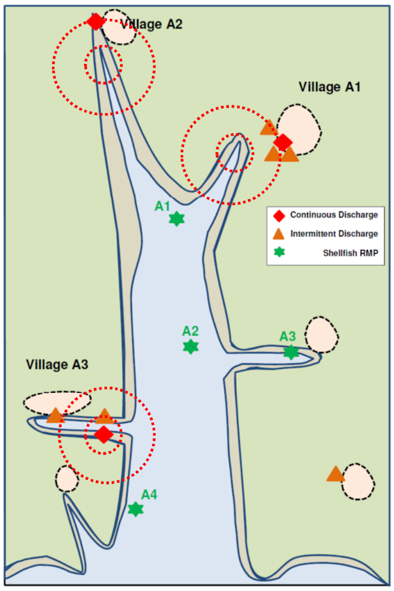
Figure 6.6 using the Scenario A catchment provides an illustration of potentially how composite zoning incorporating seasonal and spill risk factors could be used to develop ‘enhanced management’ zones. The complexity and density of wastewater discharge sources for Scenario B catchment suggests that from a zoning perspective a ‘restricted’ zone status would be probable. Additional management measures could be put into place to help provide an ‘assured’ product quality although these would impact on the cost of production and, depending on the shellfishery, its potential commercial viability.

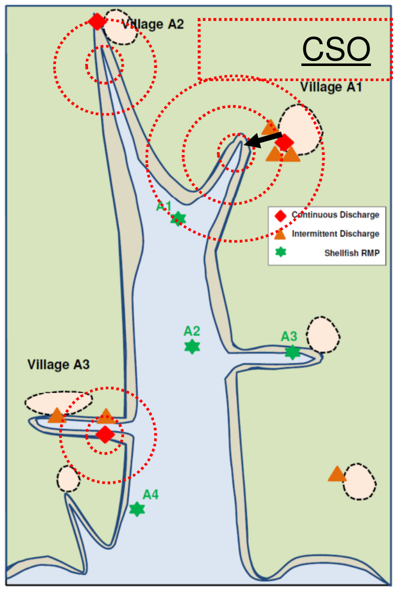
Recommendations for the development of such an evidence based approach are provided in Section 8.4.

Figure 6.6: Scenario A - Illustration of Potential Composite Zoning

a) Default zoning (based on low risk WWTP summer load)	b) Seasonal zoning (based on higher risk WWTP winter load)	a) Spill zoning (based on EDM data e.g. from Village A1)







Could this approach work here?

Possible Issues:

a) Zone range shown is diagrammatic and would need to be scaled on evidence base. (i.e. Unknown: dilution effects, environmental removal and degradation, uptake rates. Possible to proceed using NoV flesh testing and surrogate measurements) ,

b) Diffuse NoV load from catchment or vessels could undermine zoning (scope for background NoV shellfish flesh biosentinal monitoring)

Possible other management components:

a) NoV shellfish flesh testing

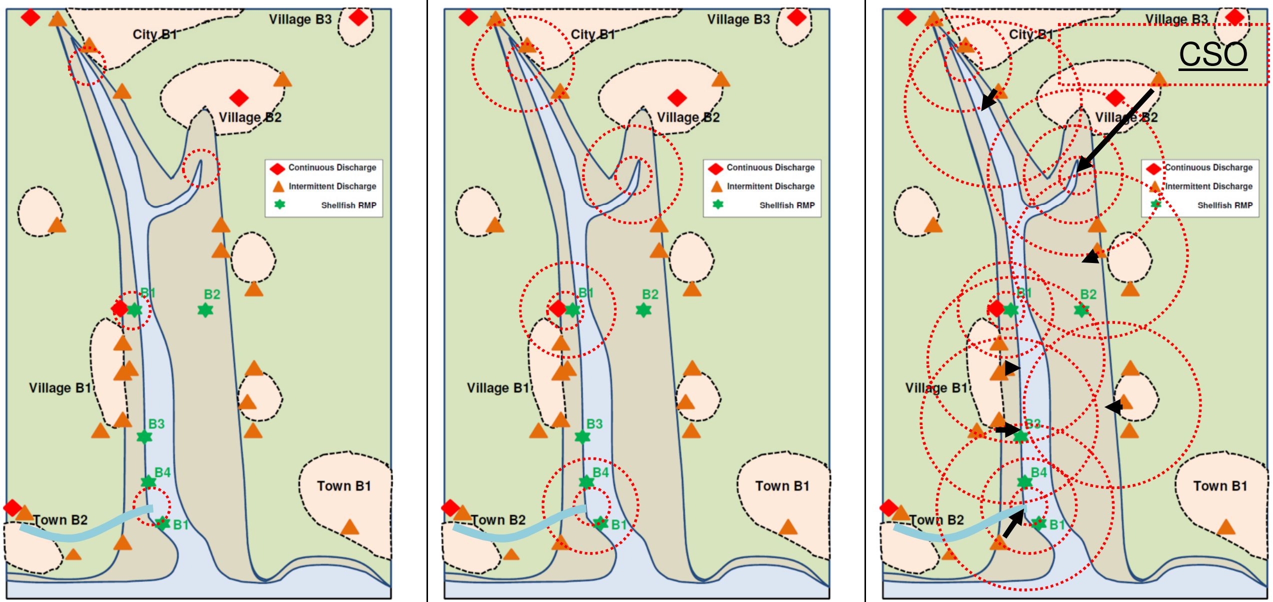
b) EDM monitoring of CSO performance

c) Receiving water monitoring of in-situ temperature and salinity

Composite zoning with Active Management (Enhanced Management) – Could work

Figure 6.7: Scenario B - Illustration of Potential Composite Zoning

a) Default zoning (based on low risk WWTP summer load)	b) Seasonal zoning (based on higher risk WWTP winter load)	a) Spill zoning (based on EDM data) e.g. from multiple sources)



Could this approach work here?

Possible Issues:

a) Zone range shown is diagrammatic and would need to be scaled on evidence base. Multiple WWTP discharges make risk from winter seasonal B zones challenging Multiple CSO discharges make risk from CSO spill unmanageable

b) Potential for significant diffuse NoV load from catchment and vessels

Potential for extensive impact on shellfishery. Unlikely to be effective

6.7 UK Application of Various Management Options

6.7.1 Regional Management Options

Section 7 considers a range of alternative shellfish and wastewater management tools which could help reduce wastewater NoV contamination impact upon shellfish quality. In many cases these management tools may be complimentary to exclusion zoning. The options for both shellfish and water industry management vary on a regional basis so that different combinations of approaches might be appropriate on a site specific basis. This sub-section considers management options from a UK application perspective.

Table 6.6 provides an overview of potential combinations of management measures which might be appropriate for various UK regions. These suggestions have been presented on the grounds of various geographical and risk profile features. Management options which might support zoning assessments include:

- Computer modelling provision of discharge dilution and time of travel predictions
- CSO reporting with Active Management system for reactive zoning

Table 6.6: UK regional overview of broad risk profiles and management options

Region *(Note 1)*	**Risk Profile** *(Note 2)*	**Management Options** *(Notes 1,2,3)*
West Coast Scotland	Small coastal communities served by small WWTPs or septic tanks. No or minimal CSOs and vessels; Limited access to computer modelling. Catchments generally rural with low NoV loading	Relay supported by NoV EPT.
Northern Ireland	Moderate and large coastal communities served mostly by secondary WWTP. Generally large catchments with risk of catchment wide NoV loading. Limited access to computer modelling. Moderate to high CSO spill risk (site specific)	Possible scope for improved wastewater treatment (e.g UV). Scope for computer modelling
Wales and most of England	Moderate and large coastal communities served mostly by secondary WWTP. Some large catchments with risk of catchment wide NoV loading. Good access to computer modelling. Moderate to high CSO spill risk (site specific)	Possible scope for improved wastewater treatment (e.g UV). Good potential for computer modelling
SW England	Small and moderate coastal communities served by UV WWTP and good stormwater storage. Moderate to high CSO spill risk (site specific) Limited access to computer modelling	Good potential for CSO spill monitoring and Active Management

Note 1: Region geographical features will include population intensity and distribution as well as coastal features (see Figure 6.1)

Note 2: Wastewater risk profile is a function as the relevant Water Utility asset profile and strategy towards shellfish waters (see Figure 6.2).

Note 3: Site specific consideration of local risk profile and management options needed. All regions may have scope for 'Alternative' management options (e.g. mitigation measures)

It is beyond the scope of this report to consider the relative benefits of other management measures unrelated to exclusion zones. It is possible that future impact assessments could consider these options on a more holistic cost-benefit basis, perhaps with a regional approach.

Any management measures will require appropriate resources to develop and in some cases a clear regulatory driver. These issues are considered in the following sub-section.

6.7.2 Regulatory Framework

Permitting, monitoring and regulatory enforcement of wastewater discharges is the responsibility of the various environmental agencies shown in Table 6.7.

Table 6.7: Relevant government agencies in regional /devolved UK

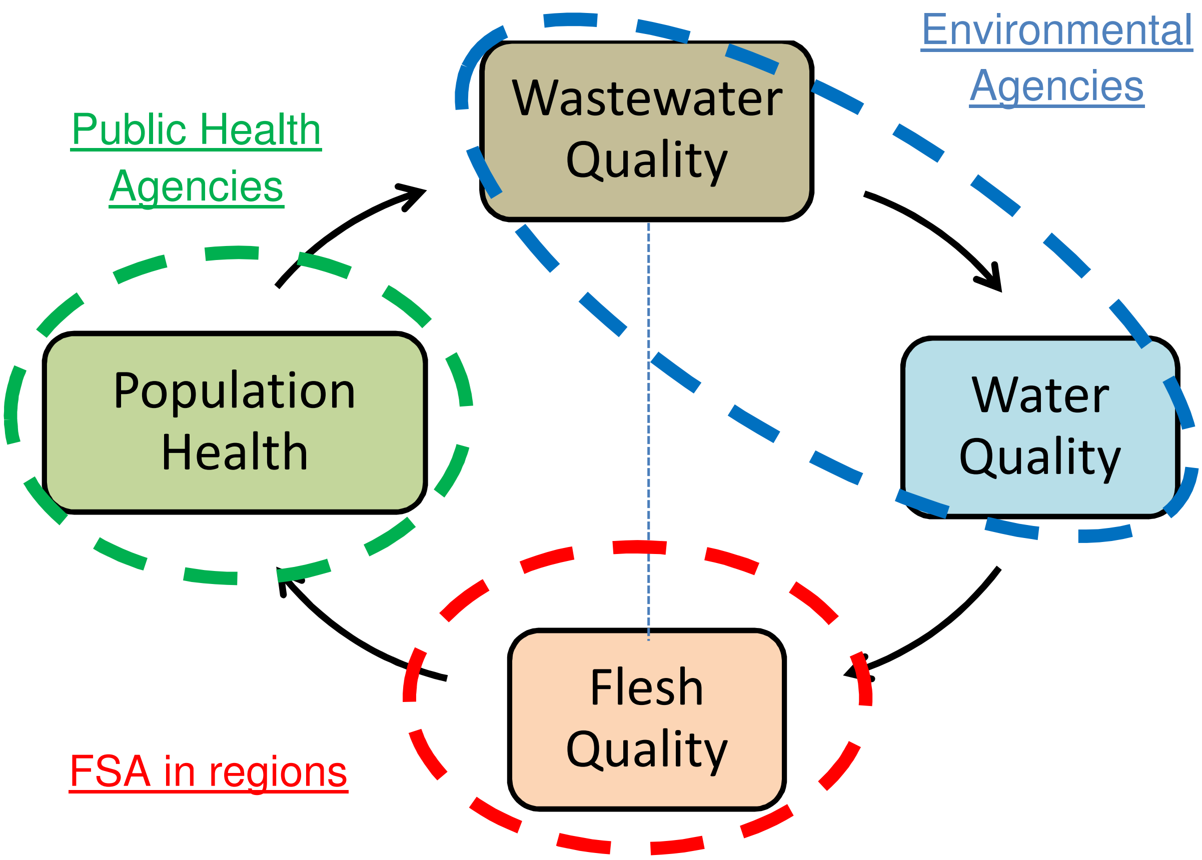
Public Health	**Food Standards**	**Environmental Agencies**
Health Protection Scotland	Food Standards Agency In Scotland	Scottish Environmental Protection Agency
Public Health Agency for Northern Ireland	Food Standards Agency In Northern Ireland	Department of Environment Northern Ireland
Public Health Wales	Food Standards Agency In Wales	Natural Resource Wales
Public Health England	Food Standards Agency In England	Environment Agency

The Shellfish Waters Directive (SWD) historically provided a driver for wastewater scheme improvements to meet guideline microbial standards based on bacterial Faecal Indicator Organisms (FIOs). Section 3 details how NoV wastewater treatment and environmental behaviour differs from that of bacterial FIOs, such that existing regulatory programmes may not be able to protect against NoV foodborne threats.

The SWD was repealed at the end of 2013 by the Water Framework Directive (WFD). The various devolved regions have the option to set their own microbial targets to meet the needs of the WFD. The new regulatory and Water Sector asset review process has become increasingly complex with the cycles of the River Basin Management Plans (RBMP) and the Water Sector asset review process not in sync, for example the 2015 – 2020 water company improvement period does not match the 2016 – 2021 RBMP round. There is no scope at present for any new direct environmental based NoV regulatory measures to protect shellfish flesh quality in support of a potential NoV shellfish standard. In the absence of a regulatory driver it is unclear how resources can be allocated to reduce catchment wide wastewater loading on a strategic basis from a NoV perspective.

As shellfish quality straddles both food and environmental regulatory regimes it is important to develop a harmonised shellfish food safety strategy. A number of government agencies have partial responsibility with roles in protecting public health, food safety and environmental quality (see Figure 6.8).

Figure 6.8: Government bodies with responsibilities influencing NoV environmental transmission through shellfish



Note 1: See Table 6.6 for devolved and regional Governmental agencies

The FSA's Advisory Committee on the Microbiological Safety of Food (Ref: ACMSF, 2014) draft report has recently recommended:

*"Given the range of risk management options set out above, Defra and the FSA should work together to develop a unified strategy for managing the risk from raw bivalves." (*Recommendation R6.7)

High level recommendations are made in Section 8.5.

6.8 UK Application – Summary

Section 6 provides a profile of the UK oyster industry and its potential exposure to various wastewater discharge sources. Options for various types of potential exclusion zoning are also considered in the context of the UK oyster industry.

- Wastewater discharges occur from multiple sources including continuous WWTP discharges, intermittent CSOs, smaller private discharges and vessels. The high NoV load within contaminated wastewater relative to the low infective dose can result in even small volume wastewater discharges having the potential to compromise production of safe shellfish products. This has implications for zoning in terms of both special and temporal coverage in addition to the practicality of implementation.

- A UK oyster database has been constructed integrating Shellfish Hygiene and Shellfish Water data relating to production areas and wastewater discharges to inform future impact assessments. This resource can be further developed with an GIS component to obtain an initial profile of oyster:wastewater discharge relationship with:
    - Continuous WWTP discharges
    - Intermittent CSO discharges

- The FSA NoV prevalence data has been re-analysed (Ref: Campos Unpublished) to assess the relationship between NoV contamination in oysters and environmental factors. This study included significant relationships with wastewater discharge 'risk factors':

- Continuous discharge magnitude. Settlement with populations of >80,000 served by >2 discharges was shown to be a potential factor in reducing NoV shellfish quality. A UK map has been produced (Figure 6.1) which highlights a stark difference between relatively low risk Scottish sites and high population areas for the rest of the UK. This concurs with the NoV regional contamination profiles obtained in the prevalence study.
- CSO Intermittent discharge frequency. Areas receiving >10 wastewater spills/yr exhibited reduced NoV shellfish quality. A UK map has been produced (Figure 6.2) which provides a similar pattern of risk to that presented by continuous discharges highlighting the general coincidence between CSO discharge number and the magnitude of continuous discharge (i.e. degree of connected population).

