# Review of knowledge on the impact of chronic microbial contamination on bivalve shellfish

**Authors: Simon Kershaw, Carlos Campos, David Kay.**

**Project WT0923 - Impact of chronic microbial pollution on shellfish**

**Cefas/CREH report to Defra**

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# Glossary

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|---|---|
| Accumulation: | Uptake and storage of FIOs within the cells of living bivalve shellfish. |
| Accumulation factor: | Measure of the intensity of the accumulation of FIOs in bivalve shellfish. This measure is given by the ratio between the concentration of FIOs in shellfish relative to the concentration of FIOs in the overlying water. |
| Bivalve filter pump: | Groups or bands of lateral cilia on filaments arranged in parallel within the mantle cavity of the bivalve. |
| Chronic exposure: | Contact of bivalve shellfish with FIOs in the overlying waters that occurs over a long time (more than 5 days). |
| Clearance: | In the context of this report, the process by which shellfish eliminate FIOs during filter-feeding when exposed to normal conditions of salinity and temperature for the species. |
| Concentration: | Amount of FIOs present in a certain amount of shellfish flesh and intravalvular liquid or water. |
| Depuration: | In the context of this report, the process used commercially by which shellfish are placed in tanks containing clean seawater to permit the purging of their microbiological contaminants under controlled conditions. In the UK, the seawater is treated by ultra-violet disinfection prior to purification to prevent possible contamination of shellfish during the process. |
| Euryhaline: | Organism capable of tolerating a wide range of salt water concentrations. |
| Faecal indicator organism | (FIO) Bacteria or groups of bacteria (usually faecal coliforms, *Escherichia coli*, enterococcus) normally residing in the intestinal tract of warm-blooded animals and used to demonstrate the potential presence or absence of microbial pathogens. |
| Intertidal | Area of the foreshore between tide marks. |
| Pseudofaeces | Particles that are filtered by the bivalve during the filter-feeding process but are wrapped in mucus and expelled without having passed through its digestive tract. |
| Seston: | All particulates, including plankton, organic detritus, and inorganic material. |

| | |
|---|---|
| Standard error of the mean (SEM) | Standard deviation of the error in the sample mean relative to the true mean. |
| Sublittoral | Area of the coast where sunlight reaches the ocean floor. Typically, extends from the low tide mark to the edge of the continental shelf. |
| Viscosity of seawater | Viscosity is defined as the internal fluid friction or the forces of drag which its molecular and ionic constituents exert on each other. In general, these forces in seawater decrease with increasing temperature. |

# Executive Summary

The Shellfish Waters Directive sets a faecal coliform guideline standard in shellfish flesh which in England and Wales many shellfish waters do not yet achieve, this is despite significant water company investment in improving coastal discharges. The EA has invested considerable resource investigating the sources of faecal indicator organisms (FIOs). However, a clearer understanding of the relationship between overlying water concentration and uptake and retention in shellfish when these animals are exposed to chronic periods of low to moderate levels of pollution is required to help develop appropriate water quality control policies for the future.

Here we review literature on the relationship between chronic microbial pollution in the water column and FIO burden in shellfish flesh. We examine the roles of water temperature and salinity in controlling the dynamics of FIO accumulation by and clearance from shellfish. We also summarise conclusions from past Defra-funded investigations into key drivers of flesh contamination.

FIO levels accumulated by shellfish may exceed those in the overlying waters within 30 minutes exposure to the pollution source. There are significant inter-species differences in the dynamics of accumulation and clearance. Cockles and mussels accumulate faster and to a greater level than oysters. Although mussels may concentrate FIOs to a higher level, the time required for FIO clearance in oysters is usually longer. These differences are consistent with higher filtration rates in the former group of species.

In the natural environment, feeding in shellfish is mainly determined by the capacity of the filter pump and the concentration of food in the water. These influence the threshold of FIO levels above which bivalves are unable to accumulate more bacteria from the overlying waters.

Usually, there are three stages during the period of accumulation: an initial phase during which there is significant variation across individuals within a shellfish bed; a period when the levels of contamination tend to be homogeneous across the bed; and finally a period during which all shellfish reach maximum levels of contamination.

