FINAL REPORT

A CRITICAL REVIEW OF THE CURRENT EVIDENCE FOR THE POTENTIAL USE OF INDICATOR SPECIES TO CLASSIFY UK SHELLFISH PRODUCTION AREAS

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Contents

1. Aim of Study

The aim of this project is to critically review and assess the available evidence on the potential use of shellfish indicator species for the microbiological monitoring of bivalve mollusc harvesting areas for the purpose of classification under EC Regulation 854/2004. Specifically, this is in support of a review and possible simplification of the official classification of shellfish production areas under these Regulations across the UK. An evaluation of the strength and robustness of the identified evidence is presented. Any knowledge gaps to support indicator species use will be identified, particularly where these might be addressed through future research work.

This is a desk-based study to collate, critically review and evaluate available UK and international literature and evidence, both published and unpublished*, on the use of indicator species for the purpose of classification. The majority of the data used was from published peer reviewed papers. A small proportion of data was also obtained from 'grey literature' (e.g. Government commissioned reports) principally produced by Seafish and Cefas and generally available by internet download.

**It was only possible to obtain relevant unpublished data from Cefas for this study.*

Definitions:

The meaning of terms used in the context of this study is as follows:

Accumulation - The final concentration of *E. coli* in shellfish observed as opposed to the process of uptake itself.

Uptake - The process of filtering and concentrating *E. coli* as part of the normal bivalve feeding process.

Removal (depuration) - The natural process of digestion and/or expelling of *E. coli*.

Filtration rate - A measure of the speed at which *E. coli* is accumulated within the bivalves.

2. Glossary

Bivalve mollusc

- Any marine or freshwater mollusc of the class *Pelecypoda* (formerly *Bivalvia* or *Lamellibranchia)*, having a laterally compressed body, a shell consisting of two hinged valves, and gills for respiration. The group includes clams, cockles, oysters, and mussels. In the context of European food hygiene legislation , the term defined as "'Bivalve molluscs' means filter-feeding lamellibranch molluscs": the requirements of the legislation for bivalve molluscs, other than depuration, also apply to echinoderms, tunicates and marine gastropods (although gastropods are also excluded from the classification requirements).

Classification of bivalve mollusc harvesting areas

- Assignment of harvesting areas to different classes based on an official monitoring programme to determine the extent of microbiological contamination in production and relaying areas. The requirements are given in Annex II, Chapter II of Regulation (EC) No 854/2004.

Coliform

- Gram negative, facultatively anaerobic rod-shaped bacteria which ferment lactose to produce acid and gas at 37°C. Members of this group normally inhabit the intestine of warm-blooded animals but may also be found in the environment (e.g. on plant material and soil).

Chemical contaminants

- Chemical compounds that can potentially harm the health of humans and the status of ecosystems.

***Escherichia coli* (*E .coli*)**

- A species of bacterium that is a member of the faecal coliform group (see below). It is more specifically associated with the intestines of warm-blooded animals and birds than other members of the faecal coliform group. Traditionally *E. coli* produce indole from tryptophan at 44°C. Now determined in the reference method on the basis of the possession of β-glucuronidase activity.

Faecal coliforms

- Coliforms (see above) which can produce their characteristic reactions (e.g. production of acid from lactose) at 44°C as well as 37°C. Usually, but not exclusively, associated with the intestines of warm-blooded animals and birds.

Faecal Indicator Organism

- In the context of this study this means bacterial indicators of faecal contamination (faecal coliforms and/or *Escherichia coli*) and of the potential presence of pathogens associated with wastewater or sewage sludge. Indicator organisms are typically used to demonstrate the potential presence or absence of groups of pathogens.

Food Business Operator

- The natural or legal persons responsible for ensuring that the requirements of food law are met within the food business under their control;

Geometric Mean

- The geometric mean of a series of N numbers is the Nth root of the product of those numbers. It is more usually calculated by obtaining the mean of the logarithms of the numbers and then taking the antilog of that mean (see Annex 3). It is often used to describe the typical values of a skewed data set such as one following a log-normal distribution (see below).

Harvesting Area

- The term Harvesting Area is used in this Guide to cover both Production and Relay Areas.

Hepatitis A virus (HAV)

- This is a 27 nm diameter virus that contains RNA as its nucleic acid. It is transmitted by the faecal-oral route and although most infections may result in mild feverish episodes, it can cause inflammation of the liver resulting in jaundice.

Norovirus

- Noroviruses are small, 27 to 32 nm, structured RNA viruses which have been implicated as the most common cause of nonbacterial gastroenteritis outbreaks. (They were formerly called Small Round Structured Viruses (SRSVs) and Norwalk-like viruses (NLVs)).

Production area

- Any sea, estuarine or lagoon area, containing either natural beds of bivalve molluscs or sites used for the cultivation of bivalve molluscs, and from which live bivalve molluscs are taken.

Representative monitoring point

- A specified geographical location from which samples are taken to represent either a single, or several, wild bivalve mollusc beds or aquaculture sites.

Sampling plan

- A formal record of the intended sampling to be undertaken in a harvesting area with respect to species, position of sampling point(s) and frequency of sampling. The components of the sampling plan are identified following the sanitary survey.

Sanitary survey

- An evaluation of the sources of faecal contamination in or near a harvesting area together with an assessment of the potential impact of these sources on the microbial status of the harvesting area.

Sewage

- A liquid that is or has been in a sewer. It usually consists of waterborne waste from domestic, trade and industrial sources together with rainfall from subsoil and surface water.

Short-term controls

- Control measures taken to reduce or negate any increased risk to public health that might arise from temporary increased contamination of harvesting areas. These controls include prohibition of harvesting, short-term reclassification and increased treatment requirement with reclassification, if necessary. The extent and period of the control measures should

address the risk from the microbial pathogens, or other contaminants of public health concern, and not simply the bacterial indicators used for monitoring purposes.

Species referenced in this report - Latin and common names:

Cerastoderma edule = Common edible cockle
Mytilus spp. = *Mytilus edulis* (common or blue mussel), *Mytilus galloprovincialis* (Mediterranean mussel) and hybrids
Ostrea edulis = Native or Flat Oyster
Crassostrea gigas = Pacific Oyster
Mercenaria mercenaria = Hard clam
Spisula solida = Thick trough shell or surf clam
Tapes philippinarum = Manila clam
Ensis spp. = Razor clams (e.g. *Ensis siliqua* = pod razor shell)
Pecten maximus = Great or King scallop
Mya spp. = Gapers (e.g. *Mya arenaria* = sand gaper)
Tapes decussatus = Palourde, native clam or carpet shell clam
Arctica islandica = Icelandic cyprine
Crassostrea virginica = Eastern oyster (also Virginia oyster or Atlantic oyster)
Chlamys opercularis = Queen scallop
Venus verrucosa = Warty venus clam
Crassostrea ariakensis = Suminoe oyster
Perna viridis = Asian green mussel
Crassostrea commercialis = Sydney rock oyster
Donax trunculus = Wedge shell
Chamelea gallina = Striped venus clam
Patella vulgata = Common limpet or common European limpet
Venerupis pullastra = Pullet carpet shell
Dosinia exoleta = Rayed Artemis

3. Executive summary

Filter-feeding molluscan shellfish (oysters, mussels, clams, etc) are grown in areas classified for sanitary quality under EU Regulation 854/2004 on the basis of *Escherichia coli* monitoring. Rationalizing monitoring programmes through the use of a single indicator-shellfish species, rather than monitoring several species, would have the benefit of reducing costs. In order to protect public health, the indicator should show an equivalent or higher level of contamination (as shown by *E. coli* accumulation under the statutory monitoring programme) than the species it represents. This study aims to evaluate the published and unpublished evidence to assess whether it can support the concept of using a single indicator species to represent multiple species and any limitations to that approach.

The number of available studies that are specific to the question of indicator species and their potential application is quite limited. Whilst there are some differences between findings of studies (field and microcosm), the significance and practical implications of these differences are comparatively minor and would not, we would suggest, prevent an indicator species approach being taken in the UK. The following recommendations are made on the assumption that species are co-located both geographically and with respect to depth in the water column:

- *Mytilus* spp may be used as an indicator in many situations typically encountered in the UK. In particular, it may be used to represent *C. gigas, O. edulis, Tapes spp.* and *M. mercenaria.*

- The data would support the use of *C. edule* to represent *Mytilus* spp. or any of the above species that could be represented by *Mytilus* spp. where the monitoring of *C. edule* is practical. It is known, however, that sampling of natural *C. edule* stocks can be problematic (for access, health and safety and sampling location repeatability reasons). The situations under the statutory monitoring programme where *C. edule* may be used as an indicator may therefore be limited.

- Additionally, where *O. edulis* and *C. gigas* are produced in the same area, then the findings of this review would support monitoring either species to represent both. *O. edulis* (and therefore by analogy *C. gigas*) may also be used to represent *M. mercenaria.*

- An indicator approach cannot be recommended at this stage for representation of *C. edule*, *Spisula solida, Mya arenaria* and *Ensis* spp. as either contradictory or no supporting data from the literature is available.

- There would appear to be insufficient evidence available to justify a recommendation for an indicator to represent scallops (*Pecten maximus*).

- In England and Wales, *Mytilus* spp. offer a number of practical advantages as an indicator shellfish species. They are relatively cheap to obtain, are generally more resilient than other bivalve species to environmental stressors

and may be readily deployed in a variety of ways to facilitate sampling e.g. mesh bags on fixed installations, suspended from buoys in the water column, or on the seabed. From the practical perspective, therefore, *Mytilus* spp. sampling is generally preferable to *C. edule* sampling. In addition, *Mytilus* spp. tend to be found in similar locations on repeated occasions (unlike *C. edule* beds which have a tendency to shift quite regularly).

Considerations

Class A areas: An important consideration is that, since Class A shellfish can be directly marketed without further processing, regulatory compliance needs to be demonstrated for each species independently in this particular classification category. Whilst this is ultimately the responsibility of the Food Business Operator (FBO) careful consideration would, nevertheless, be advisable on the part of the competent authority on whether the use of indicator shellfish species for Class A classified production areas would be appropriate. Similarly if compliance proves marginal between the B/C classification categories with *Mytilus* spp. as the indicator species (or is already marginal with the target species) then it may be preferable to sample the target species to achieve the best level of classification possible from the shellfish industry perspective.

Precautionary approach versus best classification for industry stakeholders: It should be emphasised that there are two separate, potentially conflicting, issues to consider here. The first being to protect public health and to that end the species showing the higher level of contamination would be selected. This is the primary aim of the legislation, EC Regulation 854/2004 (Anon, 2004b). The second, however, is to recognise the needs of the shellfish industry in terms of obtaining a level of classification that allows FBOs to run a viable business. The balance between these two issues may occasionally require a policy decision from the competent authority to achieve the best compromise where one may be necessary.

Spatial effects: Any indicator approach would need to carefully consider the potential for spatial differences in contamination across a harvesting area to ensure that they were adequately addressed e.g. it would not be appropriate to use an indicator species if this was located further from the main sources of contamination than the species being represented.

Pollution events and confirmation of microbial clearance: On a note of caution, Dore and Lees (1995) studying the removal of viruses (F+ bacteriophage) from shellfish found that removal from *Mytilus* spp. was quicker than from *C. gigas*. This would suggest that the use of mussels as an indicator species to reflect the viral content of oysters after sewage spills (where clearance rate rather than accumulation is the feature of interest) may not provide a protective approach. No equivalent published viral data is currently available for cockles.

In addition, it is clear given other study findings noted in this review (e.g. Beucher, 1993) that there are differences in the clearance rate of *E. coli* and/or faecal coliforms between bivalve species. Furthermore, a ranking of species according to their clearance ability may differ from a ranking according to their capacity for

accumulation. The use of an indicator species approach for investigative samples following pollution events (where confirmation of microbial clearance is key) would therefore need separate consideration.

4. Introduction

Filter-feeding molluscan shellfish (oysters, mussels, clams, etc) are grown in areas classified for sanitary quality under EU Regulation 854/2004 on the basis of *Escherichia coli* monitoring. By default, monitoring is undertaken for each commercial species that is present in an area. Rationalizing monitoring programmes through the use of a single indicator-shellfish species, rather than monitoring several species, would have the benefit of reducing costs. In order to protect public health, the indicator should show an equivalent or higher level of contamination (as shown by *E. coli* accumulation under the statutory monitoring programme) than the species it represents. The FSA has committed to review the classification system in general to ensure that it remains fit for purpose, utilizes available resources effectively and provides adequate protection for public health. An option to simplify the current monitoring arrangements would be to use one shellfish 'indicator' species to classify one or more others where they occur in the same location. However, to support such a change, sufficiently robust evidence is required to demonstrate that public health protection will be maintained or improved. It is also necessary to demonstrate that this finding is likely to be valid across the range of environmental conditions and pollution levels existing in harvesting areas. This study aims to evaluate the published and unpublished evidence on indicator species and to assess whether it can support the concept of using a single indicator species to represent multiple species and any limitations to that approach. This work will identify the available practical options that are supported by the evidence.

Relevant evidence can be obtained from two main approaches:

1) Studies carried out in a controlled laboratory/test environment.
2) Studies based on shellfish growing in the natural environment.

This review will consider evidence from each approach.

Finally, it is necessary, from a practical perspective, to understand the potential savings in sampling effort that could be realized from adoption of indicator species and also any potential impact on classifications awarded (e.g. for a more conservative indicator). These aspects will be evaluated using the full *E. coli* monitoring data set for England and Wales for the last five years as published on the Cefas website. Possible public health impacts arising from adoption of the available indicator species options are considered using data from the FSA's norovirus in oysters surveillance study (FSA 2012) as a benchmark.

5. Literature review

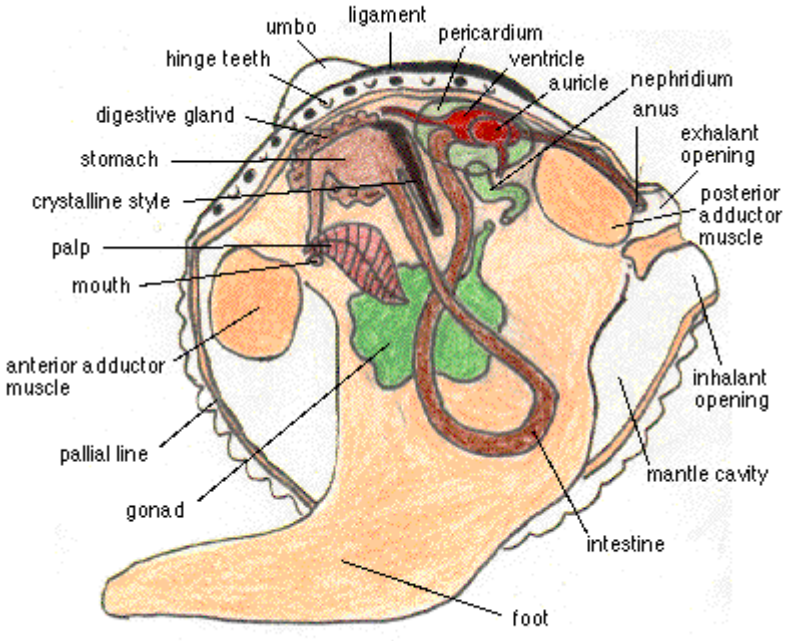
For details of search terms used see Appendix.

5.1 Bivalve filtration and feeding

Most bivalves are filter feeders, using their gills to capture particulate food such as bacteria and phytoplankton from the water. Water is drawn into the shell from the posterior ventral surface of the animal. From here it passes through the gills and is then expelled again from a point just above the intake. In burrowing species there may be two elongated and retractable siphons (inhalant and exhalant) reaching up to the surface of the seabed. Cilia on the gills capture food particles and transport them in a steady stream of mucus to the mouth.

The digestive tract comprises an oesophagus, stomach, and intestine. A number of digestive glands open into the stomach, usually via a pair of diverticula. The diverticula secrete enzymes which digest the food. Phagocyte cells are also present and these digest food particles intracellularly. An elongated rod of solidified mucus known as the "crystalline style" projects into the stomach from an associated sac. Cilia in the sac cause the style to rotate and in so doing wind in a stream of food-containing mucus from the mouth, churning the stomach contents in the process. This constant motion propels food particles into a sorting region at the rear of the stomach. This diverts heavier particles into the intestine and smaller particles into the digestive glands. Waste material collects in the rectum and is excreted as pellets into the exhalent water stream through an anal pore.

Anatomy of a typical bivalve



Cross section through a stylised bivalve - ctenidium (gill) removed

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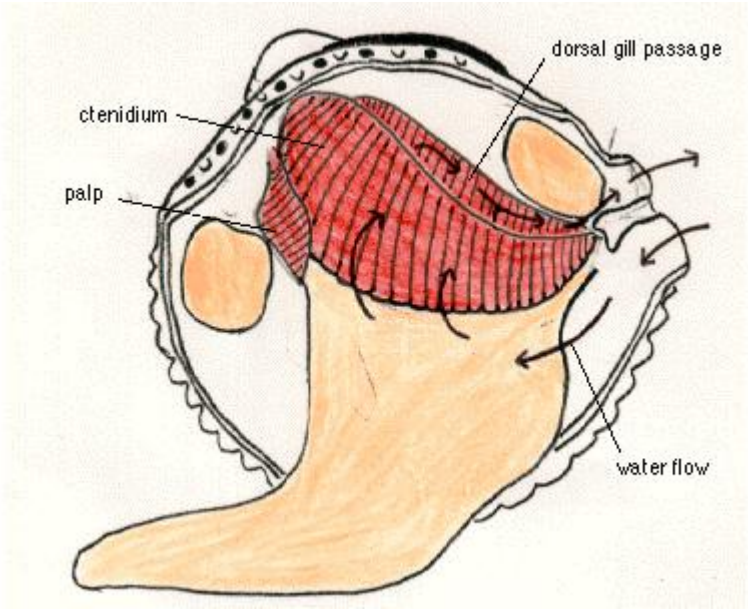
http://bio.classes.ucsc.edu/bioe122/molluscs/bivalve/bivalvia.html



Water flow through ctenidium

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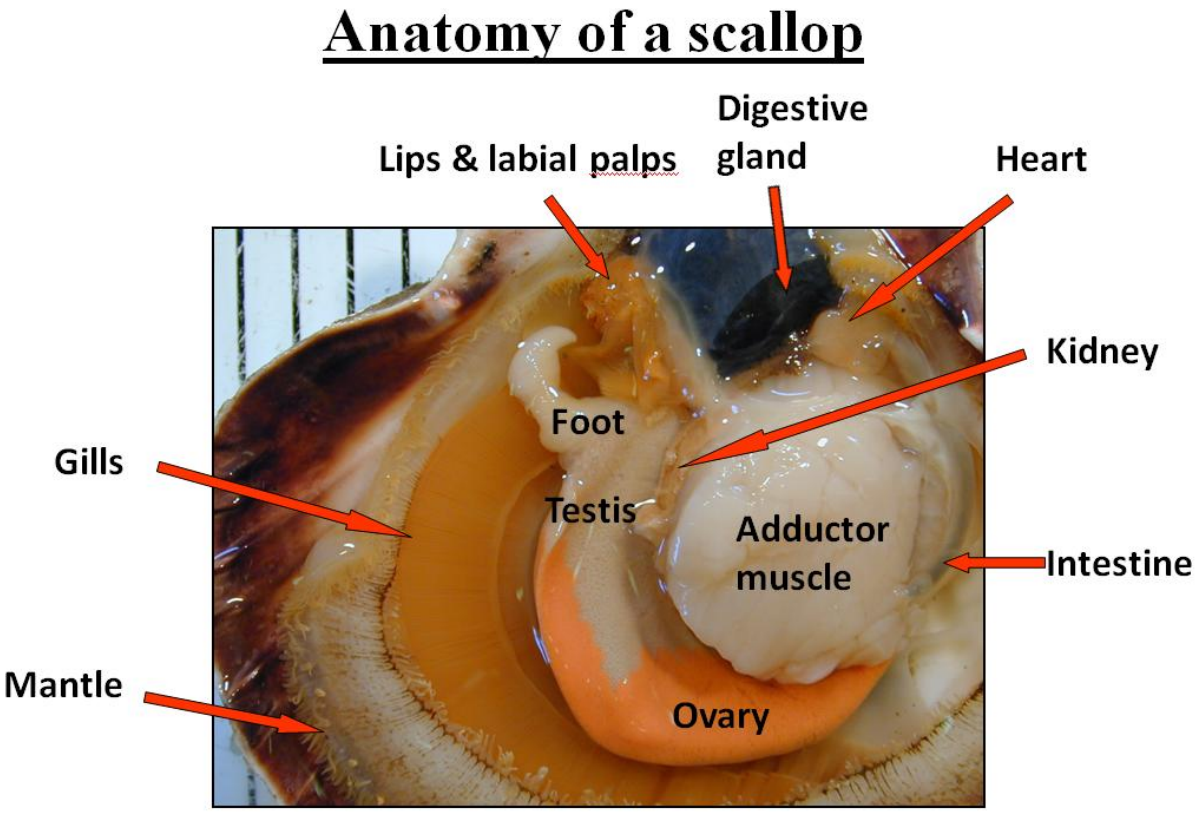
http://bio.classes.ucsc.edu/bioe122/molluscs/bivalve/bivalvia.html



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Ward and Shumway (2004) state that the particle feeding and selection mechanisms in bivalves are complex. Furthermore, processes may be species-specific based on both the physical and chemical properties of the particles themselves. They

conclude that more research is needed to fully understand the particle selection process, both in terms of the characteristics of particles that are significant for selection and the physiological and environmental factors that may influence the overall process.

Pales Espinosa (2010) reports that selection of particles involves interactions between epiparticulate carbohydrates and sugar-binding proteins (lectins) in the mucus produced by feeding organs.