- Diffuse catchment loads from septic tanks (Section 6.3.1), vessels (Section 6.3.2) and sewage sludge (Section 6.3.3) are likely to contribute to the NoV load which may impact shellfisheries. These sources are likely to be hard to control and zone individually which highlights why other countries in Europe and the US have control or zoning for freshwater inputs and marinas (see Section 2.1 and 2.2). Recommendations are made for the inclusion of these risk factors within a future impact assessment (Section 8.4). Consideration should be given to successful control measures used elsewhere to help limit impact perhaps with better awareness and notification schemes similar to those in the US.

- Exclusion zone options were considered from a UK generic adoption perspective (Section 6.4) for proximity based zoning as used in some EU countries (Section 2.1) and within Prohibition and Conditional zones as used in US affiliated countries (Section 2.3). No option provided a good fit for easy adoption within the UK. EU geographical proximity based zones are somewhat arbitrary as they have no science evidence basis for scaling. US zoning although evidence based is grounded in bacterial indicator water quality standards. As highlighted in Section 3 there are currently a number of evidence gaps which prevent the development of evidence based zoning for NoV.

- Zone Impact Scenarios (Section 6.5) were developed based upon a couple of diverse real shellfish waters. The catchments selected varied with different wastewater risk profiles and zone 'examples' developed as summarised in Table 6.5.
    - WWTP discharges (Section 6.5.2). Detailed examples were developed to show how discharge proximity to the Representative Monitoring Point (RMP) could impact on potential zoning schemes. Different types of zoning employed (proximity, shellfish NoV data based) are likely to present a range of implementation difficulties presenting problems to both industry and regulators.
    - CSO discharges (Section 6.5.3). Load calculations have been performed comparing microbial input from a CSO wastewater spill against the background treated continuous WWTP discharges. In both catchments a single 'significant' $50m^3$ untreated wastewater spill is likely to exceeded the load from other background continuous discharge sources. Scenario A is exposed to just a few infrequently operating CSOs, whereas Scenario B has many CSOs discharging directly to the estuarine shellfish water and 210 CSOs within the wider catchment. Zoning around CSOs would be complex to implement and could have a major negative impact on commercial viability for some shellfish waters.
    - Private discharges and septic tanks (Section 6.5.4). Comparative loading calculations were performed for a poorly performing private WWTP and unconnected rural population with septic tanks. In both cases the relative microbial load exceeded the background 'main' public utility WWTP consented discharges with zoning implementation implications.
    - Vessel discharge (Section 6.5.5). Comparative loading calculations performed using US NSSP guidelines and assumed 1% vessel occupancy indicated potentially greater microbial load from pleasure boats than from the combined background public WWTP continuous discharges. From a NoV seasonality perspective the potential threat level maybe somewhat reduced as peak vessel use is unlikely to correspond with high NoV wastewater load.

- Exclusion zone controls could be made to be more effective if combined with Active Management measures (e.g. CSO EDM responsive monitoring) or other alternative management approaches (Section 7). Integration within these types of 'enhanced management' zones could encompass prohibition of production immediately around WWTP discharges coupled with event triggered responsive zones around CSOs to temporarily prevent harvesting at times of increased threat (Section 6.6).

7 SHELLFISH MANAGEMENT MODELS AND TOOLS

The principal objective of this section is to evaluate whether an exclusion/proximity component could be integrated within a viral risk management matrix with the aim of establishing a generic model that can be used by harvesters to support risk management decisions.

This section explores both Shellfish Industry (Section 7.1) and Water Industry (Section 7.2) management measures which may form part of a system of control. The literature review of science based evidence (Section 3) demonstrated that there is limited scope to adequately and consistently remove all NoV sources of contamination to a precautionary level to ensure no shellfish derived foodborne illness. As there is no ‘magic bullet’ to resolve the NoV issue, a combination of measures determined on a regional and catchment basis are likely to be necessary. . A pragmatic approach is to develop a range of flexible management control measures which can be dynamically adjusted according to the level of risk. This type of approach can only be effective if shellfish and wastewater management measures are integrated and as such this section also focuses on areas of potential cross-sector co-operation. As all options will have financial implications, management measures will be evaluated against a ‘proportionate cost’ test under the Water Framework Directive (WFD).

This Section has been prepared with input from Aquafish Solutions Ltd.

7.1 Review of Shellfish Industry Management Options

7.1.1 NoV Testing – Harvest Levels, EPT Levels and Due Diligence

NoV testing using RT-PCR provides a useful tool to assess potential risk within oysters (Ref: Jothikumar *et al.*, 2005). The limitations of *E. coli* testing as an effective indicator of viral pathogenic risk, has been demonstrated by a number of researchers. Some workers have advocated complimenting NoV RT-PCR testing with other parameters such as bacteriophage analysis to better characterise viral risk (Doré *et al.,* 1998 and Flannery *et al.*, 2009.)

Over the last 10 years considerable work has been undertaken in the EU particularly by the Member State NRLs and the EURL (Cefas Weymouth) to standardise the method for regulatory purposes. In summer 2013 the RT-PCR CEN method (Ref: ISO 2013) was accepted paving the way for its potential adoption in support of regulatory monitoring.

The value of RT-PCR NoV analysis remains a contentious issue in view of the uncertainty regarding the level of inactivation by wastewater treatment (Section 3.3) and through environmental degradation (Section 3.5). The principal criticism of NoV testing is that there is still no agreed method to differentiate viable from non-viable NoV and this issue (Section 3.1.3) remains the single most important data gap which limits the application of the RT-PCR tools developed.

How NoV testing can be employed by industry and regulators is still under debate with issues including whether testing should be:

- regulatory mandatory or industry best practice,
- as standards for harvest or EPT,
- routine or in response to outbreak,
- to inform area closure or area opening ‘all clear’
- for ‘up-chain’ selection of stock or for demonstrating ‘down-chain’ quality

This sub-section considers potential regulatory harvest and final product NoV levels, area closure/opening criteria along with industry Due Diligence guidance levels which have been adopted by some operators.

Harvest Levels

A potential harvest limit of 1,000 genome copies/g has been put forward as a potential threshold which can be sufficiently treated through depuration techniques. Researchers in Ireland (Ref: Dore *et al.*, 2010) suggest that relatively high levels of NoV (>1,000 genome copies/g) are required to cause significant outbreaks of illness with Irish data being consistent with actual observed illness dose-response data (as opposed to the modelled theoretical limit for NoV infection of 18 particles). These workers propose a NoV standard as a positive public health benefit especially given the inadequacy of *E. coli* based controls.

The FSA's Advisory Committee on the Microbiological Safety of Food (ACMSF, 2014) have recently recommended to FSA:

*"The potential value of routine norovirus monitoring for better risk management during primary production should be evaluated by the FSA." (*Recommendation R6.1)

Harvest bed testing for NoV has a couple of advantages:

- *Develop Good Understanding of Spatial and Temporal NoV Variation*. Within a shellfish production area a Food Business Operator may have access to a number of shellfish harvest beds each with a different risk profile. An understanding of their relative risk exposure could allow a shellfish operator to selectively harvest according to seasonal risk removing stock from at risk sites prior to contamination whilst retaining more pristine sites for later in the season.
- *Potential to Limit Product Recall.* NoV testing in oysters direct from the shellfish bed has a key advantage over final product testing in that it potentially allows analysis in the period during depuration before dispatch of product to market or customer. This then provides scope to retain, quarantine, or even relay stock depending on levels obtained. Indeed for larger intermediary distributors, batch purchase of stock from smaller independent producers may be conditional upon NoV levels.

There are some disadvantages to harvest sampling relative to End Product testing:

- *Uncertainty about Final Product NoV level*. As considered in sub-Section 7.1.3, depuration only provides a moderate reduction in NoV levels. As depuration efficacy can be contingent on system specific conditions a batch with a marginal harvest NoV result cannot guarantee a safe product. However, in practice, operators who undertake harvest NoV testing usually also undertake some final product testing and can build their confidence about the performance of their system especially if it has a high level of environmental control (e.g. temperature setting within depuration system for a degree hour approach or possibly enhanced depuration).
- *Inability to demonstrate Final Product NoV level.* Harvest testing does not provide a specific result for a batch of product which may be placed on the market. In

consequence, if a customer is making purchase decisions based upon an End Product analytical certificate further testing would be required.

EPT Levels

End Product Testing (EPT) standards should be able to demonstrate that a seafood product is safe regardless of potential customer cooking practice. Cefas website documentation indicates that foodborne outbreaks have been related to shellfish with a NoV content as low as 152 genome copies/g. In their role as the EURL, Cefas has put forward 200 genome copies/g as a prospective final product standard for consideration.

The difficulty facing the setting of an EPT standard is the uncertainty over both NoV viability in the sample and an acceptable infective threshold. In consequence, whilst there may be an overall dose-dependent relationship it is hard to differentiate risk for individual sites and samples. For example, the NoV level in an oyster subjected to a low level of viable contamination (e.g. perhaps in an area impacted by nearby recreation craft or CSO discharge) may present a greater risk of infection than an oyster with a higher level of largely non-viable contamination (e.g. from a site near a large UV disinfected fully treated discharge).

Regulation of Area Closure and Opening

NoV testing as part of routine monitoring or in response to an 'event' or outbreak may provide a criteria for area closure as indicated by this SHD extract from 'Decisions after Monitoring' which states: *"where the results of sampling show that the health standards for molluscs are exceeded, or that there may be otherwise a risk to human health, the competent authority must close the production area concerned, preventing the harvesting of live bivalve molluscs."*

CODEX guidance would also suggest a place for NoV testing in providing a re-opening criteria. *"If there is evidence that the area has been impacted by human sewage, testing of water or bivalve molluscs for the presence of indicators of faecal contamination and/or NoV or HAV, as determined by the competent authority or an equivalent approach to ensure safety, may be an option prior to re-opening."* (CODEX, 2012).

Due Diligence – Industry Self Regulation

Long before the acceptance of the European CEN method shellfish industry leaders such as Loch Fyne Oysters developed their own internal management measures based upon testing using RT-PCR. In the absence of an agreed quantitative methodology or industry guidance NoV level, Loch Fyne undertook regular batch NoV testing to develop a database of RT-PCR cycle threshold (Ct) numbers in the context of their own internal customer health status. This allowed development of an internal quantitative 38Ct threshold as an internal management control.

Although the Loch Fyne work was not a scientifically controlled epidemiological study it was a highly effective measure which has been refined through active use and has stood the test of time. From a Scottish shellfish perspective the Loch Fyne approach has been a successful Due Diligence measure.

A number of other operators in the UK have begun to use the Loch Fyne 38Ct guideline level as a *de-facto* Due Diligence threshold. Implementation has been left to each operator to develop in a manner suited to their individual business. Some operators have sampled harvest (pre-depuration) oysters in order to monitor quality within the various beds as this allows differentiation between potential source sites. This can be used as a seasonal tool whereby the intensity of sampling can be increased through the autumn and winter as the level of threat increases. Other operators may test specific batches post-depuration to ensure a Quality Control approach which may be useful where an analytical certificate can demonstrate specific product quality to a prospective customer.

Before adoption of the CEN method the use of Ct numbers was somewhat open to criticism as individual laboratories and PCR instruments would all give a slightly different response so that 38Ct was not always transferable. However, during the period when the Integrin laboratory in Scotland was the only testing facility, this semi-quantitative level could have been considered internally relatively consistent. With the acceptance of the CEN method NoV output is now able to be reported in a quantitative manner with Ct number translated into genome copies/g (of Dt). For industry leaders such as Loch Fyne, their comprehensive testing regime has allowed them to build up comparative Ct to genome copies/g data allowing an inter-calibration of the two measures. This allows them to continue to measure internal performance against their old 38Ct threshold and to understand how this may compare to any new regulatory standard which may be based upon the CEN method.

As NoV testing is expensive it is unlikely that all oyster producers will embrace regular harvest site or sampling for EPT – especially for smaller operators where batch sizes may be small and therefore cost prohibitive. Ironically, if NoV testing were more widely conducted the overall unit price would be likely to come down making sampling more affordable. In the absence of a regulatory NoV standard it is likely that some customers will still be making purchase decisions on the basis of NoV testing results. This is likely to differentiate the market allowing some operators to sell to such customers thereby potentially creating a premium price for such product.

7.1.2 Relaying

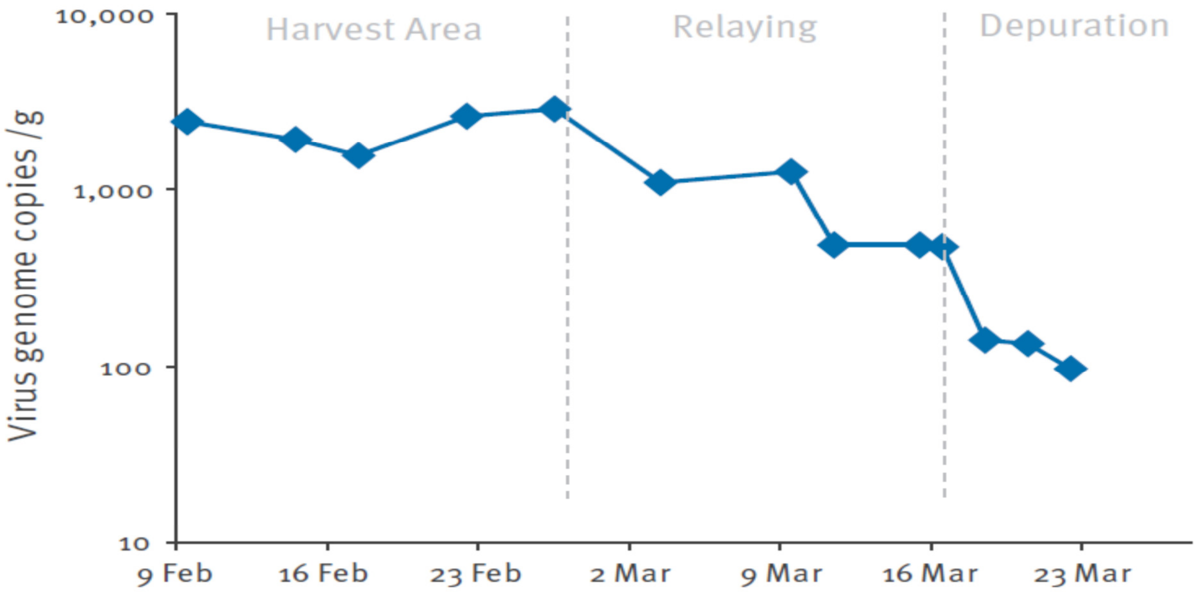
Relaying of shellfish stock from an area of reduced classification status to a designated relay area of higher classification status is an uncontrolled decontamination process. In consequence, an extended duration of >2 months is required under the regulations before the higher classification status of the relay area can be applied to relayed stock. The advantage of relaying as a management option from a NoV perspective is that a large quantity of shellfish can be relayed and retained in good quality whilst extended depuration reduces shellfish throughput and will gradually reduce shellfish quality as they metabolise biological resources.

Kingsley and Richards (Ref: 2003) working with the Eastern oyster and hepatitis A virus (HAV) showed extended retention of HAV for 6 weeks within a depuration setting whilst daily feeding with phytoplankton resulted in a reduced viral content over 3 weeks with no isolation from week 4. The researchers proposed that extended relay periods might be required to produce virologically safe shellfish.

Relaying has been shown to be effective in reducing NoV levels. Work by the Marine Institute in Ireland (Ref: Dore *et al.*, 2010) provided a good illustration when contaminated oysters from Cork Harbour were relayed for 17 days which allowed a reduction in NoV levels from 2,900 to 492 genome copies/g as shown in Figure 7.1. Subsequent extended (4 day) depuration at 15-17°C then reduced residual NoV levels to <200 genome copies/g suggesting that a relay with extended depuration combination can be effective in reducing NoV levels.