Different shellfish species have different requirements with respect to the range of optimal salinities and temperatures for survival and growth. However, shellfish held in water at salinities close to the lower limit of normal functioning for valve closure appear to show considerable ability to accumulate FIOs in a short space of time.

FIOs are rapidly cleared from shellfish when they are exposed to clean waters. There is an initial phase of greatest clearance followed by a less evident clearance phase.

We conclude that there is a lack of data to evidence the functional relationship between the length of exposure and level of contamination, particularly when shellfish are exposed to chronic low levels of pollution which is the case in most shellfish beds in England and Wales. Data generated by laboratory scale and field experiments undertaken during the second phase of the project should therefore aim to address this to allow prediction of flesh quality around the current Shellfish Waters Directive guideline standard in flesh. This should help provide guidance on appropriate monitoring regimes for shellfish waters.

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# 1. Introduction

Bivalve molluscan shellfish (hereafter referred to as shellfish) can accumulate a variety of constituents present in their growing waters, including microbial contaminants released from point and diffuse sources of pollution impacting on those same waters. Microbiological contaminants (human pathogenic bacteria and viruses) accumulate in the soft parts of the body during the filter-feeding process. When consumed raw or lightly cooked, shellfish contaminated with pathogens may cause infectious diseases. Comparison of bacterial content in shellfish with that in the overlying growing waters is therefore of particular interest to regulators interested in ensuring adequate quality of shellfish products for human consumption.

The process of elimination of microbiological contaminants from shellfish under controlled conditions 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). However, bacterial accumulation in shellfish from waters with relatively low levels of faecal indicator organisms (FIOs), has been considerably less studied.

Currently, under Directive 2006/113/EC (codified)[1] on the quality required of shellfish waters [hereafter referred Shellfish Waters Directive (SWD)], there is a statutory guideline microbiological standard of 300 faecal coliforms per 100ml of shellfish flesh and intervalvular liquid in 75% of samples. The SWD will be repealed in December 2013 by the EU Water Framework Directive (WFD)[2]. In the UK, the Department for Environment, Food and Rural Affairs (Defra) intends to develop national standards in the UK necessary to ensure no deterioration of Shellfish Protected Areas under WFD. Evidence on FIO accumulation factors and inter-species variation of these factors is therefore now required to inform the development of new standards.

This report addresses the objectives of phase 1 of the Defra-funded research project WT0923 "*Impact of chronic microbial pollution on shellfish*", namely to review published literature on factors controlling contaminant burden in key commercial species of shellfish when exposed to prolonged (chronic) microbial pollution. Previous microcosm experiments were carried out at Cefas to determine the uptake and clearance of FIOs in shellfish following a simulated intermittent discharge and thus short term exposure of bivalve molluscs to sewage contamination. These studies indicated that the decay pattern of *E. coli* in shellfish flesh approach the hygiene 'class A' threshold (230 per

[1] Directive 2006/113/EC replaced the original Shellfish Waters Directive 7 9/923/EC.

[2] Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy

100g) by the end of a 120h period (Kay *et al.*, 2007). In the context of this study, chronic microbial pollution is therefore regarded as a period of five days during which shellfish will be exposed to microbiologically contaminated water and from which a period of clearance of these contaminants in shellfish will follow. The specific objectives of this project are to:

i. establish if there are threshold levels of water contamination which cause build-up contamination in shellfish flesh;
ii. establish how are the twin factors of length of exposure and level of contamination linked in determining the quality of harvested shellfish flesh;
iii. study the dynamics of self-cleansing during periods of good water quality;
iv. identify if there is an optimum balance between level of exposure and time of exposure; and
v. make recommendations on sampling strategy required in shellfish waters to underpin prediction of flesh microbial quality.

This report presents a focused and selective evaluation of the literature on critical factors controlling microbial contaminant burden in key commercial species of shellfish with the aim of better understanding:

- water quality threshold values necessary to ensure that target flesh values are met;
- the role of water temperature and salinity;

and to develop:

- guidance on discharge regimes to optimise shellfish quality; and
- guidance on monitoring regimes for shellfish waters.

It is outside the scope of this review to extensively appraise evidence from commercial depuration. Comprehensive appraisals on this matter have been given by Richards (1988) and Lee *et al.* (2008). Similarly, it is outside the scope of this report to provide an in-depth, quantitative analysis of physiological mechanisms determining FIO accumulation by shellfish.