Kach and Ward (2008) looked at the role of marine aggregates in the ingestion of picoplankton-size particles by suspension-feeding molluscs. Picoplankton cells are defined as those within the size range (0.2-2.0 µm). Their results indicated that the ingestion by bivalves of particles from 0.5 to 1.0 µm was significantly improved (compared with that for the individual particles) when bound in marine aggregates of larger size. The ingestion of bound bacteria of up to 0.6 µm was similarly enhanced. The degree of ingestion was found to differ, however, between bivalve species: mussels and clams demonstrated a higher degree of ingestion than scallops and oysters. The authors suggest that the differences observed may be due to variation in gill structure between species and the way in which particles are processed.

5.2 Bivalve filtration rate and uptake

The efficiency with which shellfish take up and retain particles may vary with internal and environmental conditions, including concentration and composition of suspended particles (seston) in the ambient water (Jørgensen, 1990). Other factors such as temperature and current speed may account for significant variations in water processing (Prins *et al.* 1994). The effect of water temperature on pumping rate in mussels is illustrated in Figure 1. Variation in pumping rates with temperature may also be partially attributed to changes in the viscosity of the water (Jørgensen, 1990).

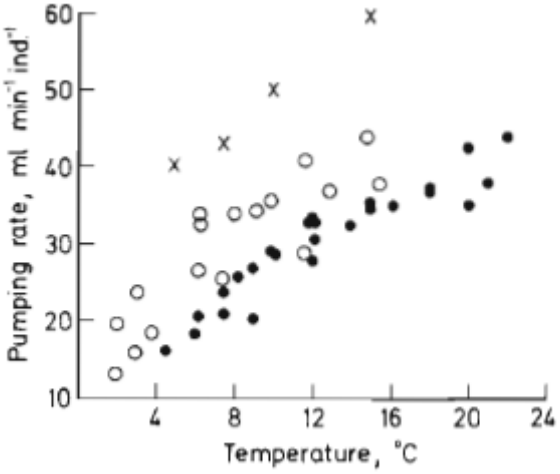

Figure 1. Relationship between temperature and pumping rate in acclimatised mussels at different times of the year. (○) February, 25 mussels, 31±0.7 (SD) mm in length, acclimatised to 6°C; (x) May, 20 mussels, 39±2mm, acclimatised to 7.5°C; (●) June, 30 mussels, 29±2.6mm, acclimatised to 12°C. Adapted from Jørgensen et al. (1990).

It has been suggested that reduced filtration in the common cockle during periods when inorganic seston levels are high could be an adaptive mechanism to reduce the high metabolic costs associated with the processing of large quantities of material of low nutritional value (Newell and Bayne, 1980). The sensitivity and tolerance towards these environmental conditions are reflected by the growth and survival characteristics of each species, particularly those inhabiting the intertidal zone (Jørgensen, 1990). Table 1 highlights differences in estimated maximum rates of water processing in mussel beds between different European environments.

Table 1. Maximum rates of water processing by mussel beds.

Location	Habitat	Pumping (Clearance) rate (m^3 m^{-2} h^{-1})	Reference
England	Intertidal	7	Dare (1976)
England	Sublittoral	12	Dare (1976)
Denmark	Sublittoral	7	Jørgensen (1980)
The Netherlands	Microcosm (continuous flow tank) supplied with natural seawater	0.4–2.7	Prins *et al.*. (1994)

McHenery and Birkbeck (1985) investigated uptake (and subsequent degradation) of *E. coli* by several species of bivalves using radiolabelled bacterial cells (Figure 2).

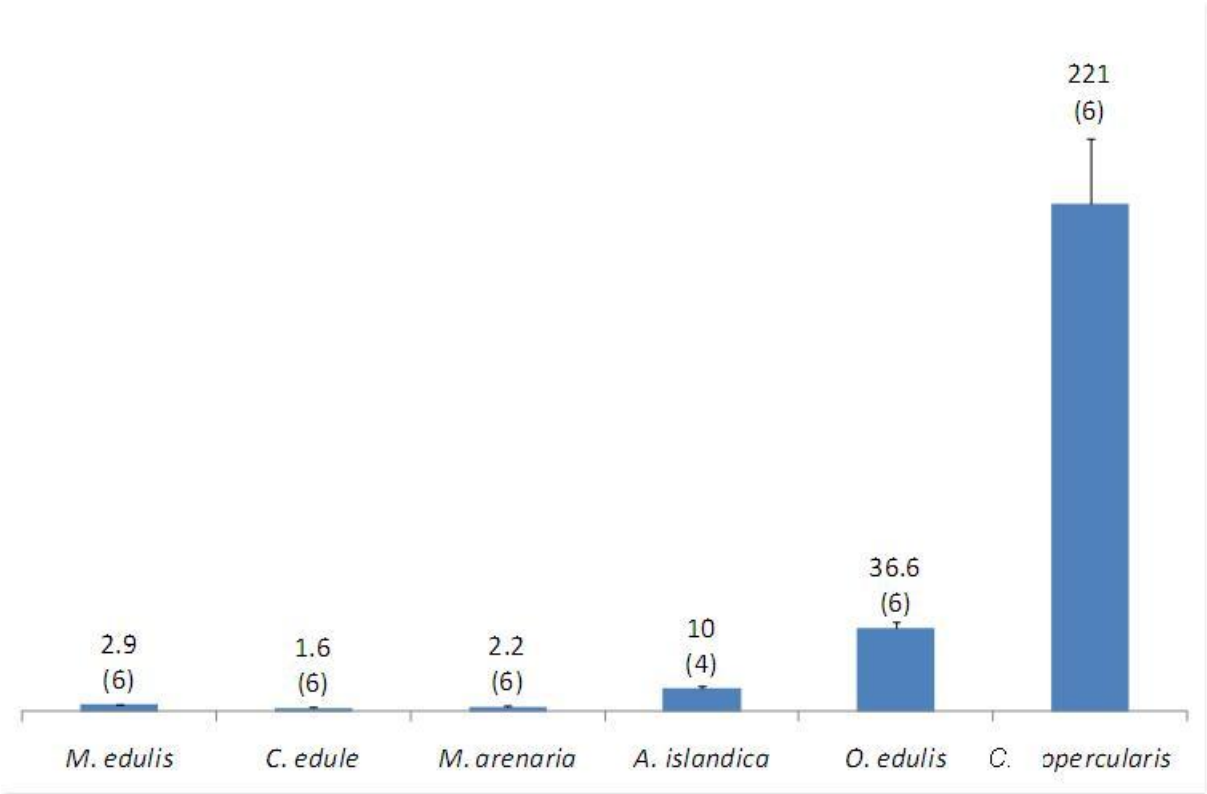

Figure 2. Time (hours + standard error of the mean followed by the number of experiments in parenthesis) to take up 90% of [^{3}H]TdR-radiolabelled *E. coli* from suspension by six species of bivalves. Data from McHenery and Birkbeck (1985). *M. edulis*=blue mussel; *C. edule*=common cockle; *Mya arenaria*=soft-shell clam; *Arctica islandica*=Icelandic cyprine (not commercially harvested in England and Wales); *O. edulis*=native oyster; *Chlamys opercularis*=queen scallop.

E. coli was rapidly taken up from suspension with no significant difference being observed between *C. edule, Mytilus edulis* and *Mya arenaria. Arctica islandica* and *Ostrea edulis* removed bacteria from suspension more slowly and *Chlamys opercularis* was reported as barely removing any at all although it appeared to be actively pumping.

Higher accumulation factors in cockles and mussels (see later section 5.5 for details) are consistent with higher filtration rates in these species reported in the literature (Table 2).

Table 2. Filtration rates as a function of size and shell length in bivalve shellfish obtained under laboratory conditions.

Species	Filtration rate (F, l h^{-1})	Quantification Method	Reference
C. edule	$11.60W^{0.70}$	Suction	Møhlenberg and Riisgård (1979)
M. edulis	$7.45W^{0.66}$	Suction	Møhlenberg and Riisgård (1979)
M. edulis	$0.0012L^{2.14}$	Suction	Kiørboe and Møhlenberg (1981)
M. edulis	$7.37W^{0.72}$	Photoaquarium	Riisgård and Møhlenberg (1979)
C. virginica	$6.79W^{0.73}$	Clearance	Riisgård (1988)
M. mercenaria	$2.5W^{0.78}$	Replacement	Coghlan and Ansell (1964)
M. mercenaria	$1.24W^{0.80}$	Clearance	Riisgård (1988)

'F' as a function of size (W, g body dry weight) or shell length (L, mm).

Suction method: samples of inhaled and exhaled water are sucked through glass tubes placed 2-4mm above the bivalve's inhalant and exhalant openings. The flow rate through the glass tubes is varied by gravity or by means of an adjustable peristaltic pump. The clearance (Cl) (volume of exhaled water cleared of particles per unit of time) is calculated according to the equation: $Cl = Fl (1-C_e/C_i)$, where Fl is the suction flow rate through the glass tubes, and C_i and C_e the concentrations of 100% retained algal cells in water collected simultaneously from inhalant and exhalant currents, respectively.

Clearance method: F is measured as the volume of water cleared of suspended particles per unit of time. The reduction in the number of particles as a function of time is monitored by taking water samples at fixed time intervals and measuring the particle concentration, usually with an electronic particle counter. Cl is determined using the equation: $Cl = (V/nt)\ ln\ (C_0/C_t)$, where C_0 and C_t is the algal concentration at time 0 and time t, V is the volume of water and n is the number of animals.

Photoaquarium method: an automatic recording apparatus that maintains constant algal concentration and allows continuous measurements of the filtration rate in bivalves. F is estimated by means of the equation: $F = (z/tn)\ (vC_s/C_e-v)-o/n$, where z is the number of algal additions, t is time, n is the number of bivalves, v is the volume of one algal addition, C_s is the algal concentration in chemostat, C_e is the algal concentration in photoaquarium, and o is the through-flow rate of fresh, particle-free seawater.

Charles *et al.* (1992a, 1992b), using radiotracer methods, found that the Mediterranean mussel (*Mytilus galloprovincialis*) was able to take up a given quantity of *E. coli* twice as quickly as Venus clams (*Venus verrucosa*). The kinetic filtration coefficient obtained for the mussels was over twice that of the clams (0.280 h^{-1} for mussels versus 0.120 h^{-1} for clams).

In summary, it can be concluded from these studies that filtration rate and uptake are affected by a number of factors including organic/inorganic particulate content of the water and the temperature. Furthermore, the effect of such factors may vary between species. *Mytilus* spp. and *C. edule* generally appear to have filtration rates and uptake either similar to, or higher than, the other species studied (*Mya arenaria.*

Arctica islandica and *Ostrea edulis, Chlamys opercularis, Venus verrucosa* and *Crassostrea virginica*).

a) Effect of temperature on pumping and uptake (feeding)

It should be noted that *E. coli* concentrations in water and shellfish are constantly changing at most sites at tidal, diurnal and annual periodicities. High and low results are possible at any time of year in many cases. Consequently, identifying any temperature-related uptake effects in the environment is problematic. Studies aimed at investigating this parameter are therefore best undertaken in a controlled laboratory environment where defined *E. coli* dosing regimes can be employed.

Temperature is confounded with season, and hence also with day-length, incident UV and the annual biological cycle of the shellfish. All these factors may act directly on the shellfish and affect their feeding rate, and they may also act indirectly through the varying availability of food particles and any variation in the *E. coli* burden.

The effects of temperature on pumping rates were studied by Jørgensen *et al.* (1990) in the blue mussel (*Mytilus edulis*) kept at temperatures ranging from 6°C to 17°C. It was found that pumping rates increased with temperature and this was linearly correlated with the decrease in viscosity of the water associated with such temperature increases.

Conversely, however, McHenery and Birkbeck (1985) in a laboratory based syetem using [^{3}H] thymidine-labelled *E. coli* found that uptake rates in the common cockle (*C. edule*) were significantly slower at 15°C than at 10°C.

Šolić *et al.* (1999), in a laboratory based study, investigated the effect of temperature on faecal coliform accumulation in shellfish using Mediterranean mussels (*M. galloprovincialis*) and native oysters (*O. edulis*) from shellfish production areas in Split, Croatia. The temperatures tested were 12°C (mean winter temperature), 24°C (mean summer temperature) and 18°C. They reported that that both temperature and the concentration of faecal coliforms in the seawater influenced faecal coliform accumulation (see Table 3). Furthermore, in mussels, as the concentration of faecal coliforms increased, the rate of uptake decreased more rapidly at the higher temperature.

Table 3. Time required for mussels and oysters to achieve maximum faecal coliform concentrations (accumulation) as obtained by Šolić *et al.* (1999).

Temperature (°C)	Levels of faecal coliforms in seawater (L^{-1})	Time (h)	
		Mediterranean mussel (*M. galloprovincialis*)	Native oyster (*O. edulis*)
12	$10–10^3$	5.54	11.13
	$10^3–10^5$	2.45	6.51
	$10^5–10^7$	1.51	4.25
18	$10–10^3$	3.33	1.67
	$10^3–10^5$	1.68	1.01
	$10^5–10^7$	1.10	0.88
24	$10–10^3$	1.85	3.97
	$10^3–10^5$	1.11	2.52
	$10^5–10^7$	0.71	1.64

In summary, from the data above it can be concluded that the effect of increasing temperature may increase the pumping rate of some species e.g. *O. edulis* and *Mytilus* spp. However, there is evidence to suggest that for some other species (e.g. *C. edule)* the opposite may apply. In any event, it is clear that the effect between species may be variable.

b) Effect of salinity on pumping and uptake (feeding)

Salinity affects both pumping rates and filter-feeding processes in shellfish (Rowse and Fleet, 1984). However, it has been noted that changes in salinity do not affect the growth of bivalves as much as variation in temperature (Laing and Spencer, 2006). Generally, valve opening of bivalves is increasingly delayed as salinity decreases (Motwani, 1956). Motwani reported that the effect was particularly pronounced in *Mytilus edulis* below 17.4‰ and this corresponds to the typical lower limit of survivability of this species in UK waters. *Mytilus* usually only feed at salinities within the range 20-35‰ (Laing and Spencer, 2006). The other bivalve species commonly exploited commercially in the UK generally prefer salinities at or above the minimum noted for *Mytilus*. Feeding rates in *O. edulis* begin to decline at 28‰ and cease at 16‰ (Rödström and Jonsson, 2000). Scallops are very intolerant of salinities lower than 30‰. Pacific oysters prefer salinity levels nearer to 25‰ (Laing and Spencer, 2006).

From the limited data available it is clear that filtration rates and uptake of bacteria vary between species. In addition to food availability, temperature and salinity are factors which influence bivalve filtering activity and the optimum range for each may vary between species. Consequently, any indicator species approach would need to ensure that the chosen indicator has an effective temperature and salinity range at least equivalent (if not wider) than the species to be represented.

5.3 Dynamics of microbial clearance

Clearance of microbial contaminants from shellfish has been relatively well studied since the installation of the first commercial scale depuration plants in the early 20th century in the UK (Dodgson, 1928; Wood, 1969) and elsewhere (Richards, 1988; Lee *et al.*, 2008) when epidemiological evidence first linked cases of human illness to shellfish consumption.

Removal of *E. coli* in *M. mercenaria* was studied by Timoney and Abston (1984) who found that *E. coli* was rapidly cleared from these clams, with the greatest clearance occurring over the first 8 hours. By this time, *E. coli* levels in the clams approximated those in the water. After 24h, *E. coli* levels in the clams had decreased by a factor of around 100 and were then higher in the water than in the clams themselves. Faeces and pseudofaeces contained the greatest concentration of *E. coli* which appeared to be closely bound to particulates. Only a small proportion of bacteria were found to be free in the water and so the authors concluded from this that the association of *E.coli* with faecal material was stable.

To understand the pattern of bacterial elimination by mussels, Plusquellec *et al.* (1990) transferred mussels previously exposed to microbial contamination for a period of 3h into a tank containing clean seawater. Steady clearance of *E. coli* was observed with a four day period being necessary to achieve complete elimination. A two log removal of *E. coli* in the shellfish was observed within 24h exposure to clean water.

Birkbeck and McHenery (1982) undertook laboratory based studies investigating the uptake of several species of Radiolabelled and unlabelled bacteria by the common mussel (*M. edulis*) along with the subsequent fate (clearance or degradation) of some polymers of the bacteria. Clearance of bacteria by the shellfish was measured by viable colony counting and scintillation counting in the case of radiolabelled bacteria. Bacteria were found to be cleared at similar, exponential, rates with 90% being cleared in a mean time of 1.93 ± 0.12 h (n = 63). The study used *E. coli, Staphylococcus aureus* and Pseudomonad 1-1-1. All three species of bacteria were rapidly removed from sea water by the shellfish and for the first 4 to 6 h, exponential clearance was observed. Clearance coefficients, defined as the times required to reduce the bacterial concentration in water by 90% (C90), were reported to be not significantly different for various bacterial species (mean C90=2.4h ±0.34, n=13). In the case of *E. coli,* the bacterial counts continued to fall between 6 and 24 h. Bacteria with cell walls which were sensitive to *M. edulis* lysozyme were found to be rapidly degraded by the mussel. By contrast, lysozyme-resistant bacteria *(Micrococcus roseus* and *S. aureus)* were found to be cleared from suspension by the shellfish but were generally then rejected intact. Birkbeck and McHenery conclude that bacteria can be degraded by *M. edulis* and selected polymers retained and presumably utilised. They further report that the range of bacteria utilised by bivalves and the food value of bacteria in relation to phytoplankton remain to be determined, as do the mechanisms by which selection occurs.

a) Effect of temperature on bacterial clearance

The effect of temperature on bacterial clearance in a number of species of bivalves has been well documented. In general, faecal coliforms and *E. coli* are rapidly removed (possibly within a few hours depending on the initial bacterial accumulation level) from bivalves during depuration and increasing temperature from 8 to 19°C has been found to increase the rate of removal (Cefas unpublished data). More importantly, it has been reported that temperature would appear to be a crucial factor in the removal of viruses. It has been demonstrated (Cefas, 2013) that with increasing temperature (from 8 to 16°C) clearance rates of *E. coli* and virus (F+ bacteriophage and norovirus) increased in *C. gigas.* However, virus removal rates were considerably slower than those for *E. coli*.

Naturally occurring marine vibrios (some of which may be pathogenic to humans) may not be reliably removed by depuration. There is some evidence to suggest that increased temperatures (20-25°C) may actually increase the numbers of vibrio within bivalve shellfish (Cefas unpublished data).

b) Effect of salinity on bacterial clearance

Rowse and Fleet (1984) examined the effect of salinity on clearance of *E. coli* in the oyster *Crassostrea commercialis*. Oysters, harvested from Georges River (NSW, Australia) were artificially contaminated in the laboratory using cultured *E. coli* added to the water. The bacterial strains used had previously been isolated from local oysters. A 5-ml volume of raw sewage was also added to the water to simulate estuarine conditions. The trials investigated *E. coli* clearance from the oysters in water of salinities within the ranges 16–20‰, 32–36‰ and 43–47‰. Their results showed that, at low salinities, clearance of *E. coli* was slow and inconsistent and oyster mortality rates were higher. In contrast, elimination of *E. coli* was rapid and consistent at 43–47‰ salinity and was similar to that for oysters tested at 32–36‰. No abnormal mortality rates were observed at these higher salinities.

5.4 Faecal indicator organism (FIO) accumulation in individual bivalve species

Whilst bacterial clearance has been comparatively well studied, bacterial accumulation in shellfish has, until quite recently, received much less attention.

Studies have shown that bivalve shellfish concentrate *E. coli* to levels several times higher than those present in the surrounding water (Prieur *et al*., 1990). Love *et al*. (2010) report that filter-feeding bivalve molluscs (shellfish) can bioaccumulate pathogenic microorganisms to levels up to 1000-fold higher than overlying waters.

Large variations in accumulation factors have been detected in environmental data because bacterial die-off in the water column varies significantly according to factors such as light intensity, water mixing, sewage content and turbidity (Campos et al., 2011). For instance, Plusquellec *et al.* (1983) reported accumulation factors for faecal coliforms and enterococci in mussels from Concarneau (France), an area directly impacted by sewage discharges, of 13.2 and 250, respectively. These

variations were thought to be associated with seasonal effects and/or differential accumulation between the two groups of indicators (Plusquellec *et al.*, 1990; Prieur *et al.*, 1990).

Burkhardt III *et al.* (1992) examined the effects of temperature and season on the ability of *Mercenaria mercenaria* to filter and retain indicator microorganisms in Naragansett Bay (USA). The study consisted of exposing shellfish to ambient seawater constantly dosed with raw wastewater to maintain constant indicator levels in overlying water. The ability of these clams to concentrate contaminants was found to be considerably reduced when seawater temperatures were below 7°C and this was attributed to reduced physiological activity. Bioaccumulation was found to cease altogether at water temperatures below 4.5°C. There was found to be a marked increase in the accumulation of faecal coliforms and *E. coli* between 4.5 and 11.5°C which corresponded with the typical water temperature range experienced in spring. The authors proposed that, aside from temperature considerations, seasonal variations in activity may also be due to the microhabitat of individual shellfish. Furthermore they suggested that shellfish in upstream reaches of a bed may show different accumulation rates from those in beds further downstream. They also postulated that, in species that typically grow in groups (e.g. mussels), individuals on the outside of a cluster may have access to more food and contaminants than those within the cluster. In addition, the method and position of growth of the shellfish determines the periods of the tidal cycle during which they are immersed in the water column. This then influences the sources of food and contaminants to which they are exposed.

The concentration of FIOs accumulated by shellfish is affected by the duration of contaminating events and usually reflects FIO concentrations of the overlying water during the preceding hours (Cabelli and Heffernan, 1970). These authors suggested that the level of equilibrium between *E. coli* contamination in the American hard clam (*Mercenaria mercenaria*) and overlying waters increases with levels of the indicator in the water.