Figure 7.1: Illustration of Successful Relay and Depuration Combination

(Source: Dore et al., 2010, Norovirus levels in oysters from the main harvest area during treatment by relaying and depuration, Ireland, 9 February–15 March 2010)



The FSA's Advisory Committee on the Microbiological Safety of Food (ACMSF, 2014) have recently recommended to DEFRA:

*"There is a need for further research into the effectiveness of depuration and relaying in reducing the viral content of shellfish species commercially harvested in the UK to try and establish ways of improving the performance of this commercial process for removal of norovirus." (*Recommendation R6.2)

7.1.3 Enhanced Depuration

Depuration is the controlled process of allowing shellfish to naturally purge contaminants within a controlled clean water environment. Although depuration is effective for the removal of bacterial contamination numerous studies have demonstrated that limited efficacy of this post-harvest control measure with respect to viruses. For this reason both CODEX and EFSA considerations of viral risk management have stressed the need for pre-harvest reduction of viral threat rather than the reliance of post-harvest decontamination. In consequence depuration is not considered within this study as a primary viral management tool. Similarly no consideration is provided in this study with regards to cooking efficacy as a post-harvest viral deactivation control method.

A recent UK stakeholder meeting to consider viral illness and shellfish related issues (FSA, 2013) reviewed UK data with respect to outbreaks and considered a number of control measures with a principal focus upon food handlers as an important vector for NoV spread. In addition, there was a desire to improve post-harvest decontamination principally through enhanced depuration despite acknowledgement of its limited efficacy from a viral control perspective. A brief overview of enhanced depuration is therefore provided.

Cefas has conducted a number of depuration studies which have included a comprehensive project on behalf of the FSA looking at the efficacy of extended depuration at optimum temperatures (Ref: Dore, 2003) which showed that Pacific oyster depuration can be enhanced at elevated temperatures (17ºC and 20ºC) without any impact on product shelf-life and at a low heating cost even for an extended depuration period of 5 days. A more recent project looking at the potential efficacy of ozone/hybrid treatment systems was also undertaken which did not demonstrate a statistically significant benefit over standard UV depuration systems (Ref: Neish, 2013).

Ifremer have also shown that oyster depuration at elevated temperatures could be further enhanced by feeding with phytoplankton. Pommepuy *et al.* (Ref: 2003) working within the EU SEAFOODPlus program, showed that oyster depuration at elevated temperatures could be further enhanced by feeding with phytoplankton within large commercial scale systems. Normal depuration provided a 1 log reduction (90%) in phage levels over 3-4 days at 22ºC which was reduced to 2 days with the addition of $2x10^9$ phytoplankton cells/oyster/day. A preliminary attempt was made in a recent SARF study to replicate the Ifremer approach for NoV using a range of pre- and probiotics (Ref: FitzGerald and Syvret, 2010). Whilst the initial findings had some encouraging output the pilot project was resource limited and the overall results were deemed inconclusive.

Whilst enhanced or extended depuration is unlikely to provide a ‘magic bullet’ to resolve all NoV contamination issues there is potential that in combination with other measures it may form part of the solution. As indicated in the previous sub-section, recent studies in Ireland have shown that a combination of relaying followed by extended depuration was effective in reducing NoV counts to safe levels (Ref: Dore *et al.*, 2010).

A desire to try and enhance depuration to improve removal rates which was expressed in the recent FSA NoV meeting with a recommendation to explore enhanced depuration (Ref: FSA, 2013). In addition, the Advisory Committee on the Microbiological Safety of Food (ACMSF, 2014) have recently recommended further work on enhanced depuration and at the time of publishing the FSA are in the process of commissioning a further study.

7.1.4 Active Management

Active Management

Active Management is the use of external measurement systems to provide information to assist in shellfish management decisions and is illustrated in Figure 7.2 The complexity of the network and the array of sensors used is likely to be site specific and could include monitoring of a number of environmental parameters such as offshore water quality via buoy mounted sensors (e.g. salinity, turbidity), or relate to onshore 'event' catchment conditions (e.g. rain gauging, river gauging and CSO spill monitoring). Recent work by Cefas (Ref: Campos Unpublished) has improved the understanding of sea temperature as 'risk factor' (Section 7.3.2) which highlights the need to monitor this parameter in order to improve site specific profiling.

Figure 7.2: Active management network strategies to limit shellfish viral contamination *(Source: Le Saux et al., 2006)*

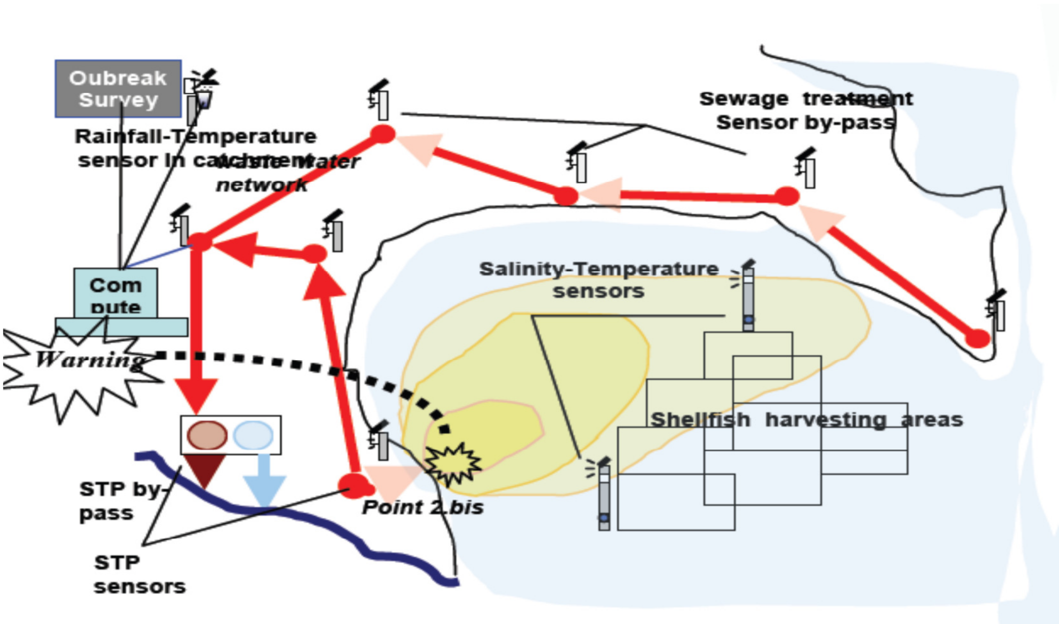


Figure 7.2 provides a system illustration which encompasses an integrated network of sensors measuring catchment, wastewater and offshore parameters. Active Management parameters can therefore include the direct wastewater spill EDM notifications (Section 7.2.2) as well as indirect measurement of the consequences of a spill event (i.e. reduced salinity as a surrogate of increased contamination potential). More importantly this type of system allows a build-up of interpretive environmental data to allow future indirect forecasting of potentially adverse 'event' impacts (e.g. CSO spill from Site A probable when rainfall intensity exceeds a set intensity). Data collation from monitoring systems and subsequent computer model analysis is considered further in Section 5.3.2.

Remote Buoy Mounted Offshore Monitoring Systems

A number of areas now operate buoy mounted monitoring systems capable of automatically transmitting their data measurements to a shore station to allow remote sensing of the marine environment.

Examples of buoy systems include:

- New Zealand shellfish management. As highlighted in the New Zealand Case Study (Section 4.3.3), offshore data buoys can be deployed to provide near-real time monitoring of water quality in response to environmental surrogate factors (e.g. salinity, turbidity) which may relate to microbial quality (Figure 3.5).
- Cefas is participating in a Defra funded Smart Buoy project with the Netherlands government and research institute to try and better understand spatial and temporal variations in water quality parameters such as suspended solid loads, nutrient and phytoplankton levels to support ecosystem health assessments.
- The Irish Marine Institute operate a number of offshore data buoys in the Smart Buoy programme although this is largely for algal bloom monitoring sensors have the capacity to measure chlorophyll a, DO sags and turbidity highs.
- Northwest Association of Network Ocean Observing Systems (NANOOS) on the US NW coast provides on-line web based real time water quality data for shellfish growers with automatic weekly email update and text alert (based on set thresholds). The website provides access to graphs and plots of temperature, DO, chlorophyll a, turbidity and pH.

Although many of these systems may be government funded networks it should be noted that the New Zealand buoy systems are exclusively set-up by shellfish operators as a direct management tool in support of their ‘Conditional’ classification status. It should also be noted that often these data buoys can provide additional marine data which could be of value in the assessment of other shellfish related risks (i.e. algal blooms status) which may provide warning of Harmful Algal Blooms (HAB) contamination or mortality events.

The New Zealand Case study (Section 4.1) shows how some shellfishery areas operate active management as part of their regulatory control. Areas subject to risk from spills or riverine threats are required to undertake comprehensive Sanitary Surveys which focus upon assessing the predictability of event impact upon microbial water quality. ‘Conditional’ areas have automatic closure upon set rainfall intensity thresholds, whilst Active Management is possible for operators who receive telemetry output from rain gauges, wastewater spill monitors and in some cases *in situ* data buoys monitoring salinity.

As described in Section 4.3 the New Zealand shellfish industry with its high earning power pays for full regulatory cost recovery. Whilst this places a high level of cost burden on industry it also provides a degree of ownership and engagement in surveillance monitoring and dynamic Active Management. Although shellfish farmers know that sewage spills or illness outbreaks can have a profound impact upon affected areas, they do have access to monitoring data with which to make informed decisions within their risk management systems.

It is probable that the New Zealand stringent regulatory regime has provided industry with access to a global market allowing a significant level of export production despite extensive distances to customers. However, the New Zealand commercial success story is not necessarily transferable to the UK in view of the markedly different risk profile as described in Section 4.1.

Whilst Active Management may be part of a shellfish operator’s internal Due Diligence to inform their own internal management the system may also be shared with the regulator to provide public health protection. Figure 7.2 shows the linkage of outbreak information being fed into the system to a central computer which implies this system may be operated by public authorities – perhaps with parallel notifications to private shellfish operators.

7.1.5 Winter Norovirus Protocol and Outbreak Closures

Active Management includes outbreak triggered responses as illustrated in Figure 7.2. This approach termed the *Winter Norovirus Protocol* has been adopted by the French Food Authority Ministry (EURL 2014a). The scheme requires that area is closed for 28 days following an outbreak and is very similar to that adopted in New Zealand (see Section 4.3). However, there is scope for evidence based early re-opening if NoV analysis in the production area, undertaken at a frequency of two-weekly basis, provides negative results. This rational is similar to that used in the US where early re-opening following an 'event' can be allowed subject to favourable bacteriophage results.

The protocol also recommends 28 day closure if positive samples are obtained following an elevated *E. coli* result, heavy rainfall or WWTP failure occurs at the time of peak NoV risk (November-April). As with the outbreak trigger re-opening is allowed if no further incident occurs and negative NoV results are obtained.

Although in principal this approach has much to recommend its adoption there are likely to be potential barriers to implementation within the UK. Key potential problems are likely to relate to differences in data availability and structural reporting mechanisms between Member States:

- It is understood that in France the Local Department regional regulatory structure is well placed to act effectively upon potential outbreak conditions. A parallel structure is not present in the UK (FSA official, personal communication).
- CSO reporting and risk profiles are also likely to differ and could be problematic in determining end points for potential closure events.

7.1.6 Shellfish Processing and Cooking

It is beyond the scope of this report to consider the efficacy of various post-harvest shellfish processing techniques on NoV deactivation. However, a brief overview of High Pressure Processing (HPP) and cooking is provided in the context of their incorporation within wider NoV management measures.

A comprehensive level of research and trials has been conducted on HPP and shown that at "*a pressure of 600 MPa applied at 6 ºC for five minutes can completely inactivate NoV in oysters*" (CODEX 2012).

Although cooking to the Tory standard (90℃ for 90s) is considered a vircidal treatment (CODEX 2012) it is recognised that most home and restaurant cooking will not attain this heat profile and fully deactivate NoV as demonstrated by recent work on NoV and bacteriophage (Ref: Flannery *et al.,* 2014). Despite this CODEX 2012 recognises that any cooking will reduce risk, although this may need to be practiced in combination with pre-harvest measures to ensure good shellfish production water quality.

"When there is a likelihood or evidence of virus contamination through epidemiological information, environmental events or direct detection of virus or viral RNA, closure of the area, destruction of contaminated bivalve molluscs and/or virucidal heat treatment before consumption of already harvested bivalve molluscs is recommended." (CODEX 2012).

Whilst it is accepted that these methods may present market challenges and opportunities to the UK oyster industry from the traditional outlets, consideration and potentially market trials could be considered to help open up this option, particularly for products with an increased risk profile.

Guidance on consumer education considerations states "*Consumers should be made aware of the risk of becoming infected with NoV or HAV after consumption of bivalve molluscs*." It is uncertain what potential impact such labelling would have from a commercial perspective. (CODEX 2012).

There are likely to be considerations for the need of advisory labelling on some or all affected shellfish products. The FSA's Advisory Committee on the Microbiological Safety of Food (ACMSF, 2014) have recently recommended:

*"The FSA should reinforce its advice on the risk of consuming raw oysters and that cooking of shellfish reduces the risk of exposure to human enteric viruses as stated in the 1998 Report." (*Recommendation R6.4.)

7.2 Review of Water Industry Management Options

7.2.1 Improved Wastewater Treatment

Section 3.3.1 details the treatment efficacy of WWTPs and current disinfection processes. Current data suggests existing technology will struggle to consistently provide a sufficient level of reduction in pathogenic load to ensure no contamination of shellfish waters. Data from some MBR plants in France have indicated good NoV removal (see Section 3.3.2) when operational practices are adjusted, although this feature is unlikely to have significant bearing to the UK where the number of these plants is somewhat limited. UV as the most commonly adopted disinfection technique used in UK WWTPs, may well be effective at NoV inactivation although current analytical techniques cannot determine this performance. Work in the US has also focussed on the development on new adsorption techniques for the selective removal of NoV which shows some promise although these methods are still in research phase (Section 3.3.2). Any potential significant capital investment in WWTPs will face a challenge under the new implementation of shellfish drivers within the WFD on the grounds of dis-proportionate costs (see Section 7.2.5).

The FSA Advisory Committee on the Microbiological Safety of Food (ACMSF, 2014) has recently recommended that permanent sewer discharges should be relocated away from oyster production areas with sufficient dilution between discharge and shellfish beds. Other permanent discharges impacting on designated shellfish beds are recommended to receive at least tertiary disinfection.

It is difficult to see how NoV water quality design standards could be devised as a scheme driver for improved wastewater treatment until a number of the technical data gaps have been overcome. In England feedback from the EA indicates a limited potential for adoption of a NoV driver to reduce NoV levels in the environment at this time.

> *"In the absence of appropriate policy drivers and treatment methods to address the implications of possible Norovirus standards (proportionate to the public health risk), while we may eventually have a standard, it will be incapable of driving measures to improve water quality…"*
>
> (EA official, personal communication)

Before any potential NoV driver could be translated into environmental management requirements a number of appropriate (and cost beneficial) measures would be required:

- *"the infective dose of Norovirus to derive the equivalent of Environmental Quality Standards*
- *the environmental pathways to generate coefficients for dilution, dispersion and degradation of Noroviruses to use in modelling and in determining discharge consents*
- *treatment efficacy; and*
- *has an analytical method that can indicate the infective viability of the NoV following treatment."*

  (EA official, personal communication)

There is a pressing need for further work to demonstrate the potential efficacy of current standard UV disinfection efficacy on NoV within UK WWTPs. Any future environmental driver for improvements will require the development of a water quality design standard which is considered further in Section 3.7.2 and has implications for evidence gap recommendations (Section 8.1).

7.2.2 Information Sharing - Wastewater Spill Notifications

Intermittent wastewater discharges from CSOs can be a significant source of NoV load to shellfish waters in the UK (Section 6.2). CSO wastewater spill information is routinely shared with regulators and the shellfish industry in NSSP countries. European calls for rapid early warning systems have also been published (Ref: Le Saux *et al.*, 2006). However, the discharge permitting process does not currently place a requirement upon the Water Utilities to routinely provide this data to either regulatory agencies or shellfish industry in near-real time (Ref: FitzGerald, 2008a). In England and Wales there is an annual reporting commitment required for identified CSO assets which impact upon designated Shellfish Waters.