# 2. Methods

Peer reviewed papers were retrieved from the following databases: Scopus (http://www.scopus.com/home.url), ScienceDirect (http://www.sciencedirect.com/) and Ingentaconnect (http://www.ingentaconnect.com/) and the Scientific Electronic Library Online - SciELO (http://www.scielo.br/). Full text articles published in the Journal of Shellfish Research were accessed from the Biodiversity Heritage Library (http://www.biodiversitylibrary.org/Default.aspx). The terms searched were "accumulation", "bivalves", "clearance", "enteric bacteria", "enterococci", "*Escherichia coli*", "faecal coliforms", "faecal indicator organisms", "feeding", "filtration", "mussels", "oysters", "retention", "salinity", "seawater", "*Streptococcus*" "temperature", "uptake", "water pumping". A number of studies were also retrieved from Cefas reference archives. Abstracts were reviewed and those considered relevant were flagged for full text review. Studies undertaken in shellfish harvesting areas or bathing waters in the proximity of shellfish waters or those reporting results from species commercially harvested in the UK were prioritised. Only studies presenting results for enterococci, faecal coliforms and *E. coli* were reviewed. It should be noted that some of the studies may not be representative of the environmental conditions influencing UK coastal waters. Non-peer reviewed studies appraised include some doctoral dissertations and project reports.

# 3. Bivalve filter feeding

Water pumping and filtration in shellfish are basically autonomous processes limited by the physical properties of the filter pump. These processes are not physiologically regulated at the organism level (Clausen and Riisgård, 1996; Jørgensen, 1990, 1996), at least between a lower critical level and upper food concentration threshold (Riisgård et al., 2011). Feeding in shellfish is, thus, a highly automatic process during which the retention of particulate matter is determined by the capacity of the pump and the concentration of food in the water (Jørgensen, 1990). When exposed to the natural range of environmental conditions for the species, filter-feeding shellfish tend to be fully open and process water at relatively constant rates. Deviations from optimal environmental conditions, such as mechanical disturbance, poor water quality or depletion of food may cause partial to complete closure of valves and hence reduced or discontinued water processing (Jørgensen, 1990). In the common mussel (Mytilus edulis) and the common cockle (Cerastoderma

edule), reduction in valve opening (gape), or closure of the valves, constitutes part of a normal activity in response to periods of depleted food or oxygen in the water or exposure (emersion) during periods of low water (Jones, 1979). However, undisturbed shellfish under natural conditions may also show lower levels of water processing than those expected under maximal capacity of the filter pump. This has been shown to be associated with the seasonal variation of suspended particulate matter in the overlying waters (Prins et al., 1994).

The efficiency with which shellfish retain particles may vary with internal and environmental conditions, including concentration and composition of 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 may also be partially attributed to changes in the viscosity of the water (Jørgensen, 1990)**.**

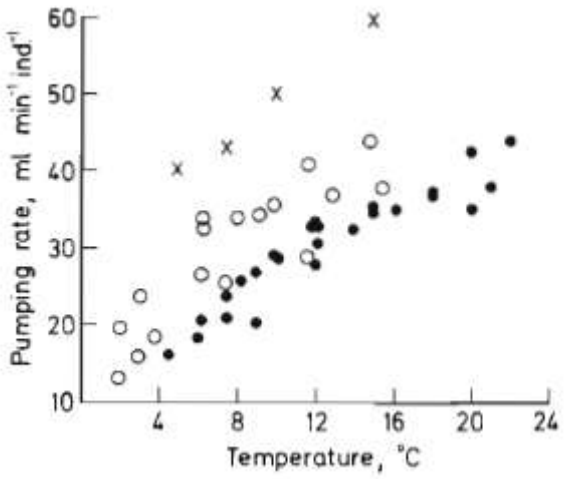


**Figure 1. Relationship between temperature and pumping rate in acclimatised mussels at different times of the year. (○) February, 25 mussels, 31±0.7 (SD) mm in length, acclimatised to 6°C; (x) May, 20 mussels, 39±2mm, acclimatised to 7.5°C; (●) June, 30 mussels, 29±2.6mm, acclimatised to 12°C. Adapted 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 (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[a] | 0.4–2.7 | Prins *et al.* (1994) |

[a] *continuous flow tank supplied with natural seawater*.