Studies using the Pacific oyster indicate that this species could reach an equilibrium between the uptake and clearance phases within 20h of active filtration. This equilibrium varied however between seasons and between individuals of the same species (Beucher, 1993).

Timoney and Abston (1984) studied the uptake and subsequent elimination of *E. coli* and *Salmonella typhimurium* in *Mercenaria mercenaria.* These were contaminated in experimental aquaria and then cleansed in depuration tanks using UV irradiated seawater. Both species of bacteria were found to be accumulated to a similar degree. The authors estimated that each clam would accumulate 1×10^6–1×10^7 cfu of *E. coli* after 15 minutes of exposure. Assuming a pumping rate of 100ml minute^{-1} at 20°C, the authors calculated that each clam would pump in the region of 7.5×10^7 organisms, 10% of which would be retained in their tissues.

Ho and Tam (2000) maintained UV-depurated green mussels (*Perna viridis*) in small (40L) tanks exposed to natural light. The mussels initially contained less than 4 *E. coli* 100g^{-1} FIL when held in sterile seawater inoculated with high concentrations of (2.6×10^3 to 2.9×10^5 100ml^{-1}) of laboratory strain *E. coli* ATCC 25922. Levels of

accumulated *E. coli* peaked within 3–5h exposure, reaching 50–100 times the seawater concentration. These subsequently declined quite quickly initially, then at a slower rate within the following 15–20h to approximately 0.2% of the peak level. The authors observed a slight uptake of *E. coli* at around 25–30h and 40–45h with levels in shellfish then remaining relatively constant.

Martins *et al.* (2006) undertook experiments investigating uptake and accumulation of *E. coli* (ATCC 25922) by native clams (*Tapes decussatus*) held in 20L aquaria. *E. coli* levels in the water in the first experiment were 3×10^8 $100ml^{-1}$ and were 9×10^4 $100ml^{-1}$ in the second experiment. Uptake and accumulation was found to differ according to the initial concentration in the water. Clam *E. coli* levels did not exceed those in the water during the first 6h of the first experiment, however, in the second experiment, levels of *E. coli* exceeded those in the water after the first 30 minutes of exposure.

Bean *et al.* (2006) carried out uptake experiments using depurated American cupped oyster (*Crassostrea virginica*) and Suminoe oyster (*C. ariakensis*). They inoculated six tanks with an *E. coli* laboratory strain (K12), to achieve final concentrations of 10^3–10^4 cells/100ml in the water. Oysters were tested for *E. coli* and *Vibrio* sp. after initial inoculation and again after 4h. *C. ariakensis* was found to contain higher baseline concentrations of *E. coli* at the beginning of the experiment. After 4h, *E. coli* levels in *C. virginica* were more than an order of magnitude higher than *C. ariakensis*. *E. coli* levels in the water decreased during the uptake phase and increased in the shellfish flesh to up to 3 times the initial concentration over the 4h time period. Rates of bacterial uptake were found to differ significantly between the two species suggesting that there is a difference in the response of *C. ariakensis* and *C. virginica* to bacterial contamination.

There is very little evidence on the effect of the interaction between the FIO concentration to which bivalves are exposed and the exposure time on the final concentration (i.e. accumulation).

Kershaw *et al*. (2012) undertook laboratory based studies and reported that exposure of mussels to *E. coli* concentrations in tank water below 10^5 $100ml^{-1}$ revealed a first peak within 3–5h exposure. This was followed by a decline phase which was firstly rapid but later slower within 15–20h and remained relatively constant thereafter. The authors suggest from this observation that the pattern of uptake can be a variable and may be dependent upon *E. coli* concentrations in the surrounding water. The concentration of *E. coli* in tank water was found to decline rapidly initially (when mussels achieved peak uptake rates) but then reduced more slowly to reach residual levels at the end of the experiment. The authors concluded from their trials overall that there is a threshold of concentrations in the water above which bivalves are unable to accumulate more bacteria.

Plusquellec *et al.* (1990), in a laboratory based study, investigated the mechanisms of uptake, retention and removal of bacteria in mussels (*M. edulis*). Depurated mussels were maintained in 100L aquaria at 19°C and inoculated with laboratory strains of *E. coli*, *Streptococcus faecalis* and *Salmonella anatum* added to a suspension of sterilised domestic sewage. Mussels exposed to 'sudden bacterial input' were found to achieve peak flesh concentrations within 30 minutes (i.e.

confirming the results obtained in clams by Martins *et al.,* 2006). After the initial uptake phase, contamination in mussel flesh remained higher than that in the overlying water and the accumulation factor remained relatively constant at around a figure of 10. The authors also tested the effect of different *E. coli* densities in the water ranging from 4 x 10^1 ml^{-1} to 3 x 10^7 ml^{-1}. At the lowest *E. coli* concentration, the mussel contamination curve was found to differ from that described previously: i.e. after 125 minutes of exposure, tank water *E. coli* levels exceeded those in the flesh and remained higher than those in mussel flesh thereafter. By contrast, the contamination pattern at higher water *E. coli* concentrations (above 1 x 10^3 ml^{-1}) was relatively similar, with levels of the indicator in mussel flesh always higher than those in the water after the initial uptake phase. These results suggest that accumulation factors in mussels may change depending on the level of bacteria in the water.

From data collected in the UK, Lees *et al.* (1995) established a relationship between geometric mean concentrations of *E. coli* in shellfish and the corresponding geometric means in seawater. For the pooled species dataset, the seawater geometric mean of 100 was considered to be equivalent to an accumulation factor of 5.9 for mussels, and of 2.6–6.9 for oysters.

Accumulation factors reported in the literature for microcosm studies are summarized in Table 4.

Table 4. Faecal indicator organism accumulation factors in various species of shellfish obtained in the laboratory.

Species	Indicator organism	Exposure period (h)	Accumulation factor	Reference
C. gigas	Faecal coliforms	27	0.8*	Beucher (1993)
C. gigas	*E. coli*	12	09–10.3*	Kay *et al*. (data unpublished)
C. gigas	*E. coli*	12	1–14*	Kay *et al.* (data unpublished)
O. edulis	Faecal coliforms	27	0.5*	Beucher (1993)
C. virginica	Faecal coliforms	Not stated	3–6$^+$	Perkins *et al*. (1980)
M. edulis	*E. coli*	12	0.9–3.4*	Kay *et al.* (data unpublished)
M. edulis	*E. coli*	12	1–7.7*	Kay *et al.* (data unpublished)
C. edule	Faecal coliforms	27	1.5*	Beucher (1993)
C. gallina	*E. coli*	72	1.6 +	Martinez-Manzanarez et al. (1991)
M. arenaria	*E. coli*	48	20 †	Cabelli and Heffernan (1970a)
M. edulis	*E. coli*	46	1.2–7*	Plusquellec *et al.* (1990)
M. edulis	Faecal coliforms	27	1.2*	Beucher (1993)
M. mercenaria	*E. coli*	48	6.5–8.5*	Cabelli and Heffernan (1970a)
M. mercenaria	*E. coli*	24	3 +	Timoney and Abston (1984)
M. mercenaria	Faecal coliforms	168	2.7 (0.02–20.4) †	Burkhardt *et al*. (1992)
M. mercenaria	*E. coli*	168	2 (0.02–17.5)†	Burkhardt *et al*. (1992)
Venus spp.	Faecal coliforms	27	0.6*	Beucher (1993)
Mytilus spp.	*E. coli*		15.2	Kershaw *et al* (2012)
C. gigas	*E. coli*		11.7	Kershaw *et al* (2012)
C. edule	*E. coli*		330	Kershaw *et al* (2012)
Mytilus spp.	*E. coli*		5.9	Lees *et al* (1995)
C. gigas	*E. coli*		2.6-6.9	Lees *et al* (1995)

**Calculated as the logarithm of the concentration of the organism in shellfish flesh divided by the corresponding logarithm of the concentration in the overlying water.*
†Calculated as the geometric mean indicator concentration of the organism in shellfish flesh divided by the corresponding geometric mean concentration in the overlying water.
+ Not stated.

5.5 Comparison of FIO accumulation between bivalve species

a) Microcosm studies

Beucher (1993) found in a number of tank experiments that the common cockle was the most contaminated species during both the uptake and clearance phases of the experiment. Oysters were the least contaminated group of species. (reported log accumulation values: *C. edule* 4.7; *M. edulis* 4.3, *C. gigas* and venus clams 3.9; *O.edulis* 3.5).

Kay *et al.* (2007) reported on a number of laboratory microcosm experiments examining the effects of sewage effluent contamination on *E. coli* levels in shellfish (*Mytilus* spp. and *C. gigas*) and relationships with concentrations in the overlying water. This work involved a contamination exposure (12 hours) to simulate the effect of intermittent sewage discharges such as combined sewer overflows. The main conclusions from the study were:

- *E. coli* uptake in shellfish flesh increased rapidly in response to the addition of sewage, to levels above 46,000 $100g^{-1}$. Accumulation levels in shellfish were consistently higher than the concentration of *E. coli* found in the overlying water.
- First order exponential decay functions were fitted to the observed phase of *E. coli* attenuation, following contamination. *Mytilus* spp. showed higher *E. coli* accumulation levels following contamination and more rapid removal than *C. gigas*.

The authors developed statistically significant ($p<0.05$) regression models in order to predict *E. coli* concentrations in shellfish flesh compared with those in the overlying water. The resulting slope coefficient for mussels was found to be almost twice that observed for *C. gigas* indicating a greater level of *E. coli* accumulation in *Mytilus* spp.

Kershaw *et al.* (2012) investigated the dynamics of *E. coli* accumulation, retention and clearance in three shellfish species (*Mytilus* spp., *C. gigas* and *C. edule*) in a series of laboratory based experiments at a temperature of 10.5°C and salinity of 30‰. Mean accumulation factors were calculated as the geometric mean indicator concentration of the organism in shellfish divided by the corresponding geometric mean concentration in the overlying water and were reported as follows: 330 (*C. edule*), 15.2 (*Mytilus* spp.) and 11.7 (*C. gigas*).

Solic et al. (2010) investigated the effect of temperature and salinity on the rate of concentration of *E. coli* in *M. galloprovincialis* and *O. edulis* using experimental conditions with different concentrations of *E. coli* in seawater. They reported that the rate of uptake of *E. coli* was significantly higher in mussels than in oysters. Furthermore they noted that, in *Mytilus*, variations in salinity had more effect than

variations in temperature in bringing about changes in uptake rate, whereas in *O. edulis* the result was reversed.

b) Environmental studies

A number of studies have investigated accumulation levels of *E. coli* in bivalve shellfish. These have been found to vary with both shellfish species and temperature (Kelly et al., 1960; Cabelli and Heffernan, 1970a; Perkins *et al*., 1980 and Bernard, 1989).

Data from clam samples collected in Italian offshore production areas suggest higher faecal coliform accumulation factors in wedge shell (*Donax trunculus*)/razor shell (*Ensis siliqua*) than those in striped venus clam (*Chamelea gallina*) (Bonadonna *et al.* 1990).

Vasconcelos *et al*. (1968) studied the uptake and elimination of indicator bacteria by *C. gigas* and *T. philippinarum* in an estuarine environment and reported that *T. philippinarum* consistently accumulated coliform and faecal coliform bacteria to a greater extent than *C. gigas*.

Lart and Hudson (Seafish 1993) reported that the ratio of *E. coli* counts varied between species of shellfish and that this variation could change according to season. Whilst noting that differences can occur they did not, however, establish any accumulation rankings. Berry and Younger (2009) attempted to address this issue using paired t-tests to compare data between species and applying a cut-off significance of 0.05 for each paired species. They used data from the England and Wales statutory monitoring programme and proposed a tentative ordering in terms of *E. coli* accumulation as follows: *(Cerastoderma edule, Tapes philippinarum, Mytilus spp.) > (Ostrea edulis, Crassostrea gigas) > Mercenaria mercenaria. Mytilus spp.* included blue mussel *(Mytilus edulis)*, Mediterranean mussel *(Mytilus galloprovincialis)* and hybrids.

Younger and Reese (2013, in press), expanding on preliminary work done by Younger and Berry (2009), undertook a study of routine monitoring data using the well-established statistical method, the Bland-Altman method comparison. The objective was to determine the ranking of species bioaccumulation of faecal indicators, the impact of different water quality levels on this ranking, and the potential to adopt indicator shellfish species for routine monitoring.

Whilst accumulation characteristics were found to vary to some extent across the range of contamination levels experienced they, nevertheless, reported an accumulation ordering as follows: *C. edule*, *T. philippinarum* and *Mytilus* spp. are broadly equivalent each showing a greater level of accumulation than the oysters *C. gigas* and *O. edulis* which both accumulate to a similar extent. Finally, *O. edulis* shows a greater level of accumulation than *M. mercenaria*. The authors conclude that the use of *Mytilus* spp. may alone provide an adequate index of faecal pollution impacting the growing areas in England and Wales.

In a similar study, Amouroux and Soudant (2011) reviewed three pairs of species using data from the French official classification monitoring programme (REMI) collected between 1989 and 2010. They reported that the following pairs showed no significant differences in their level of microbiological contamination.

Cerastoderma edule/ Tapes spp. ; Tapes spp./ Mytilus spp. ; Mytilus spp / Crassostrea gigas.

For four pairs of shellfish species, significant differences in levels of contamination are highlighted:

Cerastoderma edule / Mytilus spp. ; Cerastoderma edule / Crassostrea gigas ; Tapes spp. ;
Crassostrea gigas, Mytilus spp. /Patella vulgate
In terms of accumulation differences they reported the following:

- *C. edule* is about 2.5 times higher than *Mytilus* spp.
- *C. edule* is about 3 times higher than *C. gigas*.
- *Tapes* spp. is about 4 times higher than *C. gigas*.
- *Mytilus* spp is about 2.5 times higher than *Patella vulgata*.

Lee and Silk (2013) undertook a field study involving three bivalve species (*Pecten maximus*, *Mytilus* spp. and *Crassostrea gigas*) to determine the extent to which *E. coli* accumulation in shellfish varied between sites, sampling occasions and shellfish species. Their post-ANOVA analysis showed that the accumulation of *E. coli* in *P. maximus* was significantly higher than in the other two species. *Mytilus* spp. and *C. gigas* showed similar levels of *E. coli* accumulation.

5.6 Possible reasons for interspecies differences in accumulation

One reason suggested for differences between species is different filter-feeding throughput volumes (Cabelli and Heffernan, 1970a; Kelly *et al*., 1960; Bernard, 1989 and Perkins et al., 1980) perhaps compounded by food availability or tidal state.

Another possibility is that this may reflect different ratios of digestive tract to soft body-mass (minus shell). A pilot study reported by Younger and Reese (2013) found consistent differences in this regard between *Mytilus* spp., *C. gigas* and *O. edulis.* Fifty samples of each species (from more than one site) analysed by the Cefas Weymouth laboratory revealed a statistically significant difference (one way ANOVA p=0.000) between Pacific and native oysters with a mean of 5.1% for Pacific oysters and 7% for natives. *Mytilus* spp. averaged nearer 8% (see Figure 3). *E. coli* measurements are made on homogenized whole animals whereas the digestive tract contains the bulk of contaminants (Metcalf *et al*. 1980; Romalde *et al*. 1994). In this way the same density of bacteria on the gut lining would lead to different observed concentrations per 100g of flesh. These factors may interact to explain the water *E. coli* concentration dependent relationships observed, for example, between *C. gigas* and *C. edule* where results are similar at lower water *E. coli* levels but up to ten times different at higher levels (Younger and Reese 2013). This pattern follows the

order observed by these authors in *E. coli* concentration between the oysters (both species) and mussels. However, it shows a significant difference in body mass proportion between the two oyster species themselves which was not borne out by the *E. coli* accumulation assessment where no significant difference overall was found between the two species. The authors report, however, that this was effectively a snapshot study which required further follow up to determine whether there are any site-specific or seasonal effects.

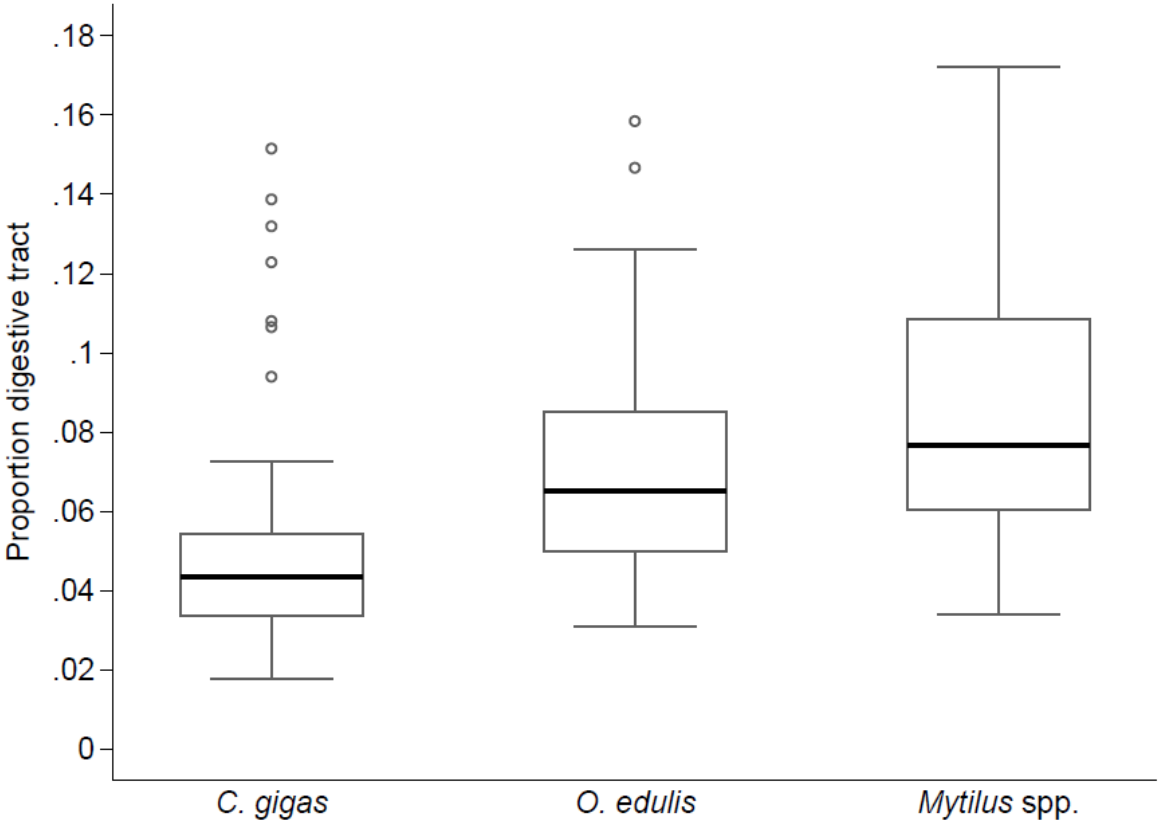

Figure 3. Boxplot showing the difference in proportion of body mass as digestive gland between Pacific oysters, native oysters and mussels.

A further factor for consideration is that the proportion of gut may change as the animal ages (Martinez & Oliveira, 2010).

Other potential reasons include differences in adsorption to the gut wall; differential digestion (mentioned earlier in the text) and different salinities within the gut due to environmental conditions exerting an effect.

5.7 Differences in virus accumulation between bivalve species

There are relatively few studies in the literature which investigate virus accumulation between different shellfish species. The following studies, however, represent the most relevant findings:

Croci *et al.* (2007) undertook an assessment of human enteric viruses in shellfish from the northern Adriatic Sea. 235 shellfish samples of various species (*T. philippinarum*, *Mytilus galloprovincialis*, *Ostrea* spp. and *Chlamys* spp.) were obtained from a number of sites and were tested for different strains of Hepatitis A virus and Norovirus. *T. philippinarum* was the species that was found to be most often contaminated. In addition, it was found to be the only species in which the legal limit for *E. coli* was occasionally exceeded after depuration. Values reported are presented in Table 5 below.

Table 5 Incidence of human enteric viruses - adapted from Croci et al (2007)

Species	Samples tested	%*E.coli*>230/ 100g	HAV	NoV	HAV and NoV
T. philippinarum	146	12	7	27	4
M. galloprovincialis	53	0	4	5	0
Chlamys spp	28	0	7	0	0
Ostrea spp.	8	0	0	0	0

Romalde *et al.* (2002) investigated the prevalence of enterovirus (EV) and Hepatitis A virus (HAV) in bivalve molluscs from a number of areas across Galicia, Spain. Shellfish samples included raft-cultured and wild mussels, as well as wild clams and cockles. The authors report that bacterial counts showed that the majority of samples (40.8%) could be classified as moderately polluted according to EU standards. However, differences in bacterial contamination were observed between cultured mussel and wild shellfish. In terms of virus results, it was found that numbers of samples positive for HAV were similar regardless of the species of bivalve analysed. In contrast, however, the number of EV positive samples was higher in wild mussels, clams and cockles than for raft cultured mussels. The authors suggest that the difference between the two types of virus is due to the higher environmental resistance of HAV. EV is less able to reach the mussels rafts as these are further offshore and is inactivated before it is able to reach them. These authors note that the correlation between *E. coli* and the two viruses, HAV and EV, was poor except in the most consistently polluted area. As samples were taken from different locations in most cases, no direct comparisons can be made between the different bivalve species of relative virus accumulation levels.