Data for the preceding couple of weeks is made available in the event of a sample failure for harvesting sites operating Long Term Classification or if a disease outbreak occurs, in order to assist with multi-agency investigations. The value of spill data is reduced when only qualitative EDMs are in place, which do not provide quantitative flow data with which to help assess the significance of the spill.

Shellfish waters are subject to direct and indirect inputs from large numbers of CSOs which presents a daunting and expensive challenge to improve spill management. The drive to provide CSO spill data to shellfish operators has been boosted via the *Cleaner Seas Forum* which with a Ministerial Chair managed to develop the success of the BeachLive scheme, where equivalent wastewater spill data is provided to recreational beach users. Recent UK ShellLive trials have been performed in Anglian Water and South West Water areas to provide near-real time data via a Seafish supported text alert system directly to shellfish operators (Ref: Bowes and Pyke, 2013). These trials require sign-up by the operator to a Memorandum of Understanding (MoU). A schematic of the text alert system is provided in Figure 7.3.

CSO spill notification to operators could provide an additional tool for increased risk management for some shellfisheries. Catchment specific correlation between rainfall event intensity/duration against spill impact could be a more useful cost–effective way forward so that indirect monitoring of rainfall could provide an improved surrogate of risk.

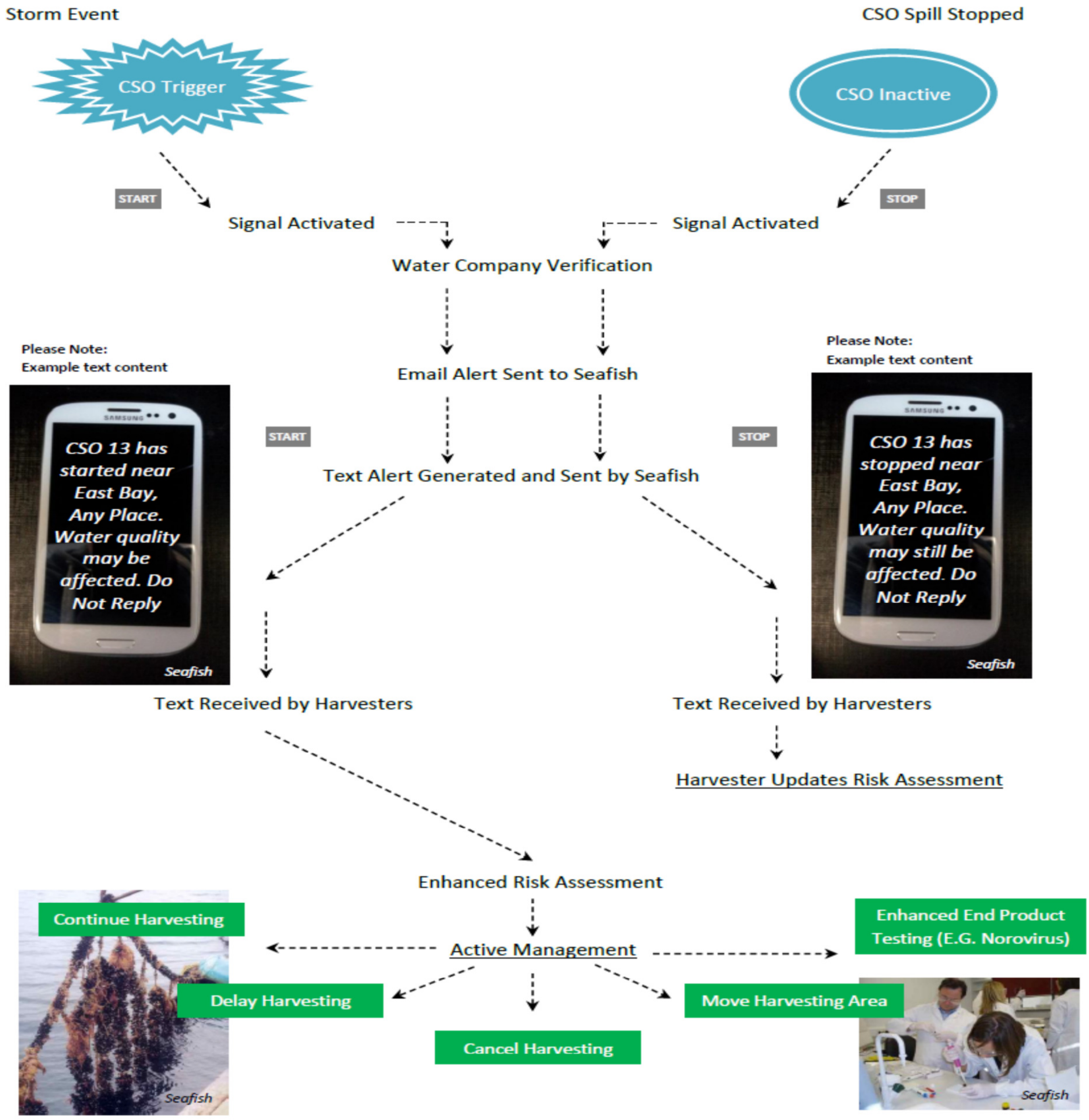
Spill notification will not prevent contamination events and therefore does not provide a comprehensive control measure. However, spill notification could assist improved risk management allowing operators to make evidence based decisions on when to harvest and what to do with potentially compromised stock.

CODEX guidance on the provision of spill data from EDM within WWTPs for CSOs and EOs states: *"Systems should be put in place to monitor sewage spills and provide prompt notification to the appropriate competent authority as well as the bivalve molluscs industry so that appropriate action (i.e., cessation of harvesting) can be taken."* (CODEX, 2012)

The FSA's Advisory Committee on the Microbiological Safety of Food (ACMSF, 2014) has recently recommended Defra and its devolved equivalents to '*control NoV risk from permanent sewer discharges and storm overflows impacting oyster areas*.' This report details the need for a review and action on CSO spill compliance and real-time monitoring/reporting on spill status. It should be noted that the previous formal report from ACMSF in 1998 also recommended real-time reporting of CSO spill status. Defra's response to ACMSF review of 1998 recommendations pointed towards current and future EDM planned installation and that 'Defra is also supporting a Seafish and Water Company trial of "real-time" warning of CSO spills.'

Figure 7.3: Schematic of CSO Text Alert System

(Source: Bowes and Pyke., 2013)



It is suggested that adaption to make current CSO EDM fit for purpose and an appropriate reporting mechanism are technically achievable although a mechanism for implementation and resourcing still needs to be found. It is understood that Seafish with SAGB are hoping to obtain EMFF funds to support a CSO manager to develop these schemes. It is possible that such an approach could enable access to resources from both sectors (e.g Foundation for Water Research and EMFF). Whilst it can be hoped that this group could help facilitate future information sharing it is unclear what tangible benefits there are for Water Industry engagement other than public perception. Recommendations for the formation of a formal cross-sector workgroup are provided in Sections 8.2 and 8.5.

7.2.3 Information Sharing – Catchment Characterisation and Enhanced Sanitary Surveys

Catchment characterisation is the process of assessing the impact of wastewater discharges on a nearby shellfishery. This could be achieved through an 'enhanced sanitary survey' with a focus upon NoV rather than faecal bacterial loading. There is increasing recognition that an understanding of environmental transmission of NoV in shellfish waters requires detailed site specific information on levels of wastewater treatment, proximity of shellfish beds to discharges, rainfall, river flows, salinity and water temperature (Ref: Campos and Lees, 2014)

Significant Spills

The definition of a 'significant' spill is set at $50m^3$ by the EA and has been subsequently used by the Water Industry for scheme design purposes. This spill threshold is somewhat arbitrary as it has no regard for the proximity to the sensitive feature or the concentration of potential pathogen. This significant spill level has formed the basis for design criteria for sewerage network systems through the use of sewerage hydrometric models to ensure bathing water schemes deliver <3 significant spills/bathing season whilst shellfish water schemes deliver <10 significant spills/year, as annual averages.

Wastewater spill information in itself is unlikely to provide an indication of significance to a specific shellfish bed at a specific time – instead it is also necessary to have an understanding of catchment characterisation to assess the impact of a particular loading between discharge and shellfish water locations.

Plume Behaviour

In consequence, there is a need to differentiate if a spill occurs what dilution is likely to be attained and how long a spill may take to impact upon shellfish water. In a tidal setting the tidal phasing of a release will clearly be fundamental as to whether plume transit may be rapid with limited dilution (e.g. on a spring ebb tide towards low water) or slow with good dilution (e.g. on a spring flood tide). Furthermore, in some cases plume trajectory and mixing can also be influenced by wind advection which may exacerbate or reduce potential impact upon specific shellfish beds. Seafish have provided guidance for catchment characterisation (Ref: Bowes and Pyke, 2013) which illustrates this potential approach. Quite often this understanding of potential plume behaviour is available for some shellfish catchments via the Water Utility which has previously computer modelled this information (see Section 7.2.4).

Viral Loading

The ShellLive SW project in the South West Water (SWW) area focussed upon assessing the magnitude of the microbial loading contributed by CSO spills and how this related to rainfall event magnitude. This involved ground-truthing qualitative EDM data with quantitative real-time flow data as well as wastewater sampling and shellfish sampling following spill events (Ref: AWS unpublished data for SWW). The study highlighted which aspects worked well from which it could be seen that there may be scope to use historical data and a period of ground truthing to develop a site specific assessment of significance for EDM data.

This approach requires co-operation with both the Water Utility and EA to access historical data and additional resources to gather new ground-truthing data. Clearly the magnitude and cost of such an exercise is dependent on the number of CSOs and complexity of the catchment.

Enhanced Sanitary Surveys

In an ideal world the Sanitary Survey would incorporate catchment characterisation and encompass both plume behaviour and viral loading aspects in order to provide an 'enhanced' Sanitary Survey.

The recent EFSA report (Ref: EFSA, 2011) highlighted that Sanitary Surveys could potentially include a viral component to enhance their use beyond the current bacterial indicator classification focus. As discussed in the New Zealand case study, enhanced Sanitary Surveys which provide a more comprehensive approach with a viral component can be undertaken to provide a useful foundation for future Active Management tools and risk management measures. It should be recognised that such enhanced Sanitary

Surveys would come at a price which may be difficult to justify or afford for all designated shellfish waters.

7.2.4 Information Sharing – Computer Modelling Output

Section 5 outlines how computer modelling can be used for a range of roles in outbreak forecast, population impact and water quality impact assessments. In New Zealand (Section 4.3) QMRA models (Section 5.1) are applied at the discharge consent stage to assess potential health impact on viral contamination of shellfish. Prohibition zones around wastewater discharges to separate shellfisheries are a standard requirement in NSSP countries and the use of EPA PLUMES models to define the extent of these zones (from a FIO perspective) is a regulatory requirement.

The Water Utilities in many regions have developed a number of coastal zone computer models for bathing water and shellfish water schemes which may have potential scope to assist regulators or the shellfish industry from a NoV perspective. These models were developed as planning tools to ensure that scheme design was adequate to attain compliance against the required regulatory bacterial indicator standards. Preliminary work to ascertain whether these tools can be ‘recalibrated’ using NoV viral variables rather than bacterial indicator inputs is explored in Section 5.3 and in Appendix B. Although these models have their limitations they do provide a ready source of information which could be beneficial to both regulators and shellfish operators if adapted for a NoV application. Gourmelon *et al,* (2010) further developed how early warning systems can be integrated with computer modelling. The GPG developed by Cefas also raises the potential value of models in quantitative assessments for Sanitary Surveys (EURL 2014b).

It should be noted that these computer models are often owned by the Water Utility and are not freely available. In consequence, it is likely that an appropriate MoU would need to be developed to ensure access for development for a different application.

7.2.5 Alternative Mitigation Measures

Changes are occurring within the UK regulatory system which could influence the way and degree to which future wastewater scheme improvements are funded to meet shellfish water requirements. Although historically the Shellfish Waters Directive has provided a significant driver for shellfish scheme improvements it is less clear whether 'disproportionate' costs in the Water Framework Directive will limit future investment. The regulatory background with respect to UK application of management options is considered further in Section 6.7.2.

Alternative Mitigation Measures

Generally alternative measures such as the 'Upstream Thinking' are non-conventional ways to achieve required standards. A potential example may be to support shellfish operators to use the Harvesters Own Sample Protocol (HOSP) thereby boosting Class B sample compliance >90% and avoiding down grading to Class C status which would require remedial action.

Whilst HOSP in itself may improve compliance status on paper it is unlikely to provide in itself business security to shellfish operators as it can provide a criteria for closure (based on concern of *E. coli* quality) without any surety about when to re-open (based on Due Diligence and potential NoV quality). This could lead to extended closure during peak market season thereby undermining commercial viability. In consequence, any HOSP measure would need to integrate appropriate viral management components.

Alternative mitigation measures could include:

- Assistance with access to regional facilities/services for common shellfish industry benefit
- Access to information sharing (see sub-sections above) to improve risk management
- Enhanced catchment no-discharge zones for selected areas to achieve relay quality areas for communal use. This could also be supported by the ACMSF 2014 recommendations for the use of local by-laws to prohibit vessel discharges into shellfish waters.

It should also be recognised that whilst some measures could be suitable from a hygiene perspective they may be unacceptable from a market perception. For example, extended cool holding tanks to maintain shellfish condition may allow product to dodge contamination events over the higher risk winter period but could undermine market benefit of perceived product character of a production area.

Secure access to high quality relay areas to reduce contamination levels in stock would allow differentiation between production and harvest. In this way good shellfish growth could be attained from higher risk estuarine areas which might be deemed to fall within a wastewater exclusion zone before relaying to a higher quality area. However, the need to transfer stock between areas would have a tangible impact on the cost of production and would need to take biosecurity considerations into account. Adverse financial impact may be mitigated by increased market value for a niche product with greater market stability giving industry the confidence to expand production. Furthermore, technological development such as intermediate containment culture cages could facilitate efficient stock transfer to an offshore setting. Parallel considerations of co-location with the offshore power sector may provide a good impetus for technological development of clean offshore culture areas such as those highlighted in Syvret *et al.* (Ref: 2013).

Any of these more progressive cross-sector co-operative approaches would require a common vision with clear economic benefits to all parties supported by political will and financial resources.

7.3 Development of Generic Industry Tool for Viral Risk Management

7.3.1 Risk Matrix – Whole System Scheme

A Risk Matrix approach to viral risk management was proposed in the SEAFOODPlus REDRISK project (Ref: Dore, 2007). This concept was used in a Scottish Aquaculture Research Fund (SARF) project assessing the potential for reduced depuration times in mussels as a means to encompass shellfish food safety beyond the regulatory requirement for *E. coli* indicator based thresholds (Ref: FitzGerald *et al.*, 2010b).

A 'whole system' approach was proposed which encompassed all stages of NoV pre-harvest contamination and post-harvest decontamination including:

- Wastewater management
- Wastewater treatment
- Environmental degradation
- Bioaccumulation
- Depuration
- Cooking

Each of these stages were then weighted according to the magnitude of log reductions (or increase) that they potentially offer. This evidence based approach (Section 3) aims to allow a HACCP based approach to key risk factors of NoV catchment health loading and CSO spill status (Table 7.1).

Table 7.1: Illustration of weighted Risk Scoring Matrix with Management Guidelines *(Source: FitzGerald et al., 2010)*

Phase	Stage	Log Factor	Low Risk	Medium	High Risk
Pre-Harvest	Catchment Health	4	No reports/Summer 4 (1x4)	Spring/Autumn 8 (2x4)	High reports/winter 12 (3x4)
	Waste Water Flow Management	3	No spill/No diffuse input 3 (1x3)	Diffuse input/misconnections 6 (2x3)	Diffuse input+CSO Spill 9 (3x3)
	Waste Water Treatment	3	Full tertiary 3 (1x3)	Secondary 6 (2x3)	Storm overflow/failure 9 (3x3)
	Environmental Degradation	2	Extended transit+sunny 2 (1x2)	Intermediate 4 (2x2)	Short transit+cloudy 6 (3x2)
	Shellfish Bioaccumulation	2	Low risk species 2 (1x2)	Moderate risk species 4 (2x2)	High risk species 6 (3x2)
Post-Harvest	Depuration	1	Enhanced depuration 1	Standard depuration 2	Reduced depuration 3
	Cooking	5	Heat treatment 5 (1x5)	Home cooking 10 (2x5)	Raw consumption 15 (3x5)

Illustrations:

Worst case scenario: *High catchment infection, CSO spill, storm overflow, cloudy, in close proximity to outfall, oysters for raw consumption =* ***60***

Best case scenario: *No catchment infection, No spills or failures, sunny day far from outfall, mussel, depuration (Note 1), heat treated =* ***20***

Potential Risk Scoring (Note 2)

Score	Action to be taken
≤ 30	None
30 to 40	Increase EPT
40 to 50	Positive Release
over 50	Quarantine Batch

Note 1: *x20 minimum score unlikely as depuration and commercial heat treatment not required*

Note 2: *Way forward - to try and 'weight' risk factors based on best available data - will help set future assessment needs*

The resultant matrix could then be used on a site specific basis allowing a Food Business Operator to adapt scoring on a seasonal (e.g. NoV loading), event (e.g. CSO spill) or proximity basis. It was suggested that matrix scores could then be applied to help inform shellfish management decisions. In essence, the model was proposed as a means to allow a pragmatic and proportionate mechanism, whereby mussels harvested in the summer at a time of no waste water spills could be differentiated, with reduced depuration times, from oysters harvested in the winter regardless of CSO spill operation, when additional measures may be required.

An illustrative scoring scheme was suggested as a foundation which could be amended as scientific knowledge improves our understanding of the weighting factors for each stage. In hindsight the model was somewhat subjective as there is such limited scientific literature with respect to NoV behaviour within the 'whole system'. The grounding of such a scheme is designed to be based on real site specific working data, through site trials would need to be developed to demonstrate that this approach could be applied globally. Another disadvantage of this scheme is that there is no way to impact assess existing shellfisheries using historical data (such as the *E. coli* system – Section 7.3.2). Despite the potential limitations to this method it should be noted that the US/Canada Risk Assessment approach currently under consideration also uses a 'whole system' approach as outlined in the Case Studies (Section 2.3.4, see Figure 2.2).

As risk score are responsive to seasonal and event conditions they could be used to inform variable management zoning schemes (Section 6). Examples of potential application the whole system risk scoring for threat based zoning is illustrated in Figures 7.5, 7.6 and 7.7 as discussed in terms of 'Enhanced Management zoning' (Section 7.3.3). Table 7.2 provides example output from this modified whole system risk scoring scheme. The 'Approved' and 'Conditionally Approved' zones have low levels of catchment loading exposure, whilst the 'Conditionally Approved' and 'Conditionally Restricted' zones are subject to CSO spill events.

Table 7.2: Example outputs from modified whole system risk score scheme

Season	CSO Spill Status	Risk Score	Action
Approved Zone Oyster production site (selling raw product) using standard depuration, with 'low risk' degradation with tertiary WWTP serving a small population (low catchment loading)			
Season	**CSO Spill Status**	**Risk Score**	**Action**
Summer	No	31	EPT
Autumn	No	33	EPT
Winter	No	35	EPT
Conditionally Approved Zone Oyster production site (selling raw product) using standard depuration, with 'intermediate' degradation with tertiary WWTP serving a small population (low catchment loading)			
Season	**CSO Spill Status**	**Risk Score**	**Action**
Summer	No	33	EPT
Autumn	No	35	EPT
Winter	No	37	EPT
Summer	Yes	39	EPT
Autumn	Yes	41	Positive release
Winter	Yes	43	Positive release
Conditionally Restricted Zone Oyster production site (selling raw product) using standard depuration, with 'intermediate' degradation with tertiary WWTP serving a substantial population			
Season	**CSO Spill Status**	**Risk Score**	**Action**
Summer	No	33	EPT
Autumn	No	39	EPT
Winter	No	45	Positive release
Summer	Yes	39	EPT
Autumn	Yes	45	Positive release
Winter	Yes	51	Quarantine batch

Risk scoring of the Table 7.1 scheme could be improved with revision of some components. For example, 'catchment health' category could better reflect NoV load if it used measures to score both flow (i.e. population size) and concentration (e.g. season). Similarly the Bioaccumulation Factor might be of more value if seasonality influence on hyper-accumulation were scored rather than species type (which has very limited data).

This system has also helped form a template for ongoing industry considerations for voluntary risk scoring scheme. The risk matrix is intended to have a science evidence base (Section 3). As such there is scope to adjust the factor weighting of components as science gaps are filled. For example, Section 7.3.2 considers Cefas risk factor developments in the use of temperature – it is conceivable that 'catchment NoV load' could be informed by air temperature, while 'Bioaccumulation' could be scaled by sea temperature. In addition, new sub-components may be added to change the relative weighting of the effected stage (e.g. dilution could be added to 'environmental degradation' stage). Recommendations to address the outstanding evidence gaps to further inform the risk factor scaling are provided in Section 8.1. In addition, recommendations are also made to trial the modified whole system risk scoring scheme alongside the *E. coli* proxy risk score scheme (see sub-section 7.3.2 below) are provided in Section 8.4.

NoV illness outbreaks comprehensively linked to specific shellfish batches could also be used to revise an areas risk score. In practice this would have a management effect similar to that of the French Winter NoV Protocol (Section 7.1.5), although 'all clear' criteria could be revised using other measured parameters. Development of this approach would require further input from both industry and regulators such as through the Norovirus and Shellfish Code of Practice Drafting Group.

The *Norovirus and Shellfish Code of Practice Drafting Group* has commenced early work to try and develop a risk based voluntary Code of Practice. Whilst initial output considered a descriptive risk tool there is scope to establish a more tangible quantitative risk scoring scheme. This may be more acceptable to EFSA as it could provide a more tangible scheme for members to adopt a definitive risk management approach. The major problem with establishing these evidence based 'whole system' approaches is access to data and information. Recommendations to the potential development of the risk scoring scheme through this group are made in Section 8.4.

7.3.2 Risk Matrix *- E. coli* Linked

Cefas Scheme

A preliminary risk score matrix was developed in the Cefas NoV prevalence study which used a 10 point scheme for sites in England and Wales of which 7 points were based on *E. coli* related terms with the remaining 2 points based on outbreak status and 1 point on catchment population density. A simpler risk score was used for Scotland based purely on a lower intensity population weighting. Whilst this scheme was only intended to help profile the prevalence survey sites, the resulting NoV data provided some degree of correlation so a further risk matrix was modified in the light of the data generated.

Although there was poor correlation between NoV and *E. coli* for individual samples there was some interdependence using *E. coli* measures as a predictor, which produced better correlation on a site by site basis, particularly when winter results were used (i.e. less scatter in data by exclusion of summer *E. coli* 'spikes' at times with low NoV).

This refinement allowed further development of risk scores for individual sites using:

- a = *E. coli* scores geometric mean (Oct-March)
- b = Seawater temperature (20-temp) with minima of 1
- c = Seasonal multiplier from 0.75-1.25 (25% reduced risk May-Aug, 25% increased risk Nov-Feb)

Risk Score = $(\log_{10}(a \times b)) \times c$

When this risk score was plotted against NoV results some strong differentiation could be seen as plotted in Figure 7.4 and summarised in Table 7.3.

Figure 7.4: Proportion of Samples Giving Total NoV Results in Different Quality Brackets (copies/g) for Different Sample Risk Scores

(Source: Lowther, 2011)

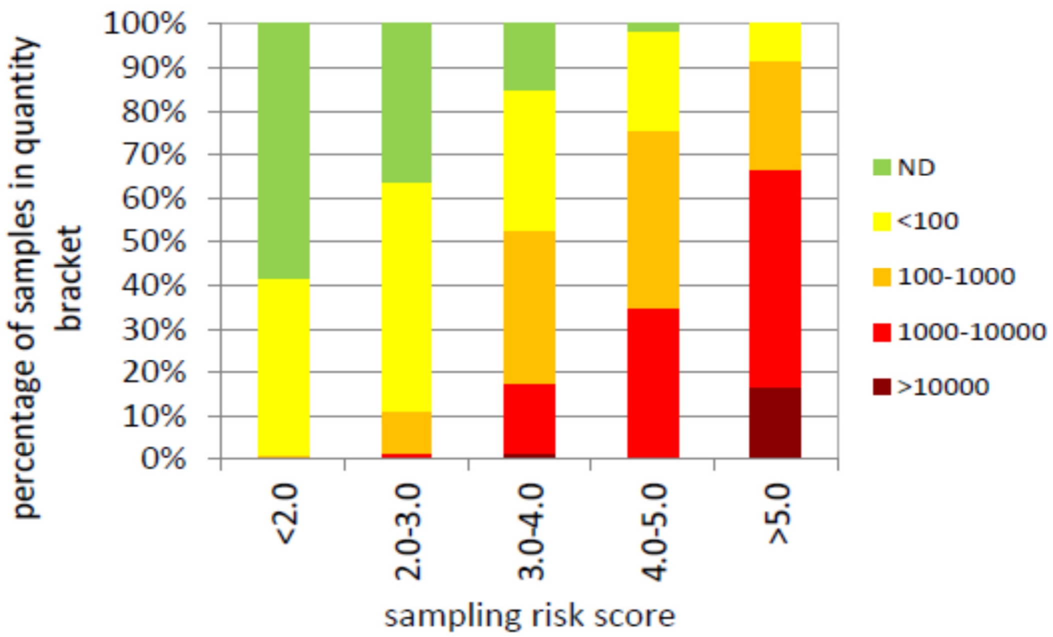


Table 7.3: Summary of Cefas Risk Scoring

(Source: Adapted using Lowther, 2011)

Risk Score	<100 genome copies/g	>1,000 genome copies/g
<2	**All <100 genome copies/g**	**-**
2-3	**~90% <100 genome copies /g**	Negligible
3-4	**~50% <100 genome copies/g**	**<20% >1000 genome copies/g**
4-5	**~25% <100 genome copies /g**	**<35% >1000 genome copies/g**
>5	**~10% <100 genome copies/g**	**~66%>1000 genome copies/g**

Lowther (Ref: 2011) calculated that the Cefas risk score provided a negative and positive predictor on 91.3% and 62.2% of occasions respectively, although it was hoped that performance could be further enhanced. The scheme was proposed as a means to help inform management decisions with the suggestion that it could be tested against new catchments to ground truth the value of the scoring basis.

The strength of this scheme is that PE, level of wastewater treatment and environmental decay are all combined with the *E. coli* factor (so long as *E. coli* is primarily derived from human source) which is a NoV proxy. Furthermore, as historical *E. coli* and temperature data exist for all classified shellfish harvest beds this scheme provides a strong simple global measure to immediately risk assess existing sites.

However, the use of *E. coli* is also the weakness of this approach as it has no ability to screen non-human *E. coli* sources for those catchments where *E. coli* load may be dominated or may increase over winter period by input from non-human sources (e.g. migratory birds). In addition, the score does not allow for year by year variation in NoV catchment health or size of catchment (with smaller catchments having potential for a more ‘spiky’ response to NoV catchment load). Most importantly the Cefas risk scoring does nothing to address the risk posed by CSO spills where viable NoV load can be increased disproportionately to corresponding *E. coli* load.

Illustrative output for the *E. coli* risk scoring scheme is shown in Figures 7.5, 7.6 and 7.7. The changing score shown has a smooth seasonal trend (in respect to factors ‘b’ and ‘c’ – However, the nature of *E. coli* analysis will tend to give a much more scattered output for component ‘a’. There will need to be consideration as to what the resultant risk scoring would mean in terms of management responses, *Optimisation of Cefas Scheme*

It may be possible that additional data requirements could help factor each of the Cefas variables to obtained to improve the risk scoring scheme:

- *a2 = Ability to 'Detune' E. coli Significance for Non-Human Source*. It is proposed to allow Microbial Source Tracking and/or Source Apportionment modelling to adjust the *E. coli* factor to the calculated human component. Clearly this assessment would be required on a catchment and seasonal basis which would have resource implications.
- *b2 = Temperature Correlation with NoV Load.* Some further work is required to link temperature to catchment health (NoV load) as indicated in the recent Cefas 'risk factors' report (see below).
- c2 = *Seasonal Weighting for NoV Load*. As above although perhaps with scope to adjust on a yearly basis according to predictions of epidemic intensity (e.g. new strain emergence).

Risk Score = ($\log_{10}$ ((a x a2) x (b x b2)) x (c x c2)

Where:
a2 = Factor for proportion of human *E. coli*
b2 = Factor for potential scaling of temperature component (based on regional/catchment NoV response)
c2 = Factor for potential scaling of population response (e.g. year on year NoV epidemic factor)

E. coli and Temperature 'Risk Factors'

The recent Cefas 'risk factors' report (Ref: Campos, Unpublished) re-analysed the 2011 FSA prevalence survey data to ascertain principal variables of interest. Continuous wastewater discharge related risk factors are considered further in Section 6.1. Temperature and *E. coli* factors were identified to exhibit a relationship with NoV data and further developed from the original 2011 analysis.

E. coli Impact - NoV and *E coli* performance against each of the environmental variables and highlighted the potential areas of similarity and difference showing that the strength and significance of the risk factors varied between NoV and the statutory *E. coli* indicator. In essence, the value of *E. coli* varied by season and by site so that the report concluded that a distinct set of measures is required to manage the risk of NoV contamination of shellfisheries.

There is a need to 'screen' the Cefas risk matrix to ensure sites do not suffer from misallocation of a risk score for catchments where the relationship with NoV is poor. For example:

- *High E. coli : NoV ratio.* A remote Scottish oyster farm in the middle of winter may suffer from both cold temperature and *E. coli* from a non-human source which could pose no NoV risk and yet still yield an adverse risk scoring.
- *High NoV : E coli ratio.* Another potentially significant limitation with the use of the developed *E. coli* : NoV relationship is again the issue of viability and the use of RT-PCR data. It is possible that shellfish samples strongly influenced by UV disinfected discharges or extensive environmental degradation may yield higher NoV (although not necessarily viable) than the corresponding *E. coli.*

Temperature Impact - A principal finding of the 'risk factors' report was that median levels of NoV in oysters grown in colder waters (<5°C) are 1 $\log_{10}$ higher than those grown in warmer waters (>10°C).

However, it is difficult to separate the degree of co-variance with population influences as some of the coldest winter sea temperatures will have been obtained from South East England where the high population loading may influence the strength of this relationship. When observing the data relationship for the 28 sites used in this analysis individually it can be seen that whilst a clear inverse relationship is generally evident a number of sites display a somewhat different relationship:

- Some sites exhibited a large temperature range with little change in NoV (presumably those with a low population loading)
- Other sites show a cluster of NoV concentrations with minimal change in temperature (presumably those subject to population loading into western coastal areas where Gulf Stream warming limits seasonal seawater temperature change

Recommendations for further work on developing the relationship between temperature, *E. coli*, NoV and wastewater NoV loading are provided in Sections 8.1 and 8.2.

7.3.3 Enhanced Management Zones

The potential applicability of zoning within the UK (Section 6) highlighted that there is no easily implemented zoning scheme which will effectively manage NoV risk. This sub-section considers a flexible zoning approach which could incorporate parallel management measures.

Rational

The current classification system based on *E. coli* flesh results takes no account of changes in microbial loading from continuous or intermittent discharges and cannot ensure '*assured'* safe shellfish production. In areas subject to high magnitudes of continuous treated wastewater discharges or high frequency of CSO spills there may be increased mismatch between the *E. coli* classification result and the NoV in flesh results.

The FSA's Advisory Committee on the Microbiological Safety of Food (ACMSF, 2014) has recently recommended that:

- *'Prevention of harvesting in areas in close proximity to sewer discharges, or regularly impacted by CSO discharges, is a sensible preventative measure and should be introduced'.*
- *'Policy should be formulated regarding preventative measures (e.g. bed closure, virus monitoring policy) following a known spill event or outbreak'.*

The measures considered within the proposed Enhanced Management Zones have the potential to meet this requirement.