Suffredini *et al.* (2012) investigated norovirus contamination in mussels, clams and oysters harvested in two class B harvesting areas of the delta of the Po River with the aim of choosing one species as an indicator. Thirty five shellfish samples were examined in each area for *E. coli* and norovirus (GI and GII). Norovirus contamination was found in 51.4% of samples. No significant differences were found between the results of the two harvesting areas and the three shellfish species. The authors report as follows: '*on the basis of the average C(t) values, the recovery rate (from 0.46 to 1.15%) and the distribution of positive results in the samplings, that mussels seem to be a suitable indicator species to monitor viral contamination in these areas'.* They argue that, although the Ct values were lower in the clams (indicating a higher level of contamination), the higher average recovery values in the mussels (0.8% in mussels compared with 0.23% in clams and 0.58% in oysters)

could be advantageous in reducing the potential for false negative results attributable to low recovery efficiency. In addition, they point out that mussels also showed the highest frequency of positive results (60.9%) – mussels were positive in 14 out of 15 sampling occasions where norovirus was detected. This is compared with figures of 45.8% for clams and 47.8% for oysters, although differences were reported as being not statistically significant.

Dore and Lees (1995) investigated the removal of *E. coli* and male-specific F+ bacteriophage during UV depuration for 48 h in oysters (*C. gigas*) and mussels (*Mytilus* spp.). These were contaminated by short-term (1 to 3 weeks) and long-term (more than 6 months) exposure to sewage in the marine environment. They reported that the time taken to reduce levels of *E. coli* by 90% was 6.5 h or less in all cases. However, the time needed to reduce levels of F+ bacteriophage by 90% was considerably longer: 47.3 and 41.3 h (after short- and long-term exposures, respectively) in mussels and 54.6 and 60.8 h (after short- and long-term exposures, respectively) in oysters. The slower clearance of virus from oysters compared with mussels in this instance raises a potential issue of concern, particularly in relation to monitoring after sewage spill events. No published data comparing these two species could be found to confirm whether other viruses such as norovirus and Hepatitis A virus might also take longer to clear from *C. gigas* than *Mytilus* spp.. However, this issue would need further consideration if *Mytilus* spp. were to be used as the indicator species to represent *C. gigas* to demonstrate virus clearance after such pollution episodes.

5.8 Data for other pathogens

Cryptosporidium

Gomez-Couso *et al*. (2003) reported that there was no statistically significant relationship between the presence of Cryptosporidium oocysts and the faecal coliform contamination detected in the samples in either the different species of mollusc or the month of sampling. The samples used in their study were sourced mainly from Galicia, Spain, with a smaller number from Italy, Portugal, UK and Ireland. Oocysts were reported as being present in 34.5% of samples from class A areas and 36.4% of samples from class B areas. No oocysts were found in the two samples that were taken from class C areas. The depuration process was reported as being ineffective in totally removing oocyst contamination. A total of 241 samples were examined, including *Mytilus galloprovincialis*, clams (*Tapes decussatus, T. philippinarum, Venerupis pullastra, Dosinia exoleta)*, *Ostrea edulis* and *Cerastoderma edule*. The study does not appear to compare Cryptosporidium content between species at the same site but does state that the highest degree of contamination was found in *Ostrea edulis* (54.8%), followed by *Mytilus galloprovincialis* (32.7%), clams (29.4%) and *Cerastoderma edule* (20.8%). This review focuses principally on finding the best shellfish indicator species on the basis of *E. coli* accumulation. Consequently, given the findings of Gomez-Couso *et al* (2013), it would appear that the outcome of this review would have no relevance for the assessment of Cryptosporidium contamination in shellfish. It would also imply that classification on the basis of *E. coli* will provide no assessment of risk from *Cryptosporidium*.

Vibrios

McGhee *et al*. (2008) investigated rates of bioaccumulation, depuration, and post-harvest decay of *E. coli* and *Vibrio* spp. between two species of oyster *Crassostrea virginica* and *C. ariakensis*. They report that uptake rates of *E. coli* in C. ariakensis were significantly lower than those for *C. virginica*. Depuration of *E. coli* was found to be variable between the two species and post-harvest decay rates of *E. coli* for *C. ariakensis* were significantly lower than in *C. virginica*. Various authors point out that *E. coli* is not a reliable indicator of *Vibrio* spp. contamination in shellfish (e.g. Lhafi and Kuhne 2007). Consequently, the findings of the current review would not appear to be relevant for any vibrio contamination assessment.

5.9 Effect of shellfish disease on uptake

The agreed search terms for this literature review did not return any studies investigating the effect of shellfish disease on *E. coli* accumulation in bivalve species.

5.10 Effect of chemical contaminants on uptake

The agreed search terms for this literature review did not return any studies involving work on the comparative effect of chemicals on the accumulation of *E. coli* by bivalve shellfish. There were, however, a number of studies relating to the effects of chemicals on the bivalves themselves and these were as follows:

Canesi *et al*. (2005) investigated the effects of the brominated flame retardant tetrabromobiphenol-A (TBBPA) (often found in the environment) on cell signalling and function of *Mytilus* haemocytes (immune cells). TBBPA is reported as having high acute toxicity to aquatic organisms such as algae, molluscs, crustaceans and fish; however, little is known on the mechanisms of action of this compound in the cells of aquatic species. The results demonstrate that TBBPA in vitro activates the immune function of mussel haemocytes.

Cheng (1989) reported that in vivo exposure to certain heavy metals, as well as alterations in salinity and temperature, will compromise the internal defence mechanisms of molluscs although no interspecies comparison was investigated.

Colwell and Sayler (1977) looked at the effects and interactions of polychlorinated biphenyl (PCB) with estuarine micro-organisms and shellfish. They found that the accumulation and retention of salmonella organisms by oysters and soft-shell clams increased under conditions of PCB stress.

El-Shenawy (2004), studying *Tapes decussatus* found that there was a significant correlation between reduction of metal concentration in clam tissue and enhancement of valve movement, as well as activity and increasing respiration rate.

Hannam (2010) investigating the effect of PAH exposure in scallops (*Pecten maximus*) reported an immunosuppressive effect of phenanthrene. The overall level of phagocytosis and cytotoxic capability following the LPS challenge was found to be

lower in phenanthrene exposed scallops. The author reports that this may have consequences for disease resistance in this commercially-exploited species.

Given the above, it is clear that chemical contaminants may have an adverse effect on bivalves and it may be reasonable to assume that any effect might differ between species. This in turn may impact upon the *E. coli* (and pathogen) uptake characteristics of each bivalve species differentially. Consequently, any indicator species approach identified in this study may not be relevant where chemical contaminant levels in the environment are at a level that they affect the biological functioning of the shellfish.

6. Critical evaluation and ranking of most relevant *E. coli* accumulation studies

As part of the evaluation process for the current critical review, references were sifted to determine the most relevant studies to be further investigated. The sift criteria for this process were as follows:

- Comparison of at least two species.
- The relevance of the species to the UK monitoring programmes.
- The relevance of the environmental conditions, and/or tank variables studied, to the UK monitoring programmes.

The current species classified in the UK are:

C. gigas, O. edulis, Mytilus spp.*, C. edule, Tapes philippinarum, Tapes decussatus, Spisula solida, Mya arenaria, Pecten maximus* and *Ensis* spp.

After undergoing this initial sift process, the most relevant studies that were identified were as follows (in alphabetical order):

- Amouroux, I., Soudant, D. (2011). Comparison of microbiological contamination level between different species of shellfish.

- Berry, R., Younger, A. (2009). Interspecies comparison of *E. coli* accumulation in bivalve shellfish using data obtained from official control monitoring under EU Regulation 854/2004.

- Beucher, M. (1993). Etude de l'accumulation, de la retention et du relargage de bacteries enteriques par l'huitre *Crassostrea gigas*.

- Kershaw S, Campos C, Reese A, Mitchard N, Kay D, Wyer M. (2012). Impact of chronic microbial pollution on shellfish.

- Lee R. J. and R. Silk (2013). Sources of variation of *Escherichia coli* concentrations in bivalve molluscs.

- Solic M., S. Jozic and N. Krstulovic (2010). Interactive Effects Of Temperature And Salinity On The Rate Of Concentration Of *Escherichia coli* In Mussels (*Mytilus galloprovincialis*) and oysters (*Ostrea edulis*).

- Vasconcelos G. J., W. Jakubowski and T. H. Ericksen (1968). Bacteriological changes in shellfish maintained in an estuarine environment.

- Younger, A.D and Reese, R.A. (2013 - In press). *Escherichia coli* levels compared between bivalve mollusc species across harvesting sites in England and Wales.

These studies are summarised in tabular form at Appendix Table 1. The most relevant virus studies are summarised at Appendix Table 2.

A scoring system was then devised to enable these studies to be ranked in terms of the strength of their findings. All studies analysed bacterial counts on the log scale, so assumed an underlying lognormal distribution that might be truncated at lower or upper limits of detection/quantification. As a general comment, the scientific procedures were much better performed than the statistical design and analysis in most cases, and the results appear robust despite any reservations about the analyses.

Scoring was based on the following criteria (poor/no = 0, acceptable = 1, excellent/yes = 2)

- The use of natural contamination (i.e. sewage) preferred over laboratory *E. coli* reference cultures in any experimental procedures.
- Standard reference MPN method (or validated equivalent) preferred over other alternative methodologies
- *E. coli* enumeration preferred over faecal coliforms
- Continuous dosing better than batch dosing
- Statistical methodology appropriate and supports findings of the study?
- Is the number of sampling occasions sufficient to remove any bias in the data?

Table 6 below summarises the outcomes of the scoring process.

Table 6. Summary of scoring outcomes

Study	The use of natural contamination (sewage?) & route of exposure	Standard reference MPN method?	*E. coli* enumeration?	Continuous dosing?	Statistical methodology relevant and supports findings?	Number of sampling occasions	Total score
Amouroux & Soudant (2010)	Yes(2) - natural	Yes(2)	Yes(2)	Yes(2)	Chi-squated & Hodges-Lehman non-parametric comparison of median (2)	1000+ min 30 prs(2)	**12**
Beucher (1993)	Yes(2) - microcosm	No other MPN (1)	No faecal coliform(1)	No(0)	Regression without interpretation (1)	5 occasions (test times) (1)	**6**
Berry & Younger (2009)	Yes(2) - natural	Yes(2)	Yes(2)	Yes(2)	t-tests without adjustment for multiple testing (1)	1000+ min 10 prs(2)	**11**
Kershaw *et al* (2012)	Yes(2) - microcosm	Yes(2)	Yes(2)	Yes(2)	Tobit regression relating flesh to water levels (2) Species ordering is a side-effect (1)	144 shellfish, 396 water (2)	**11/12**
Lee & Silk (2013)	Yes(2) – field exposure in bags	Yes(2)	Yes(2)	Yes(2)	Anova but did not treat duplicate samples correctly. Hence flawed by pseudoreplication but results do appear robust (1)	8 occasions, tested in duplicate (1)	**10**
Solic (2010)	No - pure culture E.coli(0) - microcosm	Yes(2)	Yes(2)	No(0)	Anova – over-interpreted but main effects robust (1)	12 occasions, tested in triplicate (2)	**7**
Vasconcelos (1968)	Yes(2) – field exposure in baskets	APHA MPN(1)	No faecal coliform(1)	Yes(2)	Duplicate results used as replicates, so pseudoreplication. Computed (log)means plotted but no significance tests. Qualitative results consistent with data and other studies (1)	8 experiments over 1 year samples, tested in duplicate (1)	**8**
Younger & Reese (2013)	Yes(2) - natural	Yes(2)	Yes(2)	Yes(2)	Bland-Altman which directly addresses the question of comparison of paired random variables. Allows for relationship to vary with level of contamination. Results adjusted for multiple comparisons(2)	1000+ min 10 prs (2)	**12**

Below is a more detailed summary of each study with key findings. In addition, each study is evaluated against the scoring criteria listed above.

6.1 Amouroux and Soudant (2011) reviewed three pairs of species using data from the French official classification monitoring programme (REMI) collected between 1989 and 2010. A total of 1,525 pairs of samples were extracted. Sample points

covered 84 locations, mainly in Brittany, France. In order to ensure statistical significance of results, only pairs with more than 30 results were used. They reported that the following pairs showed no significant differences in their level of microbiological contamination.

Cerastoderma edule/ Tapes spp.*, Tapes* spp.*/ Mytilus* spp.*, Mytilus* spp*. / Crassostrea gigas*.

For four pairs of shellfish species, significant differences in levels of contamination are highlighted:

Cerastoderma edule / Mytilus spp.*, Cerastoderma edule / Crassostrea gigas, Tapes* spp*./Crassostrea gigas, Mytilus* spp. */Patella vulgata*

Two types of statistical approach were employed depending on the limit of quantification (LOQ). For non-quantifiable data, i.e. data below the LOQ of the method used, the data pairs of species showing significant differences were examined. Previous results are confirmed by contingency tables which indicate for example that for 593 results "< LOQ" in *Crassostrea gigas*, results in *Tapes* spp. were quantified. For 348 results, both were "< LOQ" and 72 indicate a result "<LOQ" for *Tapes* spp. while results had been quantified for *Crassostrea gigas*. Data above the LOQ were compared using orthogonal regression so that the two species were treated symmetrically.

In terms of accumulation differences they report the following:

- *C. edule* is about 2.5 times higher than *Mytilus* spp.
- *C. edule* is about 3 times higher than *C. gigas*.
- *Tapes* spp. is about 4 times higher than *C. gigas*.
- *Mytilus* spp is about 2.5 times higher than *Patella vulgata*.

The authors found there to be a significant difference in the microbiological contamination level between taxa. They add that this difference does not allow for modelling of microbiological contamination, but identifies species that can be considered as sentinel indicators for other species. They suggest these results confirm the existence of groups of shellfish. *C. edule* is a sentinel species for group 2 (burrowing bivalves), and either *Mytilus* spp. or *C. gigas* can be used to represent group 3 (non burrowing bivalves). *C. edule* can be used as indicator for all commercial species present in the area (*Mytilus* spp, *C. gigas*, and *Tapes* spp).

Study evaluation: As data were obtained from the statutory classification monitoring programme it would be expected to be subject to a high degree of control and standardisation in terms of sampling and analytical procedures. Contamination would be from natural animal and human sources. Whilst such a programme is not intended specifically to compare interspecies differences in accumulation, the data gathered can nevertheless be considered representative and directly relevant to the purpose of this study i.e. use of an indicator species in a statutory monitoring

context. The species are relevant to the UK situation and the environmental conditions are also relevant given that these samples were from northern France. It should be noted, however, that growing practices in France are sometimes different from those used in England and Wales. For example, as a farming method Mytilus spp*. are often grown in France on vertical wooden poles ('Bouchots'), whereas this technique is not used in England and Wales. Mussels at different heights on the pole might be subjected to different sources and/or degrees of contamination in the water column.*

In summary, this would appear to be a robust study of high relevance to the UK situation.

6.2 Berry and Younger (2009) used paired t-tests to compare data between species, applying a cut-off significance of 0.05 for each paired species. They used data from the England and Wales statutory monitoring programme to demonstrate that different bivalve species grown at the same site may differ in the levels to which they accumulate faecal contamination, measured as *E. coli* content, to the extent that they may fall into different classification categories. The *E. coli* counts were logged; hence the t-tests compared geometric means. The study proposed a tentative ordering in terms of *E. coli* accumulation as follows: *(Cerastoderma edule, Tapes philippinarum, Mytilus spp.) > (Ostrea edulis, Crassostrea gigas) > Mercenaria mercenaria. Mytilus spp.* included blue mussel *(Mytilus edulis)*, Mediterranean mussel *(Mytilus galloprovincialis)* and hybrids. The most consistent difference of *E. coli* accumulation was found between *Mytilus spp.* and *C. gigas*. Average *E. coli* accumulation in *Mytilus* spp. varied across the pairings tested from 1.4 to 3.4 times greater than in *C. gigas*.

Study evaluation: As data were obtained from the statutory classification monitoring programme it would be expected to be subject to a high degree of control and standardisation in terms of sampling and analytical procedures. Contamination would be from natural animal and human sources. Whilst such a programme is not intended specifically to compare interspecies differences in accumulation, the data gathered can nevertheless (as per Amouroux and Soudant) be considered representative and directly relevant to the purpose of this study. The species and the environmental conditions are relevant to the UK situation. The statistical analysis using t-tests would test only for a difference in (log)mean and would be affected by not adjusting for multiple tests (eg by Bonferroni) and by not adjusting for LOQ values.

In summary, this would appear to be a robust study of high relevance to the UK situation.

6.3 Beucher (1993) studied the influence of the levels of faecal coliforms and seston in the overlying water and season on the accumulation and clearance of this indicator in Pacific oysters (*C. gigas*). Oysters were maintained in tanks containing seawater (capacity 6,000 litres) and to which 1,000 litres of effluent were dosed. The author reports that flesh contamination reached the "equilibrium" stage within 20h of active filtration. The time required to reach this "equilibrium" was reported to be strongly influenced by season and varied between individuals of the same species. Similar individual variation was detected during the clearance phase. *C. edule* was

reported as being the most contaminated species during both the accumulation and clearance phases of the experiment. Oysters were the least contaminated groups of species. The most active species were *C. edule* and *Mytilus* spp. and the less active species were *Venerupis* sp. and *O. edulis*. Figure 4 below summarises the findings of their study

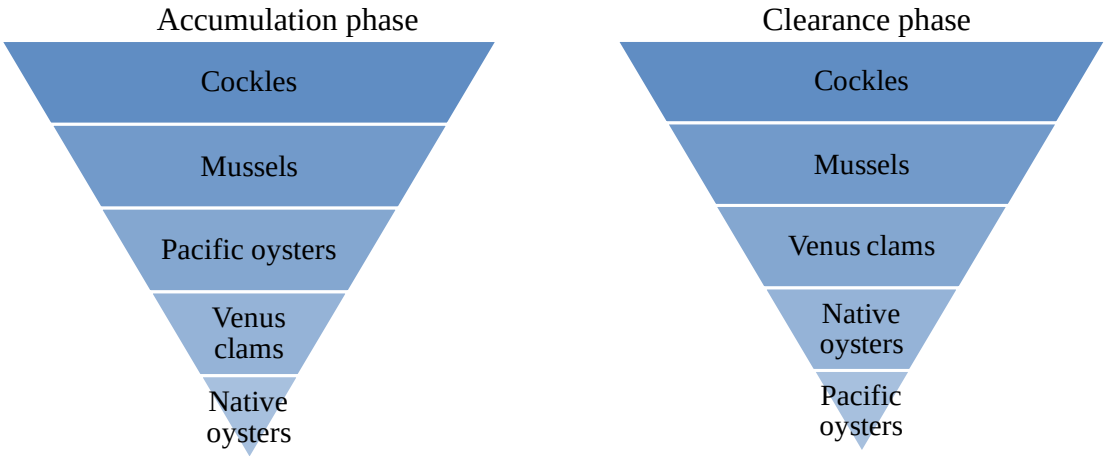

Figure 4. Inter-species patterns of faecal coliform contamination during accumulation and clearance phases obtained by Beucher (1993).

Study evaluation: This is a microcosm study comparing accumulation between species over a 24 hour period. A natural source of faecal contamination (sewage) is used but this is applied as a single dose. MPN is not standard and faecal coliforms are the determinand of choice. Beucher uses straightforward statistical methods over a longer period (24 hours) than Solic (3 hours) so has more time points (hourly intervals). Beucher notes that the accumulation is faster in the first hour. He fits linear regression after the 1st hour and tests with R^2 which is standard but not sufficient. Beucher does not test for nonlinearity (e.g. with quadratic term) and does not consider using a logistic curve to get S shape growth. The study reports very high R^2 values which is unusual with natural data. The statistical methodology is basic and the models are not interpreted.

In summary, the statistical analysis is weak and the findings, though believable, should be viewed as more qualitative than quantitative.

6.4 Kershaw et al. (2012) investigated the dynamics of *E. coli* accumulation, retention and clearance in *Mytilus* spp., *C. gigas* and *C. edule* in a series of microcosm experiments using a target temperature of 10.5°C and salinity of 30‰.

To simulate shellfish exposure to prolonged or 'chronic' microbiological pollution, six flow-through tank seawater microcosms were established in which shellfish were exposed to six different target concentrations of *E. coli* in seawater ranging from 1 to 330 cfu/100ml. Levels of *E. coli* in sewage, tank water and shellfish flesh were measured prior to, during and following exposure.

Linear regression models of *E. coli* levels in shellfish versus water showed that 52% of the variance observed for *E. coli* levels in mussels and Pacific oysters and 60% of

the variance in *E. coli* levels in cockles could be explained by the variation of *E. coli* levels in the water.

On exposure to sewage, shellfish demonstrated relatively rapid accumulation of *E. coli* up to a peak 'equilibrium' state in each tank. Following the end of dosing, a relatively rapid clearance phase was observed. Over the range of concentrations studied, maximum levels accumulated in shellfish during the exposure phase were found to be proportional to the level of water contamination. Overall, *C. edule* accumulated *E. coli* to a higher level than *Mytilus* spp. and *C. gigas*. Mean accumulation factors calculated as the geometric mean indicator concentration of the organism in shellfish divided by the corresponding geometric mean concentration in the overlying water were reported as being 387 for *C. edule*, 19 for *Mytilus* spp. and 14 for *C. gigas*. At the end of eight days sewage exposure, *Mytilus* spp. and *C. gigas* were found to be more efficient at clearing *E. coli*. *C. edule* was found to be less efficient, particularly after being exposed to more contaminated water.

Investigations were carried out in the field to verify whether the results returned in the laboratory studies could be confirmed in the natural environment. Levels of *E. coli* were monitored in shellfish collected from netlon bags laid in the intertidal zone in Swansea Bay, Wales and in adjacent water samples.

The authors report that the relative ordering of inter-species *E. coli* accumulation was consistent with that obtained in the microcosm studies. However, *E. coli* levels in water samples were not significantly correlated with the measured *E. coli* levels in shellfish flesh.

Study evaluation: This was a microcosm subject to a high degree of control and standardisation in terms of temperature and salinity. Sampling and analytical procedures were in accordance with standard reference procedures. Contamination was continuous and from a natural human source (sewage). Levels of contamination were generally, however, quite low and therefore not representative of the full range of water quality conditions likely to be experienced in harvesting areas across the UK. The species and the environmental conditions are relevant to the UK situation.

In summary, this would appear to be a robust study of relevance to the UK situation.

6.5 Lee and Silk (2013) undertook a field study to determine the extent to which *E. coli* concentrations varied between sites, sampling occasions and three species of bivalve mollusc (*Crassostrea gigas, Mytilus* spp. and *Pecten maximus*). The three species were co-located in each of three geographically separate commercial shellfisheries across England and Wales. Samples were taken in duplicate at monthly intervals over an eight month period. The authors reported that Analysis of variance (ANOVA) showed that the effects of site, sampling occasion, species and site/sampling occasion interaction were all significant. The proportion of variation due to site, however, was markedly greater than that due to the other factors. In terms of a comparison between species of *E. coli* accumulation, Post-ANOVA analysis showed that the concentration of *E. coli* in *P. maximus* was significantly higher than in *Mytilus* spp. and *C. gigas* which showed similar levels of *E. coli*.

Study evaluation: This was a limited study in terms of the number of sampling occasions and environmental conditions encountered. It was, however, subject to a high degree of control and standardisation in terms of sampling and analytical procedures. Contamination was from natural animal and human sources. The species and the environmental conditions are relevant to the UK situation.

In summary, this would appear to be a robust study of relevance to the UK situation.

6.6 Solic et al. (2010) investigated the effect of temperature and salinity on the rate of concentration of *E. coli* in mussels (*Mytilus galloprovincialis*) and oysters (*Ostrea edulis*). Their experiments were carried out in Split, Croatia in 3000 L tanks and were performed at temperatures and salinities reported to be within the normal range experienced by shellfish in this part of the Adriatic Sea (temperature: 12, 18 and 24°C; salinity: 37‰ and 32‰). Similarly, *E. coli* concentrations used were also reported as being within the locally typical range. The authors report that the rate of *E. coli* concentration in mussels and oysters depended on the concentration of *E. coli* in the surrounding seawater. Furthermore, the rate of filtration of seawater was found to be strongly influenced by temperature and salinity. The rate of *E. coli* concentration was found to be significantly higher in mussels than in oysters. In mussels, variations in salinity had more effect than variations in temperature in bringing about changes of the concentration rate, whereas in oysters the result was found to be reversed. Finally, temperature was found to have a more marked effect in changing concentration rate at a salinity of 37‰ than at 32‰, whereas in oysters the opposite was found to be the case.

Study evaluation

This is a microcosm study that uses a batch dosing technique of pure culture E. coli over a relatively short time period of up to three hours, sampling at four time points. From this point of view the study is less useful than others in terms of replicating the conditions experienced by shellfish in the natural environment. Conclusions therefore need to be viewed with caution. Methodology used is reference MPN and experimental design in terms of parameter control and number of samples taken appears to be satisfactory. In terms of statistical analysis, the choice of response variable is, however, not clear and an unwarranted formula is used to estimate a parameter which is then used as a response. Interaction terms over-interpreted in ANOVA and regression results.

In summary, the general comparison between M. galloprovincialis and O. edulis appears justified (M. galloprovincialis shows greater accumulation), but claims for interaction and specific effects are not demonstrated as robust, and no "significance level" is claimed.

6.7 Vasconcelos et al. (1968) studied the accumulation and elimination of indicator bacteria by *C gigas* and *T. philippinarum* in an estuarine environment. Both species were found to respond rapidly to changes in the bacteriological quality of the water, but clams were found to consistently accumulate coliform and faecal coliforms to a greater extent than oysters. The authors report that accumulation ratios were greater in this field study than in previous microcosm studies, however, the lab

studies used pure cultures of *E. coli* rather than a natural source of contamination. Degree of accumulation was also reported to be higher in the summer compared with the winter months.

Study evaluation

This is an environmental study carried out in Washington, USA that uses naturally contaminated shellfish. Methodology used is MPN but not the EU reference method and the determinand is faecal coliforms rather than E. coli. Experimental design in terms of sampling procedures and number of samples taken appears to be good. On a note of reservation, however, both Tapes philippinarum and C. gigas were held in the experimental floats in baskets. Experience in the UK programme would suggest that C. gigas might cope better under such conditions than T. philippinarum which are natural burrowers. The behavioural response of T. philippinarum under these conditions might therefore be atypical. The acclimatisation period from harvesting (nearby) and suspension in the apparatus was only 24 hours and this may not be sufficient time for recovery of the shellfish. Relocated bivalves used for ongoing monitoring purposes are left in situ for 2 weeks typically under the classification programme in England and Wales. Salinity range was relevant and temperatures generally representative of the UK situation (although seawater temperatures of up to 23°C we reported in July/August which is higher than that typically recorded in the UK). Statistical analysis is basic and descriptive, without significance tests of differences. The qualitative ordering of (log)means is consistent across the experiments and consistent with other studies.

In summary, whilst there are some issues with the strength of this study in terms of relevance to the UK the findings, nevertheless, concur with those of the other 'sifted' studies.

6.8 Younger and Reese (2013) expanding on work done by Younger and Berry (2009), used a well-established statistical method (Bland-Altman method comparison) on the same routine monitoring data. The objective was to determine the ranking of species bioaccumulation of faecal indicators, the impact of different water quality levels on this ranking, and the potential to adopt indicator shellfish species for routine monitoring. They recognised that each shellfish species will have an optimum temperature and salinity range for peak accumulation rates. Under conditions where any chosen indicator species stops pumping and the others continue would be of greatest concern.

Data were selected for analysis where possible from class A, B and C sites that: a) contained two or more separately-monitored species and b) shared at least ten results sampled on the same day. The data did not record exactly how close samples for different species were collected, and we have to assume they were Sites meeting these criteria covered six species: *C. edule, O. edulis, M. mercenaria, T. philippinarum, C. gigas* and *Mytilus* spp.. The selected data (from the statutory monthly monitoring programme) had been collected between 1991 and 2009 from 46 sites across England and Wales. Analysis of covariance was used to test whether data from different sites could be justifiably combined, or if the inter-species relationship varied between sites.

Bland-Altman has the advantage of making a graphical comparison across the range of values, and can detect a varying relationship where other methods compare single parameters. Whilst accumulation characteristics were sometimes found to vary to some extent across the range of contamination levels experienced, nevertheless, an accumulation ordering was established as follows: *C. edule*, *T. philippinarum* and *Mytilus* spp. are broadly equivalent, and each shows a greater level of accumulation than the oysters *C. gigas* and *O. edulis* which both accumulate to a similar extent. Finally, *O. edulis* shows a greater level of accumulation than *M. mercenaria*. See Table 7 for detailed study findings. The authors conclude that the use of *Mytilus* spp. may alone provide an adequate index of faecal pollution impacting the growing areas in England and Wales.

Table 7. Matrix comparing ratios of *E. coli* contamination in shellfish commercially harvested in England and Wales.

Shellfish type	*O. edulis*	*C. gigas*	*C. edule*	*T. philippinarum*	*M. mercenaria*
***Mytilus* spp.**	1.5 (n=596)	1 to 2 (n=1837)	= [.8] (n=113)	= [1.4] (n=64)	No data
O. edulis		=1 (n=227)	= [.2]* (n=11)	= [.5]* (n=11)	2.4 (n=153)
C. gigas			1 to 0.1 (n=145)	0.3-0.1 (n=253)	No data
C. edule				= [.9] (n=148)	No data
T. philippinarum					No data

*Adapted from Younger and Reese (2013). Note: The tabulated values are either the constant average ratio or the ratios at low and high ends of the range. *Data inconclusive due to low number of samples. Square brackets [ ] denote non-statistically significant ratio observed in study = denotes accumulation ratios are the same (i.e. no statistically significant difference)*

Younger and Reese suggest that *Mytilus* spp., *C. edule* and *T. philippinarum* are broadly equivalent for *E. coli* accumulation in that no statistically significant difference in accumulation was found between these particular species. In many situations, accessing natural stocks of *T. philippinarum* and *C. edule* requires the use of specialised dredges which is impractical and/or too expensive for local authority sampling officers to use routinely. From experience in the England and Wales statutory monitoring programme, deploying bags of these species from buoys is not feasible. They tend to die when held in bags for any length of time since this is not a natural environment for these burrowing species.

Mytilus spp., however, have been found to survive quite well for extended periods of time in bags. Use of bagged *Mytilus* spp. may be an attractive option when natural stocks cannot be sampled.

Although some observations from the England and Wales programme (unpublished data) suggest that *C. edule* and *T. philippinarum* can, on occasion, return more high-valued results than *Mytilus* spp. the results from this study suggest that this may be random sampling error. An alternative explanation is that *C. edule* and *T. philippinarum* might be able to tolerate a lower salinity than *Mytilus* spp. (as pollution rises after rainfall events) and therefore may continue to filter and accumulate whilst the *Mytilus* spp. may be less physiologically active. From the authors' experience in statutory depuration plant inspection work, however, this explanation would seem less likely. The salinity minima used in this context for each species (Cefas 2010) are very similar and the value for *Mytilus* spp. is actually the lowest (19‰ for *Mytilus* spp. compared with 20.5‰ and 20‰ for *C. edule* and *T. philippinarum* respectively). These values are derived from various studies carried out by Seafish (Seafish 1994). A precautionary approach would be to undertake further parallel sampling of these species alongside *Mytilus* spp. to determine whether the use of *Mytilus* spp. as an indicator shellfish species is appropriate on a site-specific basis.

Study evaluation: As data were obtained from the statutory classification monitoring programme it would be expected to be subject to a high degree of control and standardisation in terms of sampling and analytical procedures. Contamination would be from natural animal and human sources. Whilst such a programme is not intended specifically to compare interspecies differences in accumulation, the data gathered can nevertheless be considered representative and directly relevant to the purpose of this study. The species and the environmental conditions are relevant to the UK situation.

In summary, this would appear to be a robust study of high relevance to the UK situation.*

**The statistical methodology used was reviewed by two separate Cefas scientists unconnected with the original study and confirmed to be fit for purpose.*

General note on environmental studies

In environmental studies there can no control over temperature, salinity and other covariates. Species pairs in each comparison made will be subject to the same conditions, having come from the same location, and at least some comparisons will usually indicate that responses are not exactly parallel for each species. However, over a large number of samples from a number of sites on several occasions, environmental covariates would be expected to cover a wide range of conditions and hence even out most, if not all, potential bias towards any particular species.

General conclusions that can be drawn from the above studies are as follows:

- Filter-feeding molluscs react quite quickly to reach an equilibrium with a constant concentration of *E. coli* in the water
- Cleansing is a rather slower process
- Actual rates vary with temperature, salinity, *E. coli* concentration and species
- Other factors, as yet untested, could include season, size, maturity and sexual physiology of the animals, amounts of food particles and silt suspended

(relating *E. coli* burden to the solid matter rather than water volume), tidal factors and time spent out of water

- Results between species from field samples therefore show wide variation but with a definite species signal within the noise.

The concept of using indicator species seems robust, in that species X may be expected to accumulate at least as much as species Y in the same area for the same range of conditions.

7. Norovirus surveillance study assessment

The current critical review also included an assessment of data from the FSA funded report entitled 'Investigation into the prevalence, distribution and levels of norovirus titre in oyster harvesting areas in the UK.' (FSA 2012). This applied a standardised, quantitative norovirus detection method to provide monthly surveillance data on 39 oyster harvesting locations across the UK over a 2 year period. Samples were analysed for GI and GII norovirus and were also tested for *E. coli*. Norovirus was detected in 76.2% (643/844) of samples tested, with similar prevalence in the 2 species of oyster tested; 76.1% (468/615) for Pacific oysters (*Crassostrea gigas*) and 76.4% (175/229) for native oysters (*Ostrea edulis*). There was a marked seasonality with a positivity rate of 90% (379/421) for samples taken between October and March compared with 62.4% (264/423) for those taken between April and September. This study found a significant correlation between *E. coli* and norovirus levels on a production site basis rather than by sample. The findings support the use of *E. coli* as an indicator organism for classification purposes.

This review used data from the surveillance study and, as a first stage, matched it with data obtained from mussel (*Mytilus* spp.) and oyster (*O. edulis* and *C. gigas*) sampling (same day and same or closest site) under the statutory classification monitoring programme in England and Wales. Data was then assessed in two main ways: Geometric means of shellfish *E. coli* data (samples analysed by Cefas) were calculated for data obtained from surveillance study sites and data from adjacent oyster and mussel sites (analysed by local Health Protection Agency or Public Health Wales laboratories) under the classification monitoring programme. This allowed direct comparison of average levels of contamination between species. All data were then ranked and Spearman's Rank correlation coefficients calculated to allow comparison of these *E. coli* data alongside oyster norovirus GI, GII data.

Geometric mean results are summarised in Table 8 below:

Table 8. Geometric mean data by site

Study site	Geometric mean				Distance between surveillance site and nearest mussels (km)
	Surveillance data - Oysters	Statutory monitoring data			
		Mussels	*C. gigas*	*O. edulis*	
1	64	**152**	52		0
2	69	**84**		50	0
3	126	**269***		128	3.5
4	288	**574***	407		1
5	**86**	83*	63		0.6
6	87	**292***	97		0.5

7	697	**1278**	n/a		0
8	107	**496***	150		1.1
9	194	**253***	152		0.1
10	227	**241***	208		0.1
11	96	**197**	118		0
12	**360**	189*		232	3
13	**139**	127*	80		2

Results highlighted in red indicate the highest geometric mean for each site – 10 out of 13 were mussels. * Indicates where mussel site is not at the same location as the oyster site.

Percentage compliance figures for results are summarised in Table 9 below.

Table 9. Percentage compliance with 4600 *E.coli*/100g data by site

Study site	Percentage compliance with 4600 *E. coli*/100g				Distance between surveillance site and nearest mussels (km)
	Surveillance data - Oysters	Statutory monitoring data			
		Mussels	*C. gigas*	*O. edulis*	
1	100	**95.8**	100		0
2	100	**95.5**		90	0
3	100	**82.6***	100		3.5
4	100	**95.8***	100		1
5	100	**94.7***	100		0.6
6	100	**95.2***	100		0.5
7	94.1	**70.6**	n/a		0
8	100	**100***	**100**		1.1
9	95	**90***	100		0.1
10	100	**95.8***	100		0.1
11	100	**90.9**	100		0
12	94.4	**89.5***	94.7		3
13	100	**92.3***	100		2

Results highlighted in red indicate the lowest percentage compliance with the class B threshold of 4600 *E. coli*/100g for each site (or nearest site) – 12 out of 13 were *Mytilus* spp. At site 8, *Mytilus* spp. and *C. gigas* showed equal compliance.
* indicates where *Mytilus* spp. site is not at the same location as the oyster site.

7.1 Interpretation of geometric mean and percentage compliance figures

The geometric mean results effectively provide an indication of the general level of contamination but do not allow an assessment of legislative compliance (number of failures of a particular classification threshold), particularly if the data is 'peaky' in nature with intermittent high results set against a background of much lower results. The percentage compliance figure therefore provides confirmation of performance against the legislative limits and may be used in preference to the geometric mean value where legislative compliance is the key requirement i.e. in a statutory monitoring programme. Both figures combined provide the most useful information

on public health impact but it is possible to have a site showing better legislative compliance (higher percentage value) suggesting lower levels of contamination but a higher geometric mean value (indicating a higher general level of contamination).

Correlation coefficients: All data was ranked (i.e. in order of lowest to highest result) and a Spearman's Rank correlation coefficient calculated. Summary results are shown in Table 10 below (detailed outputs at Appendix):

Table 10. Spearman's Rank Correlation Coefficient outcomes by site

Site Number	Species	Norovirus GI	Norovirus GII
1	Cg mp	0.362 (0.082)	0.341 (0.103)
	Cg sv	0.390 (0.059)	0.558 (0.005)
	M mp	0.699 (0.000)	0.667 (0.000)
2	Oe mp	0.305 (0.167)	0.336 (0.126)
	Oe sv	0.404 (0.062)	0.374 (0.087)
	M mp	0.371 (0.090)	0.266 (0.231)
3	Oe mp	0.215 (0.323)	0.263 (0.225)
	Oe sv	0.340 (0.112)	0.428 (0.041)
	M mp	0.576 (0.004)	0.584 (0.003)
4	Cg mp	0.286 (0.175)	0.446 (0.029)
	Cg sv	0.449 (0.028)	0.621 (0.001)
	M mp	0.603 (0.002)	0.515 (0.010)
5	Cg mp	0.391 (0.098)	0.389 (0.099)
	Cg sv	0.340 (0.154)	0.542 (0.017)
	M mp	0.446 (0.056)	0.347 (0.145)
6	Cg mp	-0.006 (0.979)	-0.261 (0.253)
	Cg sv	0.108 (0.640)	-0.269 (0.238)
	M mp	0.395 (0.077)	-0.065 (0.778)
7	Cg mp	N/A	N/A
	Cg sv	-0.212 (0.415)	-0.008 (0.975)
	M mp	0.056 (0.831)	-0.006 (0.981)
8	Cg mp	0.337 (0.284)	0.294 (0.353)
	Cg sv	0.208 (0.516)	0.221 (0.491)
	M mp	0.558 (0.060)	0.333 (0.290)
9	Cg mp	-0.076 (0.749)	0.052 (0.828)
	Cg sv	-0.091 (0.702)	-0.016 (0.948)
	M mp	-0.220 (0.352)	0.077 (0.748)
10	Cg mp	0.214 (0.315)	0.222 (0.297)
	Cg sv	0.005 (0.981)	-0.059 (0.784)
	M mp	-0.238 (0.262)	-0.293 (0.165)
11	Cg mp	-0.152 (0.498)	-0.012 (0.957)
	Cg sv	-0.026 (0.908)	-0.089 (0.695)
	M mp	-0.658 (0.001)	-0.277 (0.213)
12	Oe mp	0.313 (0.192)	0.033 (0.894)
	Oe sv	0.154 (0.542)	0.176 (0.486)
	M mp	-0.210 (0.389)	-0.044 (0.859)
13	Cg mp	-0.149 (0.627)	0.139 (0.650)
	Cg sv	-0.361 (0.225)	-0.071 (0.818)
	M mp	-0.156 (0.610)	0.076 (0.805)

M mp = Mytilus spp. E.coli data from statutory monitoring programme
Cg mp = Crassostrea gigas E.coli data from statutory monitoring programme
Oe = Ostrea edulis E.coli data from statutory monitoring programme
Cg or Oe sv= Crassostrea gigas or Ostrea edulis E.coli data from surveillance study
GI = Norovirus genotype I data from surveillance study
GII = Norovirus genotype II data from surveillance study

N.B. Individual correlation assessments carried out on the same number of results taken on the same day over the same date range.

7.2 Interpretation of Spearman's Rank correlation coefficient outcomes

Focussing on the results for the sites where *Mytilus* and *C. gigas* were taken at the same location it would seem that there is a mixed picture in that at some locations, notably sites 1 and 3, mussel *E. coli* values correlate very well with those for norovirus but at others this is not the case. This, however, is very similar to the situation with respect to *C. gigas* where correlations are also variable.

7.3 Conclusions from surveillance study assessment

Essentially, according to the correlation coefficient outcomes, neither *Mytilus* or *C. gigas* would appear to be ideal in terms of representing norovirus risk on the basis of *E. coli* content on a year round basis. The surveillance report notes better correlation when seasonality is taken into account. However, as the scope of this review does not consider seasonality in terms of an indicator approach the analysis was not re-run to check for seasonal correlations. Geometric mean results are consistently higher and percentage compliance with the class B threshold of 4600 *E. coli* /100g consistently lower in *Mytilus* spp. than *C. gigas* at same-location sampling sites. This suggests *Mytilus* spp. offers an improved level of public health protection in terms of representing the microbial contamination risk and supports the use of *Mytilus* spp. as a protective indicator under a statutory monitoring programme.

8. Discussion

Accumulation of microorganisms by filter-feeding molluscs is a dynamic process affected by many variables (e.g. temperature, food availability, salinity, mollusc age, season, reproductive state, health of the molluscs and the impacts of toxins and other contaminants, etc). Studies carried out in a laboratory based environment can control or otherwise allow for the main variables for comparative studies, however, it is prohibitively costly to study the full possible range, interactions and impact of such multiple variables. Alternatively, the conditions for studies undertaken in the natural environment, although representative, may not be optimal for each species compared, and cannot be readily manipulated to explore the impact of each variable.

8.1 Potential problems in interpretation of the results of single dose contamination studies

A key problem with many tank-based laboratory studies is that they have employed a batch wise mode of contamination i.e. sewage or cultured microorganisms have been introduced as a single inoculum at the beginning of the experiment. In addition, many of these use laboratory adapted strains that do not survive well in seawater whereas there is evidence that *E. coli* strains originating from STWs exhibit greater environmental resistance. In batch wise inoculation trials the bacterial content of bivalves will increase through filter-feeding, reach a plateau, then decline as the food source becomes exhausted (depuration phase). Since different bivalve species filter at different rates in this dynamic process it is very hard to interpret the results of such batch studies unless full uptake and removal curves are given. In the example given in figure 5 below (using artificial data), samples of each species taken at the 9 hour point would lead to the conclusion that species 1 shows the higher level of accumulation. Samples taken at 12 hours, however, would give the opposite outcome. Overall in this example, species 2 achieves a higher level of accumulation albeit at a slower rate.