A reactive zoning scheme could possibly be based upon a NoV risk scoring scheme in parallel with existing regulatory classification. The Enhanced Management zoning rationale could be similar to the shellfish classification scheme used in US affiliated countries using the NSSP approach (Section 4.1.2). This would be based on NoV risk rather than faecal coliform water quality. The scheme would need to be reactive based on Active Management principles (Section 7.1.4) and would require differing levels of shellfish management action in response to changing risk levels.

A summary of zone characteristics is provided in Table 7.4.

Table 7.4: Summary of oyster enhanced management zone characteristics

Zone	**Interpretation**	**Implication**
Approved (see Figure 7.5)	Area consistently produces assured shellfish product with low or no NoV in flesh. Area likely to the Class A	Assured product to market with no additional mandatory management measures. (Although depuration still recommended)
Conditionally Approved (see Figure 7.6)	Area can produce assured shellfish product with low or no NoV in flesh but is subject to PREDICTABLE periods of 'restricted' quality. Area likely to the Class A or B	Enhanced Sanitary Survey required to provide site specific management operational criteria based on environmental variables. Product of 'approved' quality with periods of 'restricted' quality under 'conditional' status.
Restricted	Area can produce shellfish product at 'restricted' level with low or moderate NoV in flesh. Area likely to be Class B	Additional post-harvest enhanced depuration, processing or batch testing may be required to produce assured product
Conditionally Restricted (see Figure 7.7)	Area can produce shellfish product at 'restricted' level with low or moderate NoV in flesh but is subject to PREDICTABLE periods of 'prohibited' quality. Area likely to the Class B or C	Enhanced Sanitary Survey required to provide site specific management operational criteria based on environmental variables. Product of 'restricted' quality with periods of 'prohibited' quality under 'conditional' status.
Prohibited	Area likely to the Class B or C	Area used for production but cannot be relied upon to provide assured product. Shellfish relayed prior to harvesting or cooked/processed

Approved Status – These zones are likely to be confined to pristine sites with minimal risk of human NoV contamination. In all probability these will be a subset of existing Class A sites. It is possible that a price differential for 'assured' products might improve the economics for offshore relay areas allowing finishing of product in relatively unproductive (but clean) water with most shellfish growth undertaken in productive but higher risk inshore areas (see Figure 7.5 for illustrative application of potential risk scoring scheme).

Conditional Status – The essence of 'conditional' status is predictability. Smaller sewerage catchments with a limited number of well defined CSOs may be more suited to conditional status (see Scenario A, Section 6.6). In New Zealand (see Section 4.3.2) comprehensive Sanitary Surveys are undertaken over at least a year in order to fully characterise each catchment so that rainfall or river levels and any resultant drop in salinity are related to reduction in microbial quality. This allows closure criteria (e.g. rainfall intensity and antecedent conditions) and just as importantly re-opening conditions – furthermore, post-closure monitoring is focussed upon the re-opening period to ensure the 'all-clear' and to continually improve understanding of system response to 'events' (see Figures 6.6 and 6.7 for illustrative application of potential risk scoring scheme).

Restricted or Prohibited Status - The proximity, number and magnitude of CSOs relative to shellfish waters could also be reflected in zoning. Larger sewerage catchments will have multiple poorly defined CSOs and may be more suited to restricted or prohibited status (see Scenario B, Section 6.6). Similarly, larger riverine catchments with multiple small discharges and septic tank inputs may be harder to characterise and predict. Ultimately, the greater the complexity of the catchment to be characterised the greater the cost of assessing and uncertainty of achieving a favourable zoning. It should be noted that the 'restriction' or 'prohibition' zoning only relates to direct placing of live product on the market and not to production where additional control measures are used. However, it must be recognised that it is probable that zoning could potentially have an economic consequence in terms of either a premium price for 'assured' product or increased production costs.

Event Response

The Winter Norovirus Protocol (Section 7.1.5) is the system adopted by France to try and manage NoV risk using a combination of seasonal and event triggered responses. CSO spills and related NoV outbreaks are potential event triggers which would change NoV risk score and could impact upon the management response (Section 7.3.1).

Case studies from overseas (Section 4) have shown ways in which these types of events are used to elevate management responses. In the event of a CSO Spill (within ‘Enhanced Management Zone’) the initial regulatory response could be designed to be proportionate to risk. E.g.:

- Winter High Risk Score – Closure for evaluation whilst NoV shellfish sample analysed. Following analytical results EHO/FBO consult and evaluate risk and connectivity to shellfishery. Closure as a result of a unambiguous linked NoV outbreak event with additional EHO measures potentially including genotyping of patient stool sample analysed.
- Autumn Moderate Risk Score – Temporary closure whilst EHO/FBO consults and evaluates risk and connectivity to shellfishery. Positive Release of batches with quarantine of batches in the event of worsening risk score.
- Summer with Low Risk Score – Increased Due Diligence level with appropriate EPT and surrogate monitoring (or NoV testing).

After each ‘event’ closure, the predictability of the ‘conditional’ zone is reassessed and refined (see Section 4 Case Studies).

Figures 7.6 and 7.7 illustrate potential industry and regulatory actions which might be undertaken in response to ‘events’. Within the ‘Conditional’ zones it is suggested that events could either trigger a change in the default zoning status (e.g. from ‘assured’ to ‘restricted’) or the need for additional shellfish management measures to maintain ‘assured’ quality status.

7.3.4 Generic Model Implementation

The wider issue of UK implementation of management options are considered in Section 6.7. This sub-section considers the specific issue of implementing risk scoring to support evidence based zoning.

The implementation route-map Figure 7.10 considers a possible mechanism whereby the simple *E. coli* proxy risk scoring is used as an initial screening and generic impact assessment tool. Whole system risk scoring could then be an option to implement enhanced management options on a site specific basis.

The risk scoring scheme described in Section 7.3.3 is illustrated for 'Approved', 'Conditionally Approved' and 'Conditionally Restricted' zones in Figures 7.5, 7.6 and 7.7 respectively. A flow chart of how these potential 'Enhanced Management Zones' could be determined, monitored and adjusted is provided in Figure 7.8. The integration of any Enhanced Management Zoning into a wider process for 'assured,' processed or product provided with consumer advisory labelling is provided in Figure 7.9.

Theoretically risk scoring could provide a mechanism to implement 'enhanced management zoning' as considered through Section 7. In practice, there are likely to be challenges in nationally developing generic models and difficulty in affording implementation at a local level. Key risk scoring uncertainties include:

- *Access to information.* Whilst various data sources are available in the public and private sectors it is not easy to obtain relevant data and difficult for FBOs to source - especially when it is not immediately obvious what is specifically required.
- *Access to monitoring data.* In the absence of relevant data how will responsive risk scoring be performed? (E.g. where EDM data is not available must the FBO assume a CSO spill occurs ever time it rains?)
- *Understanding monitoring data*. In the presence of data who and how will its significance to specific shellfisheries be established? (E.g. if EDM data shows a spill has occurred what will this mean to NoV within a shellfish water?)
- *Default scoring scheme.* How will a scheme be started when input information is not initially available? Will guidance default values be provided at a national level or will FBOs be required to perform 'worst case' scoring for unknown parameters?

Recommendations relating to developing the evidence base and trialling risk scoring schemes are provided in Sections 8.2 and 8.4.

Figure 7.5: Illustrative application of risk scoring to approved zone area

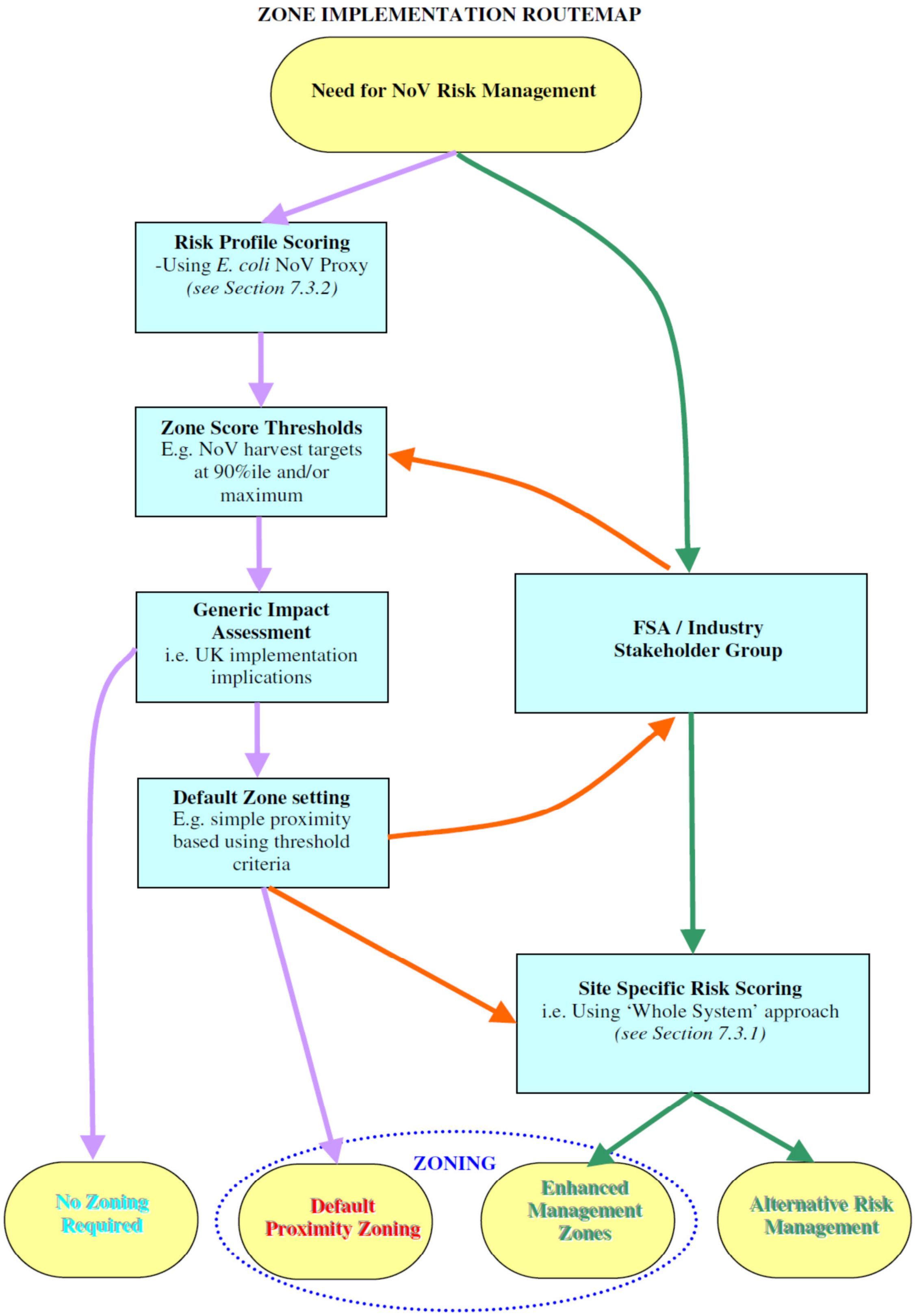
Figure 7.6: Illustrative application of risk scoring to conditionally approved zone area

Figure 7.7: Illustrative application of risk scoring to conditionally restricted zone area

Figure 7.8: Potential flow chart for enhanced management zoning model

Figure 7.9: Potential flow chart for shellfish production option model

Figure 7.10: Potential zone implementation route map



7.4 Shellfish Management Models and Tools - Summary

Section 7 considers potential management tools which could be implemented by the shellfish and water industry sectors.

- *Alternative viral risk management measures.* A number of potential alternatives to exclusion zoning are available as methods to help provide safe shellfish. In some cases options may be simpler or cheaper to implement and should be considered in the context of each countries individual setting. Alternatives available to the shellfish industry include:
  - *NoV shellfish testing* have roles as potential EPT or harvest standards, or as industry based Due Diligence tool (Section 6.1.1). It is possible that the successful industry based testing regimes developed over recent years in Scotland, coupled with the low risk profile of the region, may make this option attractive in this devolved country.
  - *Relaying* (Section 6.1.2) and *depuration* (Section 6.1.3) when used in combination have been shown in Ireland to be effective in reducing shellfish NoV contamination levels.
  - *Active Management* (Section 6.1.4) approaches use an array of monitoring systems to provide an indication of 'events' which may impact upon shellfish quality. Parameters monitored can be direct (such as CSO EDM spill monitors) or indirect (such as temperature and salinity buoy mounted systems). Comprehensive Active Management systems are described in detail within the New Zealand case study (Section 4.3)
  - *Winter Norovirus Protocol* (Section 6.1.5) is the French NoV management system which is a form of Active Management in which regulators may use a range of harvest area closure criteria including wastewater spills, high *E. coli* results and linked NoV outbreaks. Once triggered
  - *High Pressure Processing and Cooking* (Section 6.1.6) are recognised as potentially effective viracidal techniques which can provide safe, or considerably safer, shellfish products. Although favoured from a food hygiene perspective these techniques have received limited interest from industry in the UK for commercial reasons.

- *Water Industry tools* and measures (Section 7.2) may provide complimentary systems relating to wastewater and environmental management which can help in the protection of shellfish quality. CODEX 2012 and EFSA 2012 have both highlighted the need for pre-harvest limitation of contamination rather than post-harvest decontamination. Options include:
    - *Wastewater Treatment* (Section 7.2.1). Wastewater treatment within public WWTPs is unlikely in itself to provide a sufficiently high level of NoV removal to control risk. Unfortunately, UV disinfection techniques commonly employed within UK cannot demonstrate inactivation efficacy for NoV (Section 3).
    - *Information sharing - CSO Spill Monitoring and Enhanced Sanitary Survey data (Sections 7.2.2 and 7.2.3).* Could be particularly important in informing near-real time responsive systems, as well as background information on the shellfish receiving water which could inform risk scoring schemes.
    - *Computer Modelling (Section 7.2.4)* The Water Utilities hold models for many shellfish waters which could provide impact assessment and dilution output useful for any zoning considerations. This area is considered in depth within Section 5.

- It is apparent that there are no easy answers to the complex problem of NoV wastewater contamination to shellfish waters. From a HACCP perspective there is no 'silver bullet' or single Critical Control Point which will adequately limit public health risks. A combination of actions may be needed to ensure safe shellfish products e.g. shellfish management tools (relaying, enhanced depuration or NoV testing) in response to wastewater spill notifications. Co-ordinated cross sector actions (e.g. early warning systems using Water Utility CSO EDM spill information) will require a new and enhanced working relationship between two industry sectors who have historically been in conflict. Resourcing implementation of systems will be important to ensure a balanced and effective approach. Preliminary UK trials of rapid alert systems, although technically possible, will require greater support to enable shellfish industry uptake and provide operators with guidance to assess 'significance' of spill information. Furthermore, without appropriate impact assessments rapid alert systems could provide a Due Diligence rationale to instigate area closure without a clear proportionate mechanism to allow re-opening.

- A generic model has been developed to provide 'Enhanced Management Zones' (Section 7.3.3) to allow dynamic zoning around wastewater discharges (Section 6.6). Management actions within the zone would be informed by a risk scoring scheme. Two potential types of risk scoring schemes (summarised in Table 7.5) could be suitable and are proposed for further development:

    - *Whole system approaches* (Section 7.3.1). This approach was developed to assess viral risk management options in the context of a science evidence based HACCP approach. The system risk scores the stages of the environmental transmission pathway with factors weighted according to reduction (or accumulation) rates. A whole system HACCP style approach to NoV risk management based on a site specific scoring scheme is technically possible and could be used to inform a targeted and responsive zoning system. However, implementation of such a scheme is likely to be challenging (Section 7.3.4).
    - *E. coli linked (NoV proxy) risk scoring matrix* (Section 7.3.2). This was developed by Lowther, (Ref: 2011) and incorporates strong seasonal risk weighting factors. An *E. coli* NoV proxy risk scoring approach using temperature and seasonal factors is simpler and easier to implement.