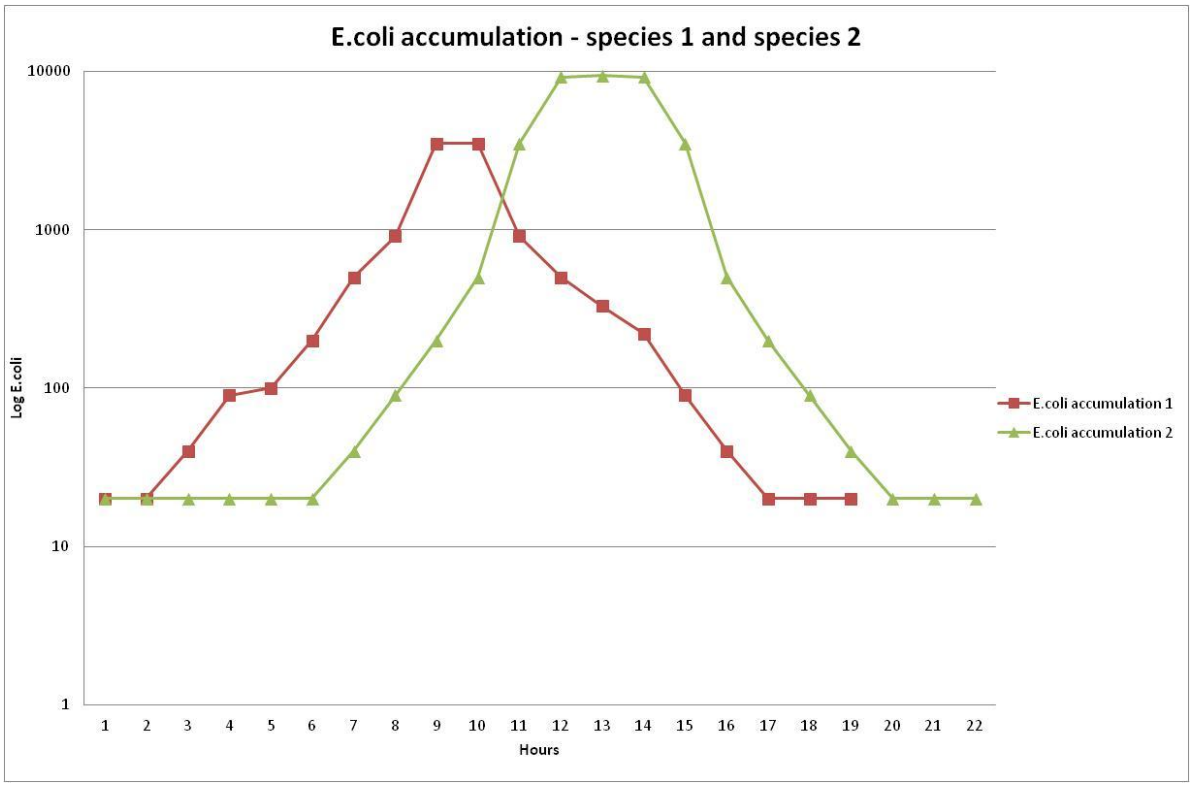

Figure 5. Example graph using artificial data showing difference in uptake and removal rates of *E. coli* between two species of bivalve.

It is important to note that the shape of the curves will change independently for each species according to temperature and salinity. Each species may have slightly different optimum range requirements for these two parameters. An alternative, preferable, approach for tank based studies is to utilize continuous contaminant dosing to achieve a steady state. Comparisons between species, with controlled variables, can then be undertaken with much more confidence. It is necessary to consider all of the above factors in considering the robustness and reliability of reported data in the literature (both published and unpublished).

There are two different aspects of accumulation relevant to the conclusions of this review. One is the accumulation factors obtained from different single species studies and the other is the comparison of accumulation by different species within single studies. In terms of the sifted studies the relevant details for these two aspects are summarised in the Tables 11 and 12 below:

Table 11. Summary table of studies comparing *E. coli* accumulation factors between bivalve species

Authors	The bivalve species studied	The experimental approach	The range of variables studied	The key findings presented (e.g. relative concentration of *E. coli* in different bivalve species, differences in uptake
Kershaw *et al.* (2012)	*Cerastoderma edule, Crassostrea gigas, Mytilus spp.*	Microcosm - Linear regression models to assess shellfish vs water relationship.	*E.coli* accumulation only experiments at 30‰ and 10.5°C	Mean accumulation factors 330 (*Cerastoderma edule*), 15.2 (*Mytilus spp.*) and 11.7 (*Crassostrea gigas*)

Table 12. Summary table of studies comparing *E. coli* accumulation ordering between bivalve species

Authors	The bivalve species studied	The experimental approach	The range of variables studied	The key findings presented (e.g. relative concentration of *E. coli* in different bivalve species, differences in uptake
Lee & Silk (2013)	*Crassostrea gigas, Mytilus* spp. and *Pecten maximus*	Field study - analysis of variance (ANOVA).	Effects of site, sampling occasion, species and site/sampling occasion interaction	*Pecten maximus*>(*Mytilus* spp = *Crassostrea gigas*)
Younger & Reese (2013)	*Cerastoderma edule, Crassostrea gigas, Mercenaria mercenaria, Mytilus spp., Ostrea edulis, Tapes philippinarum,*	Field study - review of statutory monitoring data - Bland-Altman method comparison	*E.coli* accumulation only across a range of conditions	*(Cerastoderma edule = Tapes philippinarum = Mytilus* spp.*) > (Crassostrea gigas = Ostrea edulis) Ostrea edulis = Mercenaria mercenaria*
Amoroux & Soudant (2011)	*Cerastoderma edule, Crassostrea gigas, Mytilus spp., Tapes spp.,Patella vulgata*	Field study - review of statutory monitoring data	*E.coli* accumulation only across a range of conditions	*Cerastoderma edule 2.5x > Mytilus* spp. *Cerastoderma edule 3x > Crassostrea gigas. Tapes* spp *4x > Crassostrea gigas*. *(Cerastoderma edule = Tapes* spp*), (Tapes* spp *= Mytilus* spp*), (Mytilus* spp *= Crassostrea gigas).*
Kershaw *et al.* (2012)	*Cerastoderma edule, Crassostrea gigas, Mytilus spp.*	Microcosm - Linear regression models to assess shellfish vs water relationship.	*E.coli* accumulation only Experiments at 30‰ and 10.5°C	*Cerastoderma edule> Mytilus spp.* and *Crassostrea gigas*
Solic *et al.* (2010)	*Mytilus galloprovincialis* and *Ostrea edulis*	Microcosm	Temperature and salinity on rate of concentration of *E.coli* (12, 18 and 24°C), (37 ‰ and 32 ‰).	*Mytilus galloprovincialis > Ostrea edulis*
Vasconcelos *et al.* (1968)	*Crassostrea gigas* and *Tapes japonica (aka T. philippinarum)*	Field study in estuarine environment	Faecal coliform accumulation only	*Tapes japonica (philippinarum) > Crassostrea gigas*
Berry & Younger (2009)	*Cerastoderma edule, Crassostrea gigas, Mercenaria mercenaria, Mytilus spp., Ostrea edulis,Tapes philippinarum,*	Field study - review of statutory monitoring data	*E.coli* accumulation only across a range of conditions	*(Cerastoderma edule = Tapes philippinarum = Mytilus* spp.*) > (Crassostrea gigas = Ostrea edulis) Ostrea edulis = Mercenaria mercenaria*
Beucher (1993)	*Cerastoderma edule, Crassostrea gigas, Mytilus spp., Ostrea edulis,Venerupis sp.*	Microcosm	Faecal coliform accumulation according to season (spring and summer)	*Cerastoderma edule > Mytilus* spp. *> Venerupis sp. > Crassostrea gigas > Ostrea edulis)*

The largest and most significant comparable published studies involving environmental data (also the highest ranking in this review) and comparing accumulation between species would appear to be those undertaken by Younger and Reese (2013) in the UK and Amouroux and Soudant (2011) in France. The data

derived from both Official Control monitoring programmes is subject to a high level of quality assurance. Two factors that it was not possible to address in the critical assessment process due to a lack of published information are as follows:

1. Were the sampling locations for the different species co-located (if not, were they within a reasonable distance from each other)?
2. Were the different species at a point sampled at more or less the same time?

For the Younger and Reese study we can be confident, at least in the majority of cases, that the above two questions may be answered in the affirmative as we have access to the raw data and experience of the programme itself which allow confirmation of this point. For the Amouroux and Soudant study, however, we only have access to the summary details provided on the published poster. Whilst these would suggest that the above two factors have been satisfactorily addressed, we have no additional information to confirm that this is the case. Conclusions derived in these authors' paper may therefore need to be viewed with some caution.

The statistical methodology used differed between the two studies but the findings were similar in many respects e.g. no significant differences were found between *C. edule* and *Tapes* spp.; *Tapes* spp. and *Mytilus* spp. In addition, significant differences were found between *C. edule* and *C. gigas* and *Tapes* spp. and *C. gigas*. However, a key difference between the two studies was in the observed relationship between *C. gigas* and *Mytilus* spp. Younger and Reese demonstrated a clear and consistent difference in accumulation factor between the two, whereas Amouroux and Soudant found them to be similar.

Other studies have reported findings that support both outcomes. Whilst ambient water quality or physiological properties may account for some of these differences, it is plausible that the method of growth may also be significant, i.e. shellfish grown in bags supported above the riverbed in the intertidal zone are subject to different contamination effects than those grown on the riverbed. Similarly, for shellfish grown on ropes or lantern nets, there may be a difference in contamination influence with depth in the water column (Younger *et al.*, 2003).

Amouroux and Soudant used data from sites across France where environmental conditions and growing practices are sometimes different from those used in England and Wales. For example, as a farming method *Mytilus* spp. are often grown in France on vertical wooden poles ('Bouchots') in the intertidal zone, whereas this technique is not used in the UK. Here, *Mytilus* spp. may be grown on ropes suspended from buoys or rafts in offshore or deep water areas as an alternative to the more popular mode of growth using trestles or the sea/river bed itself. *Mytilus* at different heights on the Bouchot pole in the intertidal zone might be subjected to different sources and/or degrees of contamination in the water column than *Mytilus* grown on the sea bed, trestles or ropes in deeper water areas.

Differences in the range of water quality encountered between the two studies may also be at least a partial explanation. Younger and Reese found that the difference in accumulation increased with the level of water contamination (also previously reported by Plusquellec *et al.*, 1990). France has more class A waters with lower levels of contamination and, consequently, would detect a lower degree of difference

between the two species. There is also a big difference in the numbers of paired samples: Younger and Reese had n=1837 for this pair and a mean ratio of 1.78, while Amouroux and Soudant had n=105 and a ratio of 1.3. The confidence interval from the Amouroux and Soudant data covers zero but is quite asymmetric [0.05-+0.259], so the result is in fact consistent with that of Younger and Reese but too low powered to show significance. It is also worth noting that Younger and Reese's "limits of agreement" also included equality, so there is a population difference but not necessarily on individual measurements.

Ambient water *E. coli* levels may also explain one apparent contradiction in the Amouroux and Soudant study in that accumulation in *C. edule* is reported to be >*Mytilus* spp., however, at the same time *C. edule* is reported to be = *Tapes* spp and *Tapes* spp.= *Mytilus* spp. On this basis it might be reasonable to expect that *Mytilus* = *C. edule* (as was found by Younger and Reese). Differences in water *E. coli* levels between the datasets for the pairings may perhaps be an underlying factor here.

Kershaw *et al*. (2012) also reported a significant difference in accumulation between *Mytilus* and *C. edule* with the latter accumulating to a much greater degree than *Mytilus* but, again, this study was carried out at relatively low ambient water *E. coli* concentrations (1-330cfu *E. coli*/100ml) Younger and Reese did report some reservations over the statistical findings in relationship between *Mytilus* and *C. edule (*and *Tapes spp).* Kershaw *et al.* (2012) reported that cockles accumulated *E. coli* to a higher level than *Mytilus* and *C. gigas*. The mean accumulation factors reported (330 (*C. edule*), 15.2 (*Mytilus*) and 11.7 (*C. gigas*)) suggest that mussels accumulate to a slightly higher degree than Pacific oysters. The very marginal nature of the difference between *Mytilus* and *C. gigas* may at least partially explain the contrary findings between studies reported above. The difference between *C. edule* and *Mytilus* is large and supports the findings of Amouroux and Soudant (although the difference noted in this study between the two species was less marked).

In agreement with the findings of Amouroux and Soudant but in contrast to the Younger and Reese (2013) and Berry and Younger (2009) study findings, Lee and Silk (2013) found, in a structured experimental field study, that *Mytilus* spp. and *C. gigas* effectively showed no significant difference in *E. coli* accumulation. The authors conclude on the basis of their findings that *Mytilus* could be used to represent *C. gigas (*as results are similar*)* but not *P. maximus.*

Vasconcelos *et al.* (1968) reported that *T. philippinarum* consistently accumulated coliform and faecal coliform bacteria to a greater extent than *C. gigas* and this is in accordance with the findings from other studies reported in this review.

Solic *et al.* (2010) reported that the rate of *E. coli* concentration was significantly higher in mussels *Mytilus galloprovincialis* than in the oysters *Ostrea edulis*. *Mytilus galloprovincialis* is a member of the *Mytilus* spp grouping used in England and Wales and this finding concurs with that of Younger and Reese (2013).

Beucher (1993) found that *C. edule* was the most contaminated species during both the accumulation and clearance phases of the experiment. *C. gigas* were the least contaminated groups of species. Again this finding concurs with that of Younger and Reese (2013).

In terms of virus studies the limited evidence available suggest that *C. edule>Mytilus* (Leguyader, 1993) and *T. philippinarum>Mytilus galloprovincialis* and *O. edulis* (Croci *et al.*, 2007). This supports the use of *C. edule* as an indicator. In contrast, Suffredini *et al.* (2012) investigating norovirus contamination in mussels, clams and oysters conclude that mussels seem to be a suitable indicator species to monitor viral contamination in these areas and could be used as an indicator species to provide adequate protective representation of oysters.

8.2 Investigative samples

On a note of caution, Dore and Lees (1995) studying the removal of viruses (F+ bacteriophage) from shellfish found that removal from *Mytilus* spp. was quicker than from *C. gigas*. This would suggest that the use of mussels as an indicator species to reflect the viral content of oysters after sewage spills (where clearance rate rather than accumulation is the feature of interest) may not provide a protective approach. No published viral data currently exist for cockles.

In addition, it is clear given other study findings noted in this review (e.g. Beucher, 1993) that there are differences in the clearance rate of *E. coli* and/or faecal coliforms between bivalve species. Furthermore, a ranking of species according to their clearance ability may differ from a ranking according to their capacity for accumulation. The use of an indicator species approach for investigative samples following pollution events (where confirmation of microbial clearance is key) would therefore need separate consideration.

8.3 Seasonal classifications

It should be noted that the study of seasonal differences between species is beyond the scope of this review. Patterns of uptake and clearance between species may be different during the course of the year, particularly when individuals may be seasonally weakened due to spawning activity. The indicator species approach may therefore require further consideration before being applied in a fishery showing a marked seasonal trend in contamination.

8.4 Spatial variation considerations

A geospatial study in France showed that faecal contamination varied significantly across a mussel farming area (Beliaeff & Cochard, 1995). The types and loads of pollution sources, the distance between these and the monitoring points, and the hydrography of the areas will all differ greatly between sites and will influence the average levels of *E. coli* seen in the bivalves. These spatial effects reinforce the need to consider production areas, and even separate parts of harvesting areas, separately with regard to monitoring and classification under European Union regulations (Anon 2004 a, b, Murray & Lee 2010). Any indicator approach would need to carefully consider the potential for spatial differences in contamination across a harvesting area to ensure that they were adequately addressed e.g. it would not be appropriate to use an indicator species if this was located further from the main sources of contamination than the species being represented.

9. Conclusions

In summary, the number of studies that this review has considered that are specific to the question of indicator species and their potential application is quite limited. Encouragingly, however, whilst there are some differences between findings of studies (field and microcosm), the significance and practical implications of these differences are comparatively minor. Furthermore, they would not, we would suggest, prevent an indicator species approach being taken in the UK. Instead of one species being used for all situations, two or more indicator species options could be available depending on the species scenario encountered.

This study did not reveal any data on indicator species approaches from countries outside of Europe.

The findings of the main studies were similar in many respects:

- All the data presented in this review suggest that the *E. coli* accumulation capacity of *C. edule* and *Mytilus* spp. either exceeds or is equivalent to *C. gigas, O. edulis, Tapes spp.* and *M. mercenaria*.
- Accumulation capacity of *C. edule* is similar to (or greater than) *Mytilus* spp.
- No significant differences were found between *C. edule* and *Tapes* spp.; *Tapes* spp. and *Mytilus* spp.
- Significant differences were found between *C. edule* and *C. gigas*; *Tapes* spp. and *C. gigas*.
- Some differences were found between studies in the relationship between *C. gigas* and *Mytilus* spp. The outcomes of all studies would, however, suggest that *Mytilus* spp. shows at least a similar (if not greater) degree of accumulation than *C. gigas*.
- Additionally, *O. edulis* and *C. gigas* show similar degree of accumulation and may be used to represent each other. Both show a higher degree of accumualtion than *M. mercenaria.*
- An indicator approach cannot be recommended at this stage for representation of *C. edule*, *Spisula solida, Mya arenaria* and *Ensis* spp. as either contradictory or no supporting data from the literature is available.
- Various authors point out that *E. coli* is not a reliable indicator of *Vibrio* spp. contamination in shellfish (Lhafi and Kuhne 2007) and so the findings of this review are not relevant to represent vibrios as a group of pathogens.

- Gomez-Couso *et al.* (2003) reported that there was no discernable relationship between the presence of *Cryptosporidium* oocysts and the microbiological contamination detected in the samples expressed as Most-Probable-Number (MPN) of faecal coliforms, the different species of mollusc, or the month of sampling. Consequently the findings of this review would also not be relevant to represent *Cryptosporidium* spp..

Public health considerations:

- Norovirus accumulation and clearance characteristics in bivalves may differ from those observed for *E. coli*. Furthermore they may differ both within and between species and therefore require further investigation. These differences have consequences for the following:

- Investigative samples: Given the differences observed between species the use of an indicator species approach for investigative samples following pollution events (where confirmation of microbial clearance is key) would need separate consideration.

- Seasonal classifications: Given the differences observed between species, the indicator species approach will require further consideration before being applied in a fishery showing a marked seasonal trend in contamination.

- Spatial variation considerations: Any indicator approach would need to carefully consider the potential for spatial differences in contamination across a harvesting area to ensure that they were adequately addressed e.g. it would not be appropriate to use an indicator species if this was located further from the main sources of contamination than the species being represented.

10. Recommendations

On the balance of the available evidence the following recommendations can be made:

- *Mytilus* spp may be used as an indicator in many situations typically encountered in the UK. In particular, it may be used to represent *C. gigas, O. edulis, Tapes spp.* and *M. mercenaria.* All the data presented in this review suggest that the *E. coli* accumulation capacity of *Mytilus* spp. either exceeds or is equivalent to these species. This recommendation is made on the assumption that species are co-located both geographically and with respect to depth in the water column.

- As two of the studies (Amouroux & Soudant and Kershaw *et al*.) suggested that *C. edule* showed greater levels of contamination than *Mytilus* spp. (Younger and Reese also advised caution with this species pairing), the data would support the use of *C. edule* to represent *Mytilus* spp. or any of the above species that could be represented by *Mytilus* spp. where the monitoring of *C. edule* is practical. In addition to accumulation differences, clearance rates from *C. edule* according to microcosm study findings (Kershaw *et al.*) were slower than *Mytilus* which is encouraging from the public health protection perspective. However, from practical experience under the classification monitoring programme for England and Wales (Younger pers comm.) sampling of natural *C. edule* stocks can be problematic (for access, health and safety and sampling location repeatability reasons). In addition, bagged *C. edule* do not survive well and so would not be suitable for routine classification monitoring purposes. Consequently, the situations under the statutory monitoring programme where *C. edule* may be used as an indicator may be limited.

- Additionally, where *O. edulis* and *C. gigas* are produced in the same area, then the findings of this review would support monitoring either species to represent both. Both species are often grown together in farmed situations but one species generally predominates and/or is easier to sample. *O. edulis* (and therefore by analogy *C. gigas*) may also be used to represent *M. mercenaria.*

- An indicator approach cannot be recommended at this stage for representation of *C. edule*, *Spisula solida, Mya arenaria* and *Ensis* spp. as either contradictory or no supporting data from the literature is available. In areas where these species exist and an indicator approach is considered desirable, then area-specific studies would be needed on a case-by-case basis to confirm which species it is best to monitor.

- There would appear to be insufficient evidence available to justify a recommendation for an indicator to represent scallops (*Pecten maximus*). The one study available suggests that *Mytilus* spp. would not be suitable to

represent these and so scallops should be monitored specifically. This would not, however, currently create a problem from the classification monitoring perspective as scallops are rarely classified in the UK. The legislation allows for end product testing of scallops in fish auctions, dispatch centres and processing establishments rather than requiring classification monitoring as a default position.

- In England and Wales, *Mytilus* spp. offer a number of practical advantages as an indicator shellfish species. They are relatively cheap to obtain, are generally more resilient than other bivalve species to environmental stressors and may be readily deployed in a variety of ways to facilitate sampling e.g. mesh bags on fixed installations, suspended from buoys in the water column, or on the seabed. From the practical perspective, therefore, *Mytilus* spp. sampling is generally preferable to *C. edule* sampling. In addition, *Mytilus* spp. tend to be found in similar locations on repeated occasions (unlike *C. edule* beds which have a tendency to shift quite regularly).

Considerations

Class A areas: An important consideration is that, since Class A shellfish can be directly marketed without further processing, regulatory compliance needs to be demonstrated for each species independently in this particular classification category. Whilst this is ultimately the responsibility of the Food Business Operator (FBO) careful consideration would, nevertheless, be advisable on the part of the competent authority on whether the use of indicator shellfish species for Class A classified production areas would be appropriate. Given that only around 1% of areas in England and Wales are currently class A, this is only a minor consideration. The majority of areas in England and Wales (c. 85%) are class B and the use of indicator shellfish species in these areas might represent a significant financial saving for local authorities. From the shellfish industry perspective and considering the greater marketability of Class A shellfish, the use of surrogate species might in any case only be appropriate if Class A compliance was considered unlikely at the outset and classification at Class B would be sufficient for local shellfish industry needs.

Similarly if compliance proves marginal between the B/C classification categories with *Mytilus* spp. as the indicator species (or is already marginal with the target species) then it may be preferable to sample the target species to achieve the best level of classification possible from the shellfish industry perspective.

Precautionary approach versus best classification for industry stakeholders: It should be emphasised that there are two separate, potentially conflicting, issues to consider here. The first being to protect public health and to that end the species showing the higher level of contamination would be selected. This is the primary aim of the legislation, EC Regulation 854/2004 (Anon, 2004b). The second, however, is to recognise the needs of the shellfish industry in terms of obtaining a level of classification that allows FBOs to run a viable business. The balance between these two issues may occasionally require a policy decision from the competent authority to achieve the best compromise where one may be necessary. Whilst it would not be in the interests of any FBO to supply an unsafe product, the link between

classification level and product safety is not a direct one as the classification categories are assigned according to faecal indicator as opposed to pathogen levels. Consequently, whilst an indicator approach may be desirable from the regulatory point of view (both in terms of public health protection and in conserving resources), in marginal compliance situations such an approach may meet with resistance from the shellfish industry stakeholders involved.

Spatial effects: Any indicator approach would need to carefully consider the potential for spatial differences in contamination across a harvesting area to ensure that they were adequately addressed e.g. it would not be appropriate to use an indicator species if this was located further from the main sources of contamination than the species being represented. These issues would normally be considered at the sanitary survey stage when assigning representative monitoring points to represent production areas and classification zones.

Pollution events and confirmation of microbial clearance: On a note of caution, Dore and Lees (1995) studying the removal of viruses (F+ bacteriophage) from shellfish found that removal from *Mytilus* spp. was quicker than from *C. gigas*. This would suggest that the use of mussels as an indicator species to reflect the viral content of oysters after sewage spills (where clearance rate rather than accumulation is the feature of interest) may not provide a protective approach. No equivalent published viral data is currently available for cockles.

In addition, it is clear given other study findings noted in this review (e.g. Beucher, 1993) that there are differences in the clearance rate of *E. coli* and/or faecal coliforms between bivalve species. Furthermore, a ranking of species according to their clearance ability may differ from a ranking according to their capacity for accumulation. The use of an indicator species approach for investigative samples following pollution events (where confirmation of microbial clearance is key) would therefore need separate consideration.

11. Impact evaluation of recommended options

Due to resource constraints exerted over recent years, the number of species sampled in England and Wales has been steadily reduced in many areas. Essentially, indicator species approaches have already been taken in these areas but based on historic monitoring experience at the same site or at least within the same production area. Such an approach has not been taken without the presence of historical data as justification. It is intended that the outcome of this review will help justify an indicator species approach without the need for site-specific historic data.

The benefits of an indicator species approach would apply principally under three scenarios:

- New sites, with multiple species – possibly only one species monitoring needed from the outset (depending on species combination).
- New sites with one species (e.g. *Ensis* spp.) that requires specialist equipment to sample and where an indicator species (perhaps deployed in bags from a readily accessible location) could be used instead.
- Existing sites – reduction in number of species monitored.

New sites

The indicator species approach could offer significant benefit in new applications with multiple species.

Estimated cost savings for new sites in England & Wales:

England and Wales classification applications over the last 12 months:

Area name	Species	Application date
Humber Horeshoe Point	*C. edule*	13/07/2012
Chichester	*Tapes* spp. , *M. mercenaria* and ***C. edule***	25/09/2012
Liverpool Bay - N of Hoyle Bank (Intershell)	Razor clams *Ensis* spp.	19/04/2012
Point Clear, Brightlingsea	***Crassostrea gigas*** and *Mercenaria mercenaria.*	01/10/2012
Exe	*Tapes* spp.	02/11/2012
Swale	*Crassostrea gigas*	02/07/2013

Potential indicator species highlighted in green

Using an indicator approach from the outset for the two applications above involving more than one species might offer an analytical cost* saving of 3 x 10 samples for provisional classification = £1980 plus ongoing monitoring on an annual basis of £1980 in each subsequent year. The industry would need to be aware that the use of an indicator species approach might result in a worse level of classification in some situations (e.g. the use of *C. edule* to represent *M. mercenaria)* depending on the species combination and ambient water quality.

**Assuming £66 per sample, source: Cefas CTL:*
http://www.cefastechnology.co.uk/shop/acatalog/commercial-standard.htm

There also might be the potential option of using bagged *Mytilus* in some species scenario situations where collecting the target species is problematic. In particular, where monitoring of the species of interest involves diver-gathering (e.g. *Ensis* spp.) or specialist equipment that is difficult and/or prohibitively expensive to use. In such situations, natural stocks or bagged indicator species deployed from a buoy could be a practical and cheaper alternative, however, there is insufficient data available from this review to be able to recommend such an approach for *Ensis* spp. at this stage. For the time being, site-specific studies and relevant comparative monitoring data would therefore be needed.

Estimated cost savings for new sites in Scotland:

Fast track site	Species
Loch Ryan	Razors
Loch Sunart	Common mussels
Arisaig: Morar Sands	Razors
Stevenston Sands	Wedge clams
Stevenston Sands Razors	Razors
Meikle Craigs	Razors
Papa Little Voe Fast Track	Common mussels
Gullane Point Fast Track	Razors
Machrie Bay	Razors
West Buckhaven	Razors
North Berwick SITE: Eyebroughy	Razors
Whalefirth Voe Lea Cru	Mussels
Loch na Cille	Common cockles
Bagh Chornaig Paloudres	Carpet clams

There would appear to be no obvious opportunity to use an indicator species approach with fast track applications over the last 12 months in Scotland.

New site applications:

Area name	Species	Application Date
Ardcastle Bay	Pacific Oyster	21/02/2013
Ardcastle Bay	**Common Mussels**	21/02/2013
Ardcastle Bay	Scallops	21/02/2013
Ardcastle Bay	Urchins	21/02/2013
Loch Kanaird; Ardmair	Pacific Oyster	21/02/2013
Loch Sunart; Liddisdale	Common Mussels	13/03/2013
Stevenston Sands	Wedge Clams	07/11/2012
Stevenston Sands	Razors	07/11/2012
Clift Sound Houss	Common Mussels	29/11/2012
Millburn:Sound of Houbansetter / Bight of Warwick: Pappa Little	Common Mussels	08/05/2013

An indicator species approach could have potentially been used with one new application in Scotland over the last 12 months (*Mytilus* spp. to represent *C. gigas* at Ardcastle Bay). This could have offered an analytical cost saving of 10 samples = £660 plus sampling costs which are generally significantly higher than analytical costs.

Estimated cost savings for new sites in Northern Ireland:

New site applications:

Area name	Species	Application Date
Paddy's Point	Pacific Oyster, Common Mussels	12/11/2012

An indicator species approach could have potentially been used with one new application in Northern Ireland over the last 12 months (*Mytilus* spp. to represent *C. gigas* at Paddy's Point). This could have offered an analytical cost saving of 10 samples = £660 plus sampling costs which are generally significantly higher than analytical costs.

Existing sites

An approximation of the resource savings that can be made by adopting this approach in existing classified sites using England and Wales as an example situation are as follows:

Key figures from E&W programme:

360 current RMPs: 150 *Mytilus* spp., 63 *C. gigas*, 54 *O. edulis,* 69 *C. edule, 2 M mercenaria, 9 Tapes* spp.*, 1 Mya arenaria, 9 Ensis* spp., 1 Pecten maximus, 2 *Spisula solida.*

Total number of samples over 5 years (1 July 2008 to 1 July 2013) = 17,734

In total there are 20 sites with two species still monitored at the same location:

Species pair	No. of sites
C. gigas and ***Mytilus* spp.**	14
Mytilus and ***C. edule***	2
***Mytilus* spp.** and *O. edulis*	1
Tapes spp. and ***Mytilus* spp**	1
***Tapes* spp.** and *M. Mercenaria*	1
C. gigas and ***C. edule***	1

Potential indicator species highlighted in green

In theory, an indicator species approach could be taken at all of these sites although classification considerations may prevent this.

Classification scenarios covered	No. of sites
B vs A	1
B vs B,	1
B vs BLT	6
B vs declassified (quarterly monitoring)	1

B vs new area (pending)	3
BLT vs BLT	7
BLT vs C	1
BLT vs new area (pending)	1

For some species, e.g. *C. edule,* classification status may not be considered important (i.e. providing they are not designated Prohibited) if they are intended for heat processing. There is, however, a market for live cockles and in this case class B (or A) is necessary. For most other species the classification level is crucial and so for marginal A/B or B/C situations an indicator species approach may not be considered by the local industry to be attractive given the indicator is likely to be showing a higher level of contamination thus increasing the likelihood of a worse classification. Recommendations for an indicator could be made by the classification programme co-ordinator or at the sanitary survey stage and it would then be for the local competent authority to discuss with the shellfish industry stakeholders the favoured approach to take.

Annual cost savings

Analytical costs - Based on an approximate cost of *E. coli* MPN test per sample of £66 the following savings can be envisaged:

Number of tests per year per site on average = 10 so overall analytical cost per site is approx £660 per annum.

Sampling costs – These will vary depending on the species gathered but at the very least will involve extra staff time and may require specialist equipment (e.g. species-specific dredges) and/or boat hire which can be expensive (c. £3-400 per day).

Overall cost saving per existing monitored sites - It is difficult to predict with any certainty which of the above sites would opt for an indicator species approach given the various local factors that would need to be taken into account. However, assuming ten of the above 21 sites opt for an indicator species approach, a saving of £6600 per annum (100 samples at £66), could be made on analytical costs with further savings being made on staff time and equipment/boat hire.

12. Further work

Specific tank experiments in a controlled environment would be worthwhile to clarify some of the contradictory findings noted between *Mytilus* and *C. gigas* and *Mytilus* and *C. edule*. This might give insights into the reasons for any differences in *E. coli* accumulation characteristics. It would also be of interest to undertake similar work for other commercially important species such as razor clams (*Ensis* spp.) and sand gaper (*Mya arenaria*). From experience under the programme in England and Wales, both of these species can be problematic for local authorities to sample. Given findings to date, direct comparison with *Mytilus* spp. and *C. edule* would give the most practical benefit.

Given progress with norovirus methodology, studies might also be worthwhile to investigate Norovirus vs *E. coli* accumulation and clearance differences between species.

13. References

Altman D.G., Bland J.M. (1983). "Measurement in medicine: the analysis of method comparison studies". Statistician 32: 307–317. doi:10.2307/2987937.

Amouroux, I., Soudant, D. (2011). Comparison of microbiological contamination level between different species of shellfish. Proceedings of the 8th International Conference on Molluscan Shellfish Safety, Charlottetown, Prince Edward Island, Canada, 12–17 June 2011.

Anon 2004a Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for food of animal origin. O. J. Eur. Commun.L226, 22–82.

Anon 2004b Corrigendum to Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. O. J. Eur. Commun. L226, 83–127.

Bean, T.J., Morris Jr., J.A., Noble, R.T., Fowler, P.K. (2006). Comparative microbial dynamics in *Crassostrea virginica* and *Crassostrea ariakensis*. NOAA Technical Memorandum NOS NCCOS 25.

Beliaeff, B. & Cochard, M. L.. (1995). Applying geostatistics to identification of spatial patterns of faecal contamination in a mussel farming area. (Havre de la Vanlee, France). Water Res. 29, 1541–1548.

Bernard, F.R. 1989. Uptake and elimination of coliform bacteria by four marine bivalve molluscs. Canadian Journal of Fisheries and Aquatic Sciences 46(9): 1592–1599.

Berry, R., Younger, A. (2009). Interspecies comparison of *E. coli* accumulation in bivalve shellfish using data obtained from official control monitoring under EU Regulation 854/2004. Proceedings of the 7th International Conference on Molluscan Shellfish Safety (ICMSS09)

Best, W.R. (1970). On the logarithmic transformation of intestinal bacterial counts. Am J Clin Nutr, 23(12): 1608-1609.

Beucher, M., (1993). Etude de l'accumulation, de la retention et du relargage de bacteries enteriques par l'huitre *Crassostrea gigas*. Memoire pour l'obtention du Diplôme de l'Ecole Pratique des Hautes Etudes.

Bonadonna, L., Volterra, L., Aulicino, F.A., Mancini, L. (1990). Accumulation power of some bivalve molluscs. *Marine Pollution Bulletin* 21(2): 81–84.

Birkbeck, T.H., McHenery, J.G. (1982). Degradation of bacteria by *Mytilus edulis*. *Marine Biology* 72: 7–15.

Bland, J.M., Altman, D.G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1 (8476): 307–10. PMID 2868172.

Burkhardt III, W., Watkins, W.D., Rippey, S.R. (1992). Seasonal effects on accumulation of microbial indicator organisms by *Mercenaria mercenaria*. *Applied and Environmental Microbiology* 58(3): 826–831.

Cabelli, V.J., Heffernan, W.P. (1970a). Accumulation of *Escherichia coli* by the Northern Quahaug. *Applied Microbiology* 19(2): 239–244.

Cabelli, V.J. and Heffernan, W.P. (1970b). Elimination of bacteria by the soft shelled clam, *Mya arenaria*. J.Fish.Res.Bd.Canada, 27, 1579-1587.

Campos, C.J.A., S. Kershaw, Lee, R.J. (2011). Environmental influences on faecal indicator organisms in coastal waters and their accumulation in bivalve shellfish. Cefas report to Defra, Project WT1001: Factors affecting the microbial quality of shellfish.

Canesi L;, L. C. Lorusso, C. Ciacci, M. Betti and G. Gallo (2005)
Effects of the brominated flame retardant tetrabromobisphenol-A (TBBPA) on cell signaling and function of *Mytilus* hemocytes: Involvement of MAP kinases and protein kinase C
Aquatic Toxicology Volume: 75 Issue: 3 Pages: 277-287

Cefas (2009). Protocol for the Collection of Shellfish under the Microbiological Classification Monitoring Programme (EU Regulation 854/2004). http://www.cefas.defra.gov.uk/media/436986/samplingprotocolforlocalauthorities[1].pdf [accessed 14 July 2011]

Cefas (2010). Cefas Protocol for Inspection and Approval of Purification (Depuration) Systems – England and Wales. http://www.cefas.defra.gov.uk/media/540968/purification%20generic%20protocol_v8%20final.pdf [accessed 16 August 2012]

Cefas (2013). Investigative trials on the purification of oysters to identify ways of reducing norovirus. http://www.cefas.defra.gov.uk/media/607899/2013%20cefas%20contract%20report%20c5224.pdf [accessed 2 September 2013]

Charles, F., Amouroux, J.-M., Grémare, A., Cahet, G. (1992a). Filtration of the enteric bacteria *Escherichia coli* by two filter-feeding bivalves, *Venus verrucosa* and *Mytilus galloprovincialis*. II. Modelling. *Marine Biology* 113: 125–131.

Charles, F., Grémare, A., Amouroux, J.-M., Cahet, G. (1992b). Filtration of the enteric bacteria *Escherichia coli* by two filter-feeding bivalves, *Venus verrucosa* and *Mytilus galloprovincialis*. I. Experimental study. *Marine Biology* 113: 117–124.

Cheng T. C. (1989). Immunodeficiency Diseases in Marine Mollusks: Measurements of Some Variables. Journal of Aquatic Animal Health. Volume: 1 Issue: 3 Pages: 209-216

Coghlan, J., Ansell, A.D. (1964). A direct method for determining the pumping rate of siphonate bivalves. *ICES Journal of Marine Science* 29: 205–213.

Croci, L., M. N. Losio, E. Suffredini, E. Pavoni, S. Di Pasquale, F. Fallacara and G. Arcangeli (2007). Assessment of human enteric viruses in shellfish from the northern Adriatic sea. International Journal of Food Microbiology. Volume: 114, Issue: 2. Pages: 252-257

Colwell R. R. and G. S. Sayler (1977). Effects and interactions of polychlorinated biphenyl (PCB) with estuarine micro-organisms and shellfish. U.S. National Technical Information Service. Springfield, Va., Report No. PB 272 103, (6A1 COL) Page: 54.

Dare, P.J. (1976). Settlement, growth, and production of the mussel, *Mytilus edulis* L., in Morecambe Bay, England. *Fishery Investigations Series II*, 28(1): 1–25.

Dodgson, R.W. (1928). Report on mussel purification. Fishery Invest, London Series II, 10, No. 1.

Dore W J and D. N. Lees (1995). Behaviour of Escherichia coli and male-specific bacteriophage in environmentally contaminated bivalve molluscs before and after depuration. Applied and Environmental Microbiology. Volume: 61, Issue: 8, Pages: 2830-2834.

EC Regulation 854/2004. Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption on requirements and responsibilities of Competent Authorities.
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:226:0083:0127:EN:PDF [accessed 22/11/10]

El-Shenawy N. S. (2004). Heavy-metal and microbial depuration of the clam Ruditapes decussatus and its effect on bivalve behavior and physiology. Environmental Toxicology. Volume: 19, Issue: 2, Pages: 143-153.

FSA (2012). Investigation into the prevalence, distribution and levels of norovirus titre in oyster harvesting areas in the UK
http://www.food.gov.uk/science/research/foodborneillness/microriskresearch/p01programme/p01projlist/p01009/ [accessed July 2013]

Gerdes, D. (1983). The Pacific oyster *Crassostrea gigas*: part I. Feeding behaviour of larvae and adults. *Aquaculture* 31(2–4): 195–219.

Gonzales-Barron, U., Kerr, M., Sheridan, J.J. and Butler, F (2010). Count data distributions and their zero-modified equivalents as a framework for modelling microbial data with a relatively high occurrence of zero counts. Int J Food Microbiology 136: 268-277.

Gomez-Couso H., F. Freire-Santos, J. Martinez-Urtaza, O. Garcia-Martin and M. E. Ares-Mazas (2003). Contamination of bivalve molluscs by Cryptosporidium oocysts: the need for new quality control standards. International Journal of Food Microbiology. Volume: 87, Issue: 1-2, Pages: 97-105.

Hannam M.L., Bamber S.D., Galloway T. S., Moody J., Jones M. B. (2010) Functional immune response in Pecten maximus: Combined effects of a pathogen-associated molecular pattern and PAH exposure. Fish & Shellfish Immunology 28 (2010) 249-252

Ho, B.S.W., Tam, T.-Y. (2000). Natural depuration of shellfish for human consumption. *Water Research* 34(4): 1401–1406.

ISO (2004). ISO TS 16649-3:2004 Microbiology of food and animal feeding stuffs – Enumeration of ß-glucuronidase positive Escherichia coli – part 3 : Most Probable Number technique using 5-bromo-4-chloro-3-indolyl-b-D-glucuronide acid. International Organization for Standardization, Geneva.

Jones, A.M. (1979). Structure and growth of a high-level population of *Cerastoderma edule* (Lamellibranchia). *Journal of the Marine Biological Association of the United Kingdom* 59: 277–287.

Jørgensen, C.B. (1980). Seasonal oxygen depletion in the bottom waters of a Danish fjord and its effect on the benthic community. *Oikos* 34: 68–76.

Jørgensen, C.B. (1990). Bivalve filter feeding: hydrodynamics, bioenergetics, physiology and ecology. Olsen & Olsen, Fredensborg, Denmark.

Jørgensen, C.B., Larsen, P.S., Riisgård, H.U. (1990). Effects of temperature on the mussel pump. *Marine Ecology Progress Series* 64: 89–97.

Kach D. J. and J. Ward (2008). The role of marine aggregates in the ingestion of picoplankton-size particles by suspension-feeding molluscs. Marine Biology, Volume: 153, Issue: 5, Pages: 797-805.

Kay, D., Kershaw, S., Lee, R., Wyer, M. 2007. Impact of intermittent discharges on the microbiological quality of shellfish. UK Water Industry Research Report 07/ww/18/1. UKWIR, London. Available at: http://www.ukwir.org/web/ukwirlibrary/91602

Kelly, C.B., Arcisz, W., Presnell, M.W. and Harris, E.K. (1960). Bacterial accumulation by the oysters, *Crassostrea virginica*, on the Gulf Coast. The Robert A. Taft Sanitary Engineering Center Tech. Rep. F60-4, Cincinatti, Ohio.

Kershaw S, Campos C, Reese A, Mitchard N, Kay D, Wyer M. (2012). Impact of chronic microbial pollution on shellfish. http://www.cefas.defra.gov.uk/media/608097/wt0923%20impact%20of%20chronic%20microbial%20pollution%20on%20shellfish%202013%20final.pdf [accessed 9 September 2013]

Kiørboe, T., Møhlenberg, F. (1981). Particle selection in suspension-feeding bivalves. *Marine Ecology Progress Series* 5: 291–296.

Laing, I., Spencer, B.E. 2006. Bivalve cultivation: criteria for selecting a site. Cefas. Science Series Technical Report no. 136. Available at: http://www.cefas.co.uk/publications/techrep/techrep136.pdf. [Accessed 30 August 2013].

Lee, R., Kay, D., Wilkinson, R.J., Fewtrell L., Stapleton, C. (2003). Impact of intermittent discharges on the microbial quality of shellfish. R&D Technical Report P2–266/TR.

Lee R. J. and R. Silk (2013). Sources of variation of Escherichia coli concentrations in bivalve molluscs. J Water Health, Volume: 11, Issue: 1, Pages: 78-83.