Table 7.5: Summary of risk scoring scheme advantages and disadvantages

Risk Scoring Scheme	**Advantages**	**Disadvantages**
Whole System *(Section 7.3.1)	-NoV science based without problems of *E. coli* representation issues -Can be responsive to events -Type of approach under consideration in US / Canada	-Hard to implement -No means to impact assess implications of scheme to UK
E. coli (NoV Proxy) (Section 7.3.2)	-Easy to implement using historical data -Easy to assess UK impact implications	-Many non-human wastewater *E. coli* sources which will undermine operation at some sites -Is not responsive to events (e.g. CSO spills)

It is recommended that both systems are assessed in parallel within a couple of trial catchments to ascertain suitability within the UK. It is possible that the *E. coli* proxy approach could be initially implemented as the default system with the option that the more comprehensive whole system scheme could be commissioned as and when appropriate data/information was available to provide a 'better fit' for more complex shellfish waters (Section 7.3 and Figure 7.10).

- A series of flow charts have been produced (Section 7.3.4) to illustrate how the proposed model could assist in potential NoV risk management. Components include:
    - *Enhanced management zone setting and revision* (Figure 7.8). A detailed consideration of how enhanced management zoning could be implement and revised in the event that a suitable risk scoring scheme can be established.
    - *Shellfish risk based product placement* (Figure 7.9). The proposed model also proposes a flexible approach and options for industry operators with differing risk profiles. Food product placement options could include:
        - fresh 'assured' product,
        - processed product (HPP or cooked)
        - fresh product with appropriate customer advisory labelling.
    - *An implementation roadmap* (Figure 7.10). This suggests a way to integrate both scoring systems to inform both simple proximity based zoning and enhanced management zones.

- Implementation of a generic shellfish model to assist shellfish zoning is likely to face a number of challenges (Section 7.3.4):
    - *Risk Scoring issues*
        - *E. coli NoV proxy approach.* Impact assessment may highlight many areas as candidates for default proximity zoning where implied NoV risk is disputed by industry.
        - *whole system approach.* It will not be easy to obtain and assess water sector information and data (e.g. survey, computer model and CSO EDM output). In the absence of appropriate data initiation of default risk scoring will be problematic.

- *Resources.* It is unclear how and who will help fund necessary generic developments, systems and local investigations. WFD mitigation measures (Section 7.2.5) could be a possibility, although it is unclear who would champion this type of approach.
- *Strategic.* Shellfish quality straddles food and environmental regulatory fields. Furthermore, NoV in shellfish needs to be placed into the wider community NoV public health context from a societal perspective. The Advisory Committee on the Microbiological Safety of Food (ACMSF, 2014) has recently recommended a unified strategy between FSA and Defra for managing risk from raw bivalves. Development of cross-sector drivers and regulation are likely to be challenging.

8 RECOMMENDATIONS

Many of the components within the following sub-sections are inter-related but have been separated for clarity (e.g. ‘evidence gap’ component within a ‘trial site’ to inform a ‘management option’).

8.1 Evidence Gaps

A number of objectives were identified from the review in Section 3. These are vital if a science evidence based approach is to be adopted within future viral risk management measures.

- *NoV Inactivation and Viability Methods*. NoV viability is the single greatest technical obstacle to effective use of the current RT-PCR technique to detect NoV in shellfish. No single method is yet available to ensure both capsid and genomic integrity. However, a number of methods can give indications of viability (see Section 3.1.3). An analytical suite to determine a probable level of viability would greatly enhance risk assessment capabilities. Whilst this is not appropriate for routine NoV testing (e.g. EPT or harvest levels) it would be valuable to help understand wastewater disinfection and environmental degradation processes:
    - *WWTP UV Efficacy.* (Section 3.3.3) It is critical to gain better confidence of NoV inactivation rates from current WWTP disinfection systems (Section 3.3). Consideration should be given to undertaking benchtop and field studies to assess comparative viability efficacy trials (e.g. LR-PCR and mucin methods) against known FIOs (i.e. faecal coliforms) and other viruses such as F+ coliphage and MNV. Testing would need to be performed using representative wastewaters in terms of solids type and concentrations. UV efficacy should therefore be considered in conjunction with wider WWTP treatment performance as it is known that this will directly impact on UV received dose (see also Section 2.3.2).
    - *Environmental Deactivation.* (Section 3.5.2) A combination of bench-top laboratory based testing in conjunction with site measurements using a suite of viability determinands and standard FIO and viral parameters (see above). Site measurements could be encompassed within the proposed site trials (see Section 8.2). It might even be possible to relate the NoV decay rate

(T_{90}) output to site specific water quality data (e.g. TSS, phytoplankton and organic content) in conjunction with UV monitoring/modelling (Section 5.2.5).

- *Shellfish Bioaccumulation.* It is recommended that further work is needed to understand sediment-microbe associations and their implications for shellfish uptake (Section 3.6). It is suggested that future assessment work should also encompass the use of viral surrogates in addition to NoV to help establish their further use in environmental monitoring (e.g. F+bacteriophage to relate to microbial water quality and previous hyper-accumulation studies).
- *'Health of Catchment'* – assessing NoV load and development of surrogate relationships (Section 3.2.5). It is suggested that a number of parallel crude/WWTP monitoring programmes encompassing both direct measurement and indirect surrogate monitoring (e.g. air vs. sea temperatures) would be a valuable next step. Public health modelling systems to provide NoV incidence predictions and early warning should be considered in parallel with any direct wastewater monitoring to help link 'cause and effect'. This component could also be linked in with a site trial (Section 8.2.)
- *Environmental Removal.* NoV readily binds to suspended solids (Section 3.5.3) and as such sedimentary processes can act as environmental 'sources' and 'sinks'. The issue of NoV reservoir dynamics during storm conditions is integrally linked with CSO spill impact. It is suggested that this work would link strongly with the environmental decay component considered above highlighting the benefit of multi-parameter synchronous site trials Section 8.2).
- *NoV Contribution from Diffuse sources.* Septic tanks, vessels and potentially biosolids could all have a significant impact on NoV loading. More work is needed to evaluate these sources and should be included in the proposed catchment inventory (Section 8.4- Stage 1).
- *A dose-response study* to adequately encompass the relationship between NoV RT-PCR concentration (in the digestive gland) and the whole shellfish portion (Section 3.1.2) and the relationship between shellfish standards and significant community impact. This is not developed as it is beyond the scope of the current project.

8.2 Site Trials

It is recommended that regulators work with industry at a series of trial sites to develop models of how enhanced management could work. Multiple objectives could be integrated through a number of key components:

- *Inform science based evidence objectives*. A number of technical evidence gap issues have been identified elsewhere in this section which could be incorporated in multi-component studies (e.g computer modelling trials, environmental degradation of NoV and shellfish NoV uptake)
- *Development of risk matrix schemes*. Parallel trials of both scoring schemes discussed in Section 7.3.4.
- *Develop balanced closing and opening criteria*. Closing areas at times of increased risk using Active Management tool (Section 7.2.4) is likely to be relatively easy (e.g. EDM spill, increased rainfall, increased HNORS reporting, NoV risk data). It will be more difficult to provide a NoV data based or degree day based opening criteria. This will be vital to engage industry buy-in (Section 7.3.4).
- *Establish regional shellfish groupings* to draw together shellfish industry, agencies, regulators and interested parties (e.g. Universities). Such groups could also form a vehicle to obtain further funding (e.g. through EMFF) to develop common work programmes (e.g. academic links for study or access to monitoring equipment to develop site specific risk profiles).

8.3 Management Options

A number of management options have been reviewed in Section 7 many of which are likely to be complimentary to certain types of zoning. This sub-section is only making recommendations with respect to Computer Modelling and Active Management as the two key measures likely to have a significant bearing on zones.

Active Management

Active Management is likely to be an important component in future risk scoring and the basis for any potential reactive zoning. It is recommended that further consideration is given to:

- *Overseas case studies* – It would be useful to asses the responsive systems used within the French Winter Norovirus Protocol. Comprehensive environmental monitoring based systems are routinely used in many US NSSP affiliated countries (e.g. US and New Zealand) which would provide an illustration of good practice. A fact finding mission would aim to gather experience from both a regulatory and shellfish/wastewater industry perspective to assess implementation issues.

- *Site specific development trials* (linked to Section 8.2) It is recommended that further work is needed to explore the relationship between rainfall intensity, CSO spills and resultant shellfish NoV quality. There may be scope at some trial sites to develop surrogate monitoring systems (e.g. temperature, salinity and rainfall) to help inform risk management measures.
- *Computer modelling*. (linked to previous recommendation) These *in-situ* monitoring systems can collect a range of parameters in a harmonised manner over an extended period of time enabling the compilation of an environmental database in parallel with NoV shellfish quality. This could perhaps also provide input for self-learning ANN computer models for the next generation of predictive systems.

Computer Modelling.

Computer models can provide output to inform the dilution and time components of proximity based prohibition zoning as performed in the US affiliated NSSP countries. It is suggested that further focussed research is undertaken to fully assess the scope for computer modelling of NoV in UK shellfish waters.

This study has shown that computer models are widely available in many English and Welsh shellfish waters (UK oyster database - Appendix A). It is suggested that the UK impact assessment also assesses in more depth the quality of existing model resources (i.e. how up to date and at what resolution in shellfish water areas).

The CSO spill tool produced in this report provides a good indication that user-friendly output can be tailored to regulators or shellfish operators to provide a useful management guide. In particular the development of the dual decay approach (see Section 5.3.3) that allows the modelling of both viable and non-viable NoV components which could be useful in areas subject to both UV disinfected and CSO impacted discharges. It would be useful to ‘ground truth computer model output with real NoV shellfish data (although this would need to be linked with the Section 8.1 science objectives in order to make links with water quality).

The science evidence issues outlined in Section 8.1 would also help inform the QMRA whole system approach to computer modelling used in New Zealand to assess wastewater discharge impact on shellfish consumption (see Section 5.1).

8.4 Implementation

Although a number of potential components are set out in this sub-section is it suggested that these actions are likely to be considered in parallel.

Stage 1 - Microbial Contamination Inventory

Before an impact assessment can be conducted it will be necessary to have a more exact understanding of the relationship between oyster fisheries and wastewater sources. This could build upon the foundation of the oyster databased constructed in this study.

Resources will need to be made available to help the regional environmental agencies to compile catchment inventories of FIO and NoV loading sources for sensitive catchments (i.e shellfish waters and Bathing Waters).

It may also be possible to maintain the cross-department links between environment, food quality and public health to consider other parallel cross-sector microbial issues which could also incorporated within the inventory (e.g. anti-microbial resistant bacteria and *Campylobacter* sp.)

Stage 2 - Impact Assessment

Various components of a UK impact assessment are likely to be required to inform consultation in Stage 3.:

- *E. coli NoV proxy risk scoring.* As highlighted in Section 7.3.2 the Cefas risk score derived from the FSA NoV prevalence survey work provides coarse screening of potential NoV impact by linking in to the Classification microbiological and sea/air temperature data sets. This should also include a preliminary 'health check' to flag potential atypical catchments where the indicator : NoV relationships may be particularly weak. This output could be incorporated within the discharge proximity tool.
- *Discharge proximity assessment tool.* It is recommended that a UK wide impact assessment is undertaken to consider NoV risk exposure of shellfishwater to

wastewater discharges at a catchment specific level. This can build on the oyster database (described in Appendix A) which compiled continuous discharge and CSO discharge information in the context of oyster shellfish waters. It is suggested that further progress with the oyster database should encompass a GIS component to allow layering of the various shellfish and wastewater source data. As indicated in Section 6.1. the information on continuous discharges population size should reflect the probable NoV loading by factoring in the level of wastewater treatment. The CSO exposure profile is also likely to be more complex as considered in Section 6.2.

Stage 3 - Review and Consultation of Default Zoning

Exclusion zoning, if employed, is likely to be problematic for many shellfish waters with associated high magnitude NoV loads. This is likely as a result of the number and diversity of contributing wastewater discharge sources (Section 6.6). It is suggested that the shellfish industry will need support to develop options to adopt the most appropriate management measures. No ‘one-size-will-fit-all’ approach will work for all UK oyster shellfisheries.

A potential route-map for developing evidence based zoning is considered in Section 7.3.4 (see Figure 7.10). This provides a possible mechanism for the FSA to implement default exclusion zone criteria using the *E. coli* NoV proxy risk scoring criteria. As can be seen from Figure 7.10 a consultation phase could allow debate as to the setting of the risk score thresholds for any default geographical zoning. This could inform an iterative impact assessment process (Stage 1 recommendations). Section 6.5 provides an outline of how a theoretical proximity zone could be established.

It is probable that those oyster production sites screened which do not require default zoning will be happy to maintain their own non-zoning management measures. In contrast, oyster fisheries proposed to require default zoning may wish to develop a more evidence based zoning approach (Stage 4).

Stage 4 – Develop Options for Evidence Based Zoning

If a precautionary default geographical zone were to be proposed it may be difficult for shellfish operators to amend this to a more equitable evidence based zoning approach. The key difficulty would be access to information, data and appropriate systems to inform responsive zoning. This could perhaps be assisted by the following group representations for the shellfish industry:

- *UK shellfish industry workgroup* – It is recommended that the *Norovirus and Shellfish Code of Practice Drafting Group* engage with and promote site specific trials (see Section 8.2) in order to develop real world risk based scoring (see Section 7.3.1) templates as exemplars for future industry uptake.
- *Shellfish / Water Industry workgroup* – It is recommended that a cross-sector working group is established to develop joint initiatives. There are a number of components which could be within the group brief:
    - *CSO Spill Reporting*. This group could formally capture the early good cooperative work undertaken by the 'ShellLive' trials (see Section 7.2.2) and could periodically report to the *Cleaner Seas Forum*.
    - *Information Sharing* If whole system risk scoring systems are to be developed (Section 7.3.1) then it is vital to provide access to Water sector data to inform enhanced Sanitary Surveys (Section 7.2.3), and computer model output (Section 7.2.4). It is probable that the right to information access (including EDM CSO data) will need to be established and considered at a higher political level in order to devise a mechanism to incentivise information dissemination or where necessary influence future policy.
    - *WFD Disproportionate Cost Considerations.* As highlighted in Section 6.7.2 there is a need to have a realistic dialogue between sectors to establish a balance between wastewater scheme improvements and the impact on the shellfish industry. Furthermore, there may also be scope to consider alternative mitigation options (Section 7.2.5).

8.5 Policy and Resources

High Level Strategic Plan

It is not currently possible to establish a policy based driver to link shellfish quality needs with environmental quality objectives (Section 6.7.3). It is therefore recommended that a high level group instigates a plan to develop an appropriate process to achieve the aim of:

> *Developing a holistic strategy to provide a NoV based driver on a catchment wide basis to limit loading to sensitive shellfish waters from all significant contributing wastewater sources*

A cross-sector steering group could ensure stakeholder input and perhaps report to the Cleaner Seas Forum. As a multi-departmental group this body may be an appropriate vehicle to divide and promote the research evidence gaps (Section 8.1). This steering group would need to engage with diverse groups representing a range of wastewater discharge sources:

- Water Industry Public Utilities responsible for WWTP Continuous discharges (Section 7.2.1) with CSOs (Section 7.2.2) and the wider issue of SUDS implementation
- Vessel organisations such as Blue Green (see Section 6.3.2)
- Private WWTPs and septic tanks (see Section 6.3.1) – via various environmental agencies

Regional Measures

Within the new devolved UK structure there is scope for increased regional powers to develop the most appropriate management measures to reflect the geographical characteristics, population and wastewater strategies adopted. Certain funding measures are also often provided on a regional basis and it is possible that corresponding public agencies may have different political and strategic priorities. It may be possible for the local shellfish industry to set-up regional co-operative bodies to negotiate with other stakeholders and develop their own local management models (Figure 7.9). They could then perhaps obtain support for their own fishery products and management options as appropriate using their own EMFF resources. Regional groups could be gathered around the proposed site specific trials (Section 8.2) which could help with adoption of working management models.

ACKNOWLEDGEMENTS

The Principal Author wishes to thank the project team for input from Martin Syvret (Aquafish Solutions Ltd), Will Gaze and Ruth Garside (ECEHH), Ann Saunders and Richard Danette (Intertek Ltd). Special thanks to Jide´ Mirikwe and David Alexander of FSA for comments and input to draft and final reports.