Lee, R., Lovatelli, A., Ababouch, L. (2008). Bivalve depuration: fundamental and practical aspects. FAO Fisheries Technical Paper 511. FAO Rome.

Lees, D.N., Nicholson, N., Tree, J.A. (1995). The relationship between levels of *E. coli* in shellfish and seawater with reference to legislative standards. *In:* Proceedings of the International Mollusc Conference, Sydney, November 1994.

Leguyader F., V. Apairemarchais, J. Brillet and S. Billaudel (1993). Use of Genomic Probes to Detect Hepatitis-A Virus and Enterovirus RNAs in Wild Shellfish and Relationship of Viral Contamination to Bacterial-Contamination. Applied and Environmental Microbiology. Volume: 59, Issue: 11, Pages: 3963-3968.

Lhafi S. K. and M. Kuhne (2007). Occurrence of Vibrio spp. in blue mussels (Mytilus edulis) from the German Wadden Sea. International Journal of Food Microbiology. Volume: 116, Issue: 2, Pages: 297-300.

Love D. C., G. L. Lovelace and M. D. Sobsey (2010). Removal of Escherichia coli, Enterococcus fecalis, coliphage MS2, poliovirus, and hepatitis A virus from oysters (*Crassostrea virginica*) and hard shell clams (*Mercenaria mercenaria*) by depuration. International Journal of Food Microbiology. Volume: 143, Issue: 3, Pages: 211-217.

Martinez-Manzanares, E., Egea, F., Castro, D., Moriñigo, M.A., Romero, P., Borrego, J.J. (1991). Accumulation and depuration of pathogenic and indicator microorganisms by the bivalve mollusc, *Chamelea gallina* L., under controlled laboratory conditions. *Journal of Food Protection* 54(8): 612–618.

Martinez, D.I., Oliveira A.J.F.C. (2010). Faecal bacteria in Perna perna (Linnaeus, 1758) (Mollusca: Bivalvia) for biomonitoring coastal waters and seafood quality. Brazilian Journal of Oceanography 58(special issue IVSBO):29–35.

Martins, F., Reis, M.P., Neves, R., Cravo, A.P., Brito, A., Venâncio, A. (2006). Molluscan shellfish bacterial contamination in Ria Formosa coastal lagoon: a modelling approach. In Proceedings of the 8th International Coastal Symposium, 1551–1555.

McGhee T. J., J. A. Morris, R. T. Noble and P. K. Fowler (2008). Comparative Microbial Dynamics in Crassostrea virginica (Gmelin 1791) and Crassostrea ariakensis (Fujita 1913). Journal of Shellfish Research. Volume: 27, Issue: 3, Pages: 559-565.

McHenery, J.G., Birkbeck, T.H. (1985). Uptake and processing of cultured microorganisms by bivalves. *Journal of Experimental Marine Biology and Ecology* 90: 145–163.

Metcalf, T.G., Moulton E., Eckerson D. (1980). Improved method and test strategy for recovery of enteric viruses from shellfish Appl Environ Microbiol, Jan, 39(1), 141 – 52.

Møhlenberg, F., Riisgård, H.U. (1979). Filtration rate, using a new indirect technique, in thirteen species of suspension-feeding bivalves. *Marine Biology* 54: 143–148.

Motwani, M.P. (1956). Experimental and ecological studies on the adaptation of *Mytilus edulis* L. to salinity fluctuations. Proceedings of the National Institute of Science of India 21B (5): 227–246.

Murray, L. & Lee, R.(2010) Overview of legislative principles and measures. In: Safe Management of Shellfish and Harvest Waters: minimizing health risks from sewage contaminated shellfish (G. Rees, K. Pond, D. Kay, J. Bartram& S. Domingo, eds). Published jointly by International Water Association and World Health Organization, London, pp. 183–204.

Newell, R.C., Bayne, B. L. (1980). Seasonal changes in the physiology, reproductive condition and carbohydrate content of the cockle *Cardium* (=*Cerasterma*) *edule* (Bivalvia: Cardiidae). *Marine Biology* 56: 11–19.

Pales Espinosa, E., D. Hassan, J.E. Ward, S.E. Shumway & B. Allam, (2010). The role of epicellular molecules in the selection of particles by the blue mussel, *Mytilus edulis*. Biological Bulletin, 219: 50-60.

Perkins, F.O., D.S. Haven, R.M. Alamo, Rhodes, M.W. (1980). Uptake and elimination of bacteria in shellfish. *Journal of Food Protection* 43: 124–126.

Plusquellec, A., Beucher, M., Le Gal, Y. (1983). Enumeration of the bacterial contamination of bivalves in monitoring the marine bacterial pollution. *Marine Pollution Bulletin* 14(7): 260–263.

Plusquellec, A., Beucher, M., Prieur, D., Le Gal, Y. (1990). Contamination of the mussel, *Mytilus edulis* Linnaeus, 1758, by enteric bacteria. *Journal of Shellfish Research* 9(1): 95–101.

Prieur, D., Mével, G., Nicolas, J.-L., Plusquellec, A., Vigneulle, M. (1990). Interactions between bivalve molluscs and bacteria in the marine environment. *Oceanography and Marine Biology: an Annual Review* 28: 277–352.

Prins, T.C., Dankers, N., Smaal, A.C. (1994). Seasonal variation in the filtration rates of a semi-natural mussel bed in relation to seston composition. *Journal of Experimental Marine Biology and Ecology* 176: 69–86.

Rees, G., Bartram, J., Kay, D. (2010). Expert consensus. In: Rees, G., Pond, K., Kay, D., Bartram, J., Santo Domingo, J. (eds). Safe Management of Shellfish and Harvest Waters. IWA Publishing, London, UK.

Richards, G.P. (1988). Microbial purification of shellfish: a review of depuration and relaying. *Journal of Food Protection* 51(3): 218–251.

Riisgård, H.U. (1988). Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. *Marine Ecology Progress Series* 45: 217–223.

Riisgård, H.U., Møhlenberg, F. (1979). An improved automatic recording apparatus for determining the filtration rate of *Mytilus edulis* as a function of size and algal concentration. *Marine Biology* 52: 61–67.

Rödström, E.M., Jonsson, P.R. (2000). Survival and feeding activity of oyster spat (*Ostrea edulis* L) as a function of temperature and salinity with implications for culture policies on the Swedish West coast. Journal of Shellfish Research 19(2): 799–808.

Romalde, J.L., Estes M. K., Szücs G., Atmar R. L., Woodley C. M., and Metcalf T. G. (1994). In situ detection of hepatitis A virus in cell cultures and shellfish tissues; Appl Environ Microbiol, Jun, 60(6), 1921 - 6

Romalde J. L., E. Area, G. Sanchez, C. Ribao, I. Torrado, X. Abad, R. M. Pinto, J. L. Barja and A. Bosch (2002). Prevalence of enterovirus and hepatitis A virus in bivalve molluscs from Galicia (NW Spain): inadequacy of the EU standards of microbiological quality. Int J Food Microbiol. Volume: 74, Issue: 1-2, Pages: 119-30

Rowse, A.J., Fleet, G.H. (1984). Effects of water temperature and salinity on elimination of *Salmonella charity* and *Escherichia coli* from Sydney Rock oysters (*Crassostrea commercialis*). *Applied and Environmental Microbiology* 48(5): 1061–1063.

Seafish (1993). Seafish Report no 419 Lart W. J. & Hudson S.A. 1993. Factors affecting Escherichia coli levels in shellfish with reference to E.E.C. Directive 91/492. Seafish Industry Authority, Hull, England.

Seafish (1994). Boulter M, Wilson P (1994). An assessment of optimum environmental criteria for the successful depuration of *Ostrea edulis, Mercenaria mercenaria, Tapes decussatus, Tapes philippinarum, Cerastoderma edule*, and

Mytilus edulis, in the UK. http://www.seafish.org/media/Publications/IR1442.pdf [accessed 4/12/12]

Solic M., Jozic S., Krstulovic N. (2010). Interactive Effects Of Temperature And Salinity On The Rate Of Concentration Of Escherichia coli In Mussels (*Mytilus galloprovincialis*) and oysters (*Ostrea edulis*). Fresenius Environmental Bulletin. Volume: 19, Issue: 8a, Pages: 1634-1639

Šolić, M., Krstulović, N., Josić, S., Curać, D. (1999). The rate of concentration of faecal coliforms in shellfish under different environmental conditions. *Environment International* 25(8): 991–1000.

StataCorp (2007). Stata Statistical Software: Release 10.1 (2009). College Station, TX: StataCorp LP.

Suffredini E, C. Magnabosco, M. Civettini, E. Rossetti, G. Arcangeli and L. Croci (2012). Norovirus contamination in different shellfish species harvested in the same production areas. J Appl Microbiol. Volume: 113, Issue: 3, Pages: 686-92

Tillett, H.E. (1987) Most probable numbers of organisms: revised tables for the multiple tube method. Epidemiology and Infection, 99: 471-476

Timoney, J.F., Abston, A. (1984). Accumulation and elimination of *Escherichia coli* and *Salmonella typhimurium* by Hard Clams in an in vitro system. *Applied and Environmental Microbiology* 47(5): 986–988.

Vasconcelos G. J., Jakubowski W., Ericksen T. H. (1968). Bacteriological changes in shellfish maintained in an estuarine environment. Proc. natn. Shellfish Ass. Volume: 59, Pages: 51-1970,51,13229

Ward, J.E. & S.E. Shumway, (2004). Separating the grain from the chaff: Particle selection in suspension- and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. 300: 83-130.

Wood, P.C. (1969). The production of clean shellfish. Ministry of Agriculture, Fisheries and Food (MAFF), Fisheries Laboratory, Burnham on Crouch, Leaflet No. 20.

Younger, A.D., Lee, R.J., Lees, D.N. (2003). Microbiological monitoring of bivalve mollusc harvesting areas in England and Wales: rationale and approach. In: Villalba, A., Reguera, B., Romalde, J. L., Beiras, R. (eds). Molluscan Shellfish Safety. Consellería de Pesca e Asuntos Marítimos de Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Santiago de Compostela, Spain. pp. 265−277.

Younger, A.D., Reese, R.A. (2013). *Escherichia coli* levels compared between bivalve mollusc species across harvesting sites in England and Wales. Journal of Shellfish Research. Aug 2013 : Volume 32 Issue 2, pages 527–532

14. Appendix

Search terms

Classification
Indicator
Production area
E. coli
“faecal coliform”
Accumulation
Uptake
Removal
Depuration
Season

Species in any combination of the following:
Oysters: native oysters (Ostrea edulis), Pacific oysters (Crassostrea gigas)
Mussels: (Mytilus edulis, M. galloprovincialis), Mytilus spp.
Clams : native clams, palourdes (Tapes decussatus), Manila clams (Tapes philippinarum), Tapes spp., American hard clams (Mercenaria mercenaria)
Cockles (Cerastoderma edule)
Great scallops (Pecten maximus)
Razor clams (Ensis spp.)
Sand gaper (Mya arenaria)
Thick Trough Shell (Spisula solida)

Inhibition
Contaminants
Toxins
Disease

Correlation coefficients - detailed outputs

All data was ranked (i.e. in order of lowest to highest result) and a Spearman's Rank correlation coefficient calculated. Summary results were as follows (at Appendix):

Site 1

	Site 1 Cg	Site 1 M	Site 1 GI	Site 1 GII
Site 1 M	0.566			
	0.004			
Site 1 GI	0.362	0.699		
	0.082	0.000		
Site 1 GII	0.341	0.667	0.545	
	0.103	0.000	0.006	
Site 1 Cg sv	0.767	0.749	0.390	0.558
	0.000	0.000	0.059	0.005

Site 2

	Site 2 Oe	Site 2 M	Site 2 GI	Site 2 GII
Site 2 M	0.775			
	0.000			
Site 2 GI	0.305	0.371		
	0.167	0.090		
Site 2 GII	0.336	0.266	0.254	
	0.126	0.231	0.255	
Site 2 Oe sv	0.801	0.555	0.404	0.374
	0.000	0.007	0.062	0.087

Site 3

	Site 3 Oe	Site 3a M	Site 3 GI	Site 3 GII
Site 3a M	0.576			
	0.004			
Site 3 GI	0.215	0.576		
	0.323	0.004		
Site 3 GII	0.263	0.584	0.791	
	0.225	0.003	0.000	
Site 3 Oe sv	0.608	0.670	0.340	0.428
	0.002	0.000	0.112	0.041

Site 4

	Site 4 Cg	Site 4a M	Site 4 GI	Site 4 GII
Site 4a M	0.433			
	0.035			
Site 4 GI	0.286	0.603		

	0.175	0.002		
Site 4 GII	0.446	0.515	0.869	
	0.029	0.010	0.000	
Site 4 Cg sv	0.525	0.235	0.449	0.621
	0.008	0.269	0.028	0.001

Site 5

	Site 5 Cg	Site 5a M	Site 5 GI	Site 5 GII
Site 5a M	0.572			
	0.010			
Site 5 GI	0.391	0.446		
	0.098	0.056		
Site 5 GII	0.389	0.347	0.310	
	0.099	0.145	0.196	
Site 5 Cg sv	0.593	0.574	0.340	0.542
	0.007	0.010	0.154	0.017

Site 6

	Site 6 Cg	Site 6a M	Site 6 GI	Site 6 GII
Site 6a M	0.269			
	0.239			
Site 6 GI	-0.006	0.395		
	0.979	0.077		
Site 6 GII	-0.261	-0.065	0.536	
	0.253	0.778	0.012	
Site 6 Cg sv	0.694	0.290	0.108	-0.269
	0.000	0.202	0.640	0.238

Site 7

	Site 7 M	Site 7 GI	Site 7 GII
Site 7 GI	0.056		
	0.831		
Site 7 GII	-0.006	0.078	
	0.981	0.767	
Site 7 Cg sv	0.296	-0.212	-0.008
	0.249	0.415	0.975

Site 8

	Site 8 Cg	Site 8a M	Site 8 GI	Site 8 GII
Site 8a M	0.499			
	0.099			
Site 8 GI	0.337	0.558		
	0.284	0.060		

Site 8 GII	0.294	0.333	0.704	
	0.353	0.290	0.011	
Site 8 Cg sv	0.696	0.668	0.208	0.221
	0.012	0.018	0.516	0.491

Site 9

	Site 9 Cg	Site 9a M	Site 9 GI	Site 9 GII
Site 9a M	0.619			
	0.004			
Site 9 GI	-0.076	-0.220		
	0.749	0.352		
Site 9 GII	0.052	0.077	0.632	
	0.828	0.748	0.003	
Site 9 Cg sv	0.691	0.730	-0.091	-0.016
	0.001	0.000	0.702	0.948

Site 10

	Site 10 Cg	Site 10a M	Site 10 GI	Site 10 GII
Site 10a M	0.379			
	0.068			
Site 10 GI	0.214	-0.238		
	0.315	0.262		
Site 10 GII	0.222	-0.293	0.754	
	0.297	0.165	0.000	
Site 10 Cg sv	0.721	0.403	0.005	-0.059
	0.000	0.051	0.981	0.784

Site 11

	Site 11 Cg	Site 11 M	Site 11 GI	Site 11 GII
Site 11 M	0.478			
	0.025			
Site 11 GI	-0.152	-0.658		
	0.498	0.001		
Site 11 GII	-0.012	-0.277	0.646	
	0.957	0.213	0.001	
Site 11 Cg sv	0.448	0.452	-0.026	-0.089
	0.037	0.035	0.908	0.695

Site 12

	Site 12 Oe	Site 12a M	Site 12 GI	Site 12 GII
Site 12a M	-0.322			
	0.179			
Site 12 GI	0.313	-0.210		
	0.192	0.389		

Site 12 GII	0.033	-0.044	0.492	
	0.894	0.859	0.033	
Site 12 Oe sv	0.239	-0.250	0.154	0.176
	0.340	0.317	0.542	0.486

Site 13

	Site 13 Cg	Site 13a M	Site 13 GI	Site 13 GII
Site 13a M	0.398			
	0.178			
Site 13 GI	-0.149	-0.156		
	0.627	0.610		
Site 13 GII	0.139	0.076	0.521	
	0.650	0.805	0.068	
Site 13 Cg sv	0.787	0.500	-0.361	-0.071
	0.001	0.082	0.225	0.818

M = Mytilus spp.
Cg = Crassostrea gigas
Oe = Ostrea edulis
Sv= Surveillance study E. coli data (oyster site)
GI = Norovirus genotype I
GII = Norovirus genotype II

Table 1. Summary table of studies comparing bacterial accumulation between bivalve species

Authors	**The bivalve species studied**	**The experimental approach**	**The range of variables studied**	**The key findings presented (e.g. relative concentration of E. coli in different bivalve species, differences in uptake**
Lee and Silk (2013)	*Crassostrea gigas, Mytilus* spp. and *Pecten maximus*	Field study - analysis of variance (ANOVA).	Effects of site, sampling occasion, species and site/sampling occasion interaction	*Pecten maximus*>(*Mytilus* spp = *Crassostrea gigas*)
Younger and Reese (2013)	*Cerastoderma edule, Crassostrea gigas, Mercenaria mercenaria, Mytilus spp., Ostrea edulis,Tapes philippinarum,*	Field study - review of statutory monitoring data - Bland-Altman method comparison	*E.coli* accumulation only across a range of conditions	*(Cerastoderma edule = Tapes philippinarum = Mytilus* spp*.) > (Crassostrea gigas = Ostrea edulis) Ostrea edulis = Mercenaria mercenaria*
Amoroux and Soudant (2011)	*Cerastoderma edule, Crassostrea gigas, Mytilus spp., Tapes spp.,Patella vulgata*	Field study - review of statutory monitoring data	*E.coli* accumulation only across a range of conditions	*Cerastoderma edule 2.5x > Mytilus* spp*. Cerastoderma edule 3x > Crassostrea gigas. Tapes* spp *4x > Crassostrea gigas*. *(Cerastoderma edule = Tapes* spp*), (Tapes* spp *= Mytilus* spp*), (Mytilus* spp *= Crassostrea gigas).*
Kershaw et al. (2012)	*Cerastoderma edule, Crassostrea gigas, Mytilus spp.*	Microcosm - Linear regression models to assess shellfish vs water relationship.	*E.coli* accumulation only Experiments at 30‰ and 10.5°C	Mean accumulation factors 330 (*Cerastoderma edule*), 15.2 (*Mytilus spp.*) and 11.7 (*Crassostrea gigas*)
Solic et al. (2010)	*Mytilus galloprovincialis* and *Ostrea edulis*	Microcosm	Temperature and salinity on rate of concentration of *E.coli* (12, 18 and 24°C), (37 ‰ and 32 ‰).	*Mytilus galloprovincialis > Ostrea edulis*

Vasconcelos et al. (1968)	*Crassostrea gigas* and *Tapes japonica (aka T. philippinarum)*	Field study in estuarine environment	Faecal coliform accumulation only	*Tapes japonica (philippinarum)* > *Crassostrea gigas*
Berry and Younger (2009)	*Cerastoderma edule, Crassostrea gigas, Mercenaria mercenaria, Mytilus spp., Ostrea edulis,Tapes philippinarum,*	Field study - review of statutory monitoring data	*E.coli* accumulation only across a range of conditions	*(Cerastoderma edule = Tapes philippinarum = Mytilus* spp*.) > (Crassostrea gigas = Ostrea edulis) Ostrea edulis = Mercenaria mercenaria*
Beucher (1993)	*Cerastoderma edule, Crassostrea gigas, Mytilus spp., Ostrea edulis,Venerupis sp.*	Microcosm	Faecal coliform accumulation according to season (spring and summer)	*Cerastoderma edule > Mytilus* spp*. > Venerupis sp. > Crassostrea gigas > Ostrea edulis)*

Table 2. Summary table of studies comparing virus accumulation between bivalve species

Authors	The bivalve species studied	The experimental approach	The range of variables studied	The key findings presented
Croci et al. (2007)	*Tapes philippinarum, Mytilus galloprovincialis, Ostrea* spp. and *Chlamys* spp	Field study - an assessment of human enteric viruses in shellfish from the northern Adriatic sea. Incidence and circulation of different strains of hepatitis A and Norovirus in shellfish were studied on 235 samples	Incidence and circulation of different strains of hepatitis A and Norovirus in shellfish	*T. philippinarum* was the species most often contaminated, as well as being the only species in which the legal limit for *E. coli* was, in some cases, exceeded after depuration.
Leguyader et al. (1993)	*Mytilus* spp. and *Cerastoderma edule*	Field study - used genomic probes to investigate hepatitis A virus (HAV) and enterovirus RNAs	Hepatitis A virus (HAV) and enterovirus RNAs	On the same site, viral (HAV and enterovirus) RNAs were found in a larger fraction of *Cerastoderma edule* than *Mytilus* spp..
Romalde et al. (2002)	Shellfish samples included raft-cultured and wild mussels, as well as wild clams and cockles. *Cerastoderma* sp. , *Mytilus galloprovincialis, Tapes* sp.	Field study - investigated the prevalence of enterovirus and hepatitis A virus in bivalve molluscs from Galicia (NW Spain)	Enterovirus and hepatitis A virus in bivalve molluscs	Differences in bacterial contamination were observed between cultured mussel and wild shellfish.
Suffredini et al. (2012)	*Crassostrea gigas , Mytilus galloprovincialis, Tapes philippinarum*	Field study in Po River, Italy. investigated Norovirus contamination	Norovirus contamination - Environmental parameters (temperature and salinity) and hydrometric levels of the tributary river were measured	No significant differences were found between results from the two harvesting areas and the three shellfish species. However, on the basis of the average C(t) values, the recovery rate (from 0.46 to 1.15%) and the distribution of positive results in the samplings, the authors conclude that mussels seem to be a suitable indicator species to monitor viral contamination in these areas.