A number of researchers and regulators from around the world were contacted as part of this study some of whom were particularly helpful in the provision of data and opinions on NoV and shellfish management issues. Overseas case study feedback was most appreciated from:

- New Zealand: Gail Greening, Graham McBride, Jim Sim, Brian Roughan (in particular thanks is given to Brian Roughan and Jim Sim for comments on Section 4.3.)
- Australia: Anthony Zammit, Phillip Geary, Felicity Brake
- US/Canada: Jane Van Doran, Gary Richards, Lee-Ann Jaykus, Gerri Ransom, Howard Kator, William Burkhardt III Jnr, Enrico Buenaventura, Chris Roberts
- European NRLs: Irene Pol-Hofstad (Netherlands) and Mario Latini (Italy).

UK Regulatory, agency, researchers and industry input was welcomed from: Jenny Howie, Douglas Sinclear, Nicola Connery, Ros Stewart, Clare Blacklidge, Kevan Connolly, Nicky Mitchard, Paul McNie, Tim Smthye, Ben Winterbourn, Shelagh Malham, Jane Swan, Rachel Hartnell, Carlos Campos and Ron Lee. Particular thanks for David James Allen and John Harris from Public Health England for discussion and comments. Thanks also to Michelle Bell, Elaine Connolly and Mandy Pyke for report comments.

It should be acknowledged that in addition to FSA funding for this project, much of the literature review components were made possible by NERC CASE PhD funding for Andy FitzGerald

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Appendix A: UK Oyster and Wastewater Proximity Database

Table A1: Summary of UK Oyster Industry Harvest Area Status[1]

			England					Wales	Scotland	Northern Ireland
			North East	South East	South	South West	North West			
No. of Shellfish Harvest Beds[2]			1	69	48	37	2	6	52	14
% PO vs. NO for Shellfish Harvest Beds			PO = 100% NO = 0%	PO = 57% NO = 43%	PO = 8% NO = 92%	PO = 59% NO = 41%	PO = 100% NO = 0%	PO = 33% NO = 67%	PO = 94% NO = 6%	PO = 64% NO = 36%
% of Shellfish Harvest Beds with a Sanitary Survey			0%	93%	94%	89%	100%	67%	92%	100%
% Classification A / B-LT / B / C		**A**	0%	2%	0%	0%	0%	0%	Seasonal Classifications	50%
		B-LT	100%	65%	86%	73%	50%	50%		0%
		B	0%	29%	10%	27%	50%	17%		50%
		C	0%	4%	4%	0%	0%	33%		0%
Number of Intertidal & Subtidal operations by species[3]	**PO**	**IT**	1	33	1	18	2	2	44	9
		ST	0	32	3	4	0	0	4	0
	NO	**IT**	0	17	0	0	0	0	1	3
		ST	0	30	44	15	0	4	2	2

Key:

PO = Pacific oyster (*Crassostrea gigas*) / NO = Native oyster (*Ostrea edulis*)

IT = Intertidal / ST = Subtidal

Notes:

1: Summary table is subject to change following final feedback from Local Authorities.

2: Where a Shellfish Harvesting Bed has both POs and NOs then this is counted as 2 beds.

3: Some Shellfish Harvesting Beds may have both Intertidal and Subtidal operations.

Appendix A: UK Oyster Harvest Database - Description

It is impossible to assess applicability of potential exclusion zoning in relation to wastewater discharges unless there is a clear understanding of the UK oyster industry profile and its relationship to corresponding wastewater loads. To assist with this a comprehensive database of oyster shellfish production areas has been generated amalgamating both Shellfish Hygiene Directive (SHD) and Shellfish Water Directive (SWD) data and other sources were available.

This EXCEL database is intended to provide the foundations for a tool, which can be maintained and updated by the FSA, its devolved administrations and partner agencies to help future impact assessments of management options.

A.1 Data Sources

The following sub-sections outline the data sources and limitations of the database.

A1.1 Shellfish Hygiene Directive (SHD)

Aquafish Solutions Ltd has assimilated classification data from the Shellfish Hygiene Directive (SHD) programmes which provide a good outline of where Pacific and native oyster shellfish operations exist. Supporting information from Sanitary Surveys has provided data on:

- Nature of the shellfisheries (i.e. production methods or wild fishery)
- Shellfishery exposure (whether stock is intertidal or subtidal)
- Nature of the receiving waters (e.g. drying intertidal, nearshore or deepwater loch)
- Proximity to discharges (diffuse and point sources)

There are a couple of limitations to these data sources:

- *Classification Variability* – Although many harvest beds remain classified and operational for a number of years some may drop in and out of production. In some cases this may be a result of decreasing fishing potential such as dropping native oyster landings which may reduce the viability of an area. In other cases a new harvest bed may be opened and run for a few years until a coupleof poor microbiological quality results compromise the classification status of the bed and threaten the commercial viability of the site leading to its abandonment. The database reflects shellfish beds which have most recently been classified in 2013 and does not include beds which may have recently been removed (e.g. native oysters in Lyhner, Pacific oysters on Exe estuary) or may be currently commercially inactive (e.g. Swansea Bay native oysters). In contrast, other areas (e.g. Milford Haven) may still have a maintained SHD classification even when not currently active. In consequence, further work on the database, perhaps with production levels, could help future impact assessments although this presents a confidentiality issue and a need for data protection.

- *Classification RMP Location* – A primary function of the Sanitary Survey is to set a Regulatory Monitoring Point (RMP) in view of the assessed sources of microbial contamination relative to the proposed harvest bed. Some regions present RMPs as National Grid Reference (NGR) co-ordinates (e.g. England and Wales and Scotland) whilst others use WGS84 (e.g. Northern Ireland). In addition, some regions (e.g. Scotland and Northern Ireland) may use a single Regulatory Monitoring Point (RMP) for a number of harvest beds, whilst in England and Wales each harvest bed tends to have its own RMP. In some cases RMPs have been relocated to new positions in view of revised data/threats. Researchers have sought to provide the most up to date listing of RMPs which in some cases required direct enquiries to EHOs.
- *Sanitary Survey Completion* – A few areas do not currently have Sanitary Surveys some of which are understood (e.g. Loch Ryan) to have received considerable investment and reduction in microbial loading in recent years.
- *Sanitary Survey Source Differentiation* – Most Sanitary Surveys provide a comprehensive catchment listing of potential wastewater discharge sources. However, Sanitary Surveys do not include an impact assessment from these sources (e.g. impact of a large more distant discharge relative to a small close discharge), there is no differentiation between the potential impact of intermittent discharge (e.g. spill frequency /volume data) and no consideration of NoV issues (i.e. focus is purely on faecal coliforms).

A1.2 Shellfish Waters Directive (SWD)

Under the Shellfish Waters Directive (SWD) environmental regulatory agencies have prepared Pollution Reduction Plans (PRPs) for each designated area which summarises historical compliance and reviews, potential contaminant sources along with any measures required, or underway, to improve water quality to meet compliance commitments (Class B status). In particular, the PRPs list continuous discharges (with discharge level of treatment, flow or PE) and intermittent wastewater discharges considered to impact on the shellfish water. The database allows the geographical positional data for shellfish monitoring point and in some cases the wastewater discharges to be compiled allowing assessment of loading magnitude in relation to shellfish water proximity.

There are a number of limitations to this data source and regional variations in implementation approach:

- *Area Designation* – Ideally the designated SWD area would encompass the equivalent SHD 'production' area which would be made up of a number of individual harvest beds. In practice, the two parallel schemes do not always mesh well and a number of mismatches can occur such as:
  - Different Naming and Boundary Criteria. Although many SHD production areas match well with equivalent SWD areas, occasionally multiple SWD areas may apply to a SHD production area. For example, Southampton Water and Solent production areas correspond to 14 SWD areas.
  - Non-SHD Designation. Areas can have SWD designation without equivalent active SHD beds (as considered in Section 6.1.1).
  - Non-SWD Designation. Conversely some SHD harvest beds (e.g. Campbeltown Loch and Burra Voe) exist without equivalent SWD designation.
- *Wastewater Load Description (Continuous Discharges)* – Some regions describe discharge magnitude in terms of Dry Weather Flow (DWF) (i.e. England and Wales), whilst others use Population Equivalent (PE) or number of houses (i.e. Scotland).
- *Discharge Location* – Although some PRPs list discharge positions these are not always provided preventing further proximity analysis at this stage. Requests have been made to various Environmental Regulators in affected regions although data is still outstanding.
- *Up to Date PRPs* – It is uncertain with the transition of the SWD to the Water Framework Directive (WFD) how the PRPs will be maintained in the future – particularly between the regions. England and Wales have not produced new PRPs since 2009, although these are planned to be updated and restructured into Action Plans as part of WFD river basin plans for June 2014 (EA official, personal communication). In Northern Ireland PRPs have yet to be produced, although drafts are expected shortly (DOENI official, personal communication). In Scotland the PRPs have been well integrated with the SHD into a common 'basin' approach that lends itself well to incorporation into the WFD. Where existing PRPs have not yet been updated discharge information is often out of date, particularly with regards to the level of wastewater treatment offered by schemes.
- *CSO Spill Selection Criteria (Intermittent Discharges)* – There are two principal issues with respect to CSOs:

- Proximity. In Northern Ireland (DOENI official, personal communication) and Scotland 2km is used. In England and Wales where different sub-regions operate a set distance is not specified.
- Impact. As the SWD is only concerned with faecal coliforms as a driver there is no consideration of NoV issues which may impact on a shellfishery for a more extended zone due to the potentially increased environmental survival of NoV in relation to faecal coliforms.

It is recommended that following submission of the new PRPs that the oyster database is updated with current discharge status and positions to allow a detailed proximity assessment from a NoV perspective. GIS layers of special data could also be linked to the database to allow superimposition of discharge, shellfish harvest, water quality data and other water use criteria (e.g. vessel moorings).

A.2 Proximity Map - Methodology Notes

The UK oyster database was used to construct some proximity maps for continuous and CSO discharges as described below.

A2.1 UK Oyster Continuous Wastewater Discharges

Individual discharges within the database are colour coded using the same 5 colour scheme used in Figure 6.1, although where a number of WWTPs discharge into a common area the amalgamated load is used to set the catchment colour coding. Only active SHD classified production areas are included with only 1 symbol applied for each designated area which presents some data limitations as highlighted in Section 6.1. When discharge PE values are not provided (e.g. England & Wales regions have PRPs which provide Dry Weather Flow) flows are converted to equivalent PE using 150L/head/day as a standard per capita flow rate. On this basis a WWTP with a Dry Weather Flow of 139 litres/second equates to a PE of 80,000.

A degree of caution is needed when interpreting this data for specific shellfisheries as considered below:

- *Proximity of Wastewater Discharges.* All SWD assessments of a discharge impact zone are based upon *E. coli* and not NoV (Section 4.1). In consequence, the PRP 'impact zone' may under-estimate the range at which NoV may compromise quality for both coastal and catchment loads.

- Coastal Proximity - Walton Backwater in SE England currently has no impacting discharges (as indicated by the PRP) and is therefore coded a 'blue' site. However, Harwich and Felixstowe discharge 5-10km from the designated area with a combined PE of ~48,000 and may have an adverse impact upon this shellfishery from a NoV perspective. Similarly, Langstone and Portsmouth Harbours have no directly identified discharges (in the PRP) and are therefore coded 'blue' sites. However, the adjacent Chichester Harbour receives major discharges ('red' site) along with 2 major long sea outfalls at 7.5km and 10km offshore again with an unknown NoV impact upon the embayment shellfisheries.
- Catchment Proximity - Strangford Lough has 3 wastewater discharges directly to marine waters with a combined PE of 2,600 yet >PE 88,000 of indirect wastewater discharges occur in the upstream riverine catchment.

- *Colour Coding Thresholds.* Any summary plot using somewhat arbitrary thresholds can be subject to site specific issues which can skew output.
  - Seasonal Variation. The PE of 80,000 threshold cited in the Cefas report does not differentiate sites which may be subject to vast seasonal variation. For example, Ashford STW serves Barnstaple with a DWF of 176 litres/second which equates (at 150L/head/day) to a PE of >100,000. However, this does not take account of infiltration or actual population, which in the case of this site with a large tourist provision is a winter resident population of 40,000. From a NoV perspective the winter resident load, when the catchment level of NoV infection is probably more relevant than the consented flow designed around summer peak flow conditions.
  - DWF and PE Data Mismatches. As indicated in Section 4.1 there is sometimes a mismatch between DWF and PE criteria from the Pollution Reduction Plans. The use of a somewhat arbitrary colour scheme with set thresholds will obviously have the potential to place areas on the threshold of two loading bands. For example, the Helford catchment has DWF criteria which indicate a PE of >800 (demarked by 'dark green'), whereas the known PE is actually <800 (demarked by 'pale green').

A2.2 UK Oyster CSO Intermittent Wastewater Discharge

The oyster database was used to generate a summary of the number of CSO discharge numbers for the UK using the same oyster production area symbol convention used for the continuous discharges. Colour coding relates to the number of CSO discharges identified in the Pollution Reduction Plans (where available). Where a production area may have a number of designated shellfish waters the CSO numbers have been summated for that area.

Some areas of uncertainty should be noted:

- All areas have used CSO identified by regulatory authorities within proximity zone thought to be of influence to faecal coliform indicator compliance (<2km for Department Of the Environment Northern Ireland (DOENI), <2km for Scottish Environment Protection Agency (SEPA), unspecified for Environment Agency (EA)).
- Some areas (e.g. Colne and Milford Haven) have multiple unspecified number of CSOs.
- East Anglian Region generally had a low number of identified CSOs – it is not known what proximity selection criteria was used.
- Scottish areas did not have CSO discharges specified – some areas (Loch Ryan, Loch Fyne and Oban) have sewerage systems which may have a potential for CSOs input although they maybe beyond the 2km proximity threshold used by SEPA.
- NI areas generally have identified CSOs within 3km of shellfish water identified by DOENI.

Appendix B: Computer Modelling of NoV in Shellfish Waters

Table B1: NoV Exclusion Zone – Modelling Input Parameter AssumptionsSummary Loading and NoV Viability

Peak Winter Conditions

Level of Treatment	*NoV Concentration (Note 1)*	*NoV Viability (Note 2)*	*Threat Reduction (Note 3)*
Crude/CSO Spill	$1x10^8$ genome copies/100ml	100%	Minimal *(Note 4)*
Secondary Treated	$1x10^6$ genome copies/100ml	100%	~2log *(Note 5)*
Tertiary Treated	$1x10^6$ genome copies/100ml	1% *(Note 6)*	~4log ($1x10^4$ gc/100ml viable)

Summer Conditions (Note 7).

Level of Treatment	*NoV Concentration (Note 1)*	*NoV Viability (Note 2)*	*Threat Reduction (Note 3)*
Crude/CSO Spill	$1x10^6$ genome copies/100ml	100%	Minimal *(Note 4)*
Secondary Treated	$1x10^4$ genome copies/100ml	100%	~2log *(Note 5)*
Tertiary Treated	$1x10^4$ genome copies/100ml	1% *(Note 6)*	~4log ($1x10^2$ gc/100ml viable)

Note 1: GI and GII grouped. Variable crude loads and relative dominance between G1 and GII (see Section 3.2.1)

Note 2: Low transmission and limited natural UV dose in front end of WWTP likely to limit decay. Therefore all viral particles assumed to be viable in absence to disinfection process.

Note 3: Relative to crude viable load (function of both secondary treatment removal and tertiary treatment deactivation)

Note 4: CSO spills may receive dilution relative to DWF crude although reduction likely to be <1log. First flush of event may liberate NoV associated with resuspended settled solids and increase titre for onset of event – therefore assume CSO same as crude. Total NoV levels (GI+GII) in settled storm water were not significantly different from those in influent samples. (Cefas, 2013)

Note 5: Reductions dependant on treatment type and will range from ~1-3 log (see Section 3.3.1)

Note 6: UV efficacy on human NoV unknown but some data for MNV (see Section 3.3.3)

Note 7: Assume a larger catchment PE where probably of low NoV infection over summer. Small catchments may yield –ve NoV samples and no NoV load if catchment health good.