Early studies of the feeding organs of shellfish were largely qualitative but emphasis later focused on quantitative determination of the uptake of particulate material from the environment and identification of this material. Introduction of electronic particle counters led to a better understanding of the rates and efficiencies at which particle removal occurred, and the interface between food availability in the ocean and responses of these animals to temporal and spatial variability. While some early workers inferred the capabilities of these animals to select particles. It was the introduction of more advanced technologies (e.g., flow cytometry, video endoscopy, confocal microscopy) that allowed more detailed studies of the mechanisms associated with particle uptake and selection by shellfish. These techniques have provided a more comprehensive analysis which clearly demonstrates that the mechanisms associated with particle feeding and selection are complex, with species-specific processes acting upon both the physical and chemical characteristics of the particles (Ward and Shumway, 2004). Selection of particles involves interactions between particle-associated chemical compounds produced by phytoplankton (Ward and Targett, 1989; Pales Espinosa, 2010).

# 4. FIO accumulation in shellfish: evidence from microcosm studies

### *4.1 Accumulation factors in shellfish*

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. No direct association was found between the level of "equilibrium" and accumulation interval when clams were exposed to *E. coli* after 6, 24 and 48h. The number of animals which achieved the "equilibrium" stage did however increase with time. The authors proposed a linear model to represent the relationship of the "equilibrium" level in shellfish relative to the concentration of *E. coli* in the contaminated water (Fig. 2).

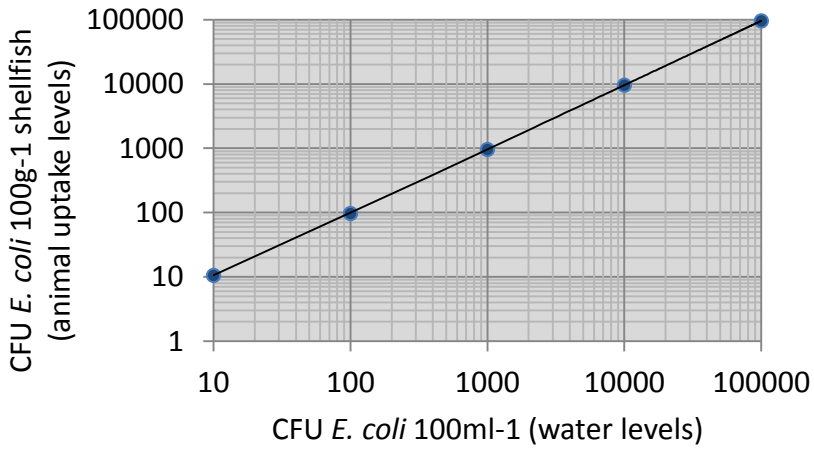


**Figure 2. Relationship between the equilibrium level of *E. coli* in *M. mercenaria* relative to the concentration of the microbial indicator in the overlying water. Linear regression model: $\log_{10}$ "equilibrium" of *E. coli* in *M. mercenaria* = 0.96$\log_{10}$ *E. coli* in the water + 0.97. Environmental conditions: seawater temperature 20°C; salinity=30‰; flow rate=2L $min^{-1}$. Modified from Cabelli and Heffernan (1970).**

The accumulation factors reported in the literature, for microcosm studies, suggest the existence of an "equilibrium" stage during the filtration period and indicate some variation in the characteristics of this stage between shellfish species (Table 2). It should however be noted that this summary includes accumulation factors calculated as both ratios of geometric means and ratios of log-transformed values.

**Table 2.**

**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 (1970) |
| *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 (1970) |
| *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) |

* 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.

It has been suggested however that the accumulation factors reported in the literature for the coliform group are more similar than those for bacterial and viral pathogens (Prieur *et al.*, 1990). Furthermore, it should be noted that the more "classical" methods for the detection and enumeration of FIOs in shellfish, such as membrane filtration (MF) and most probable number (MPN) approaches, which depend on cultivation techniques, have different sensitivities with respect to the recovery of different groups of organisms (Jagals *et al*., 2000). Gronewold and Wolpert (2008) argued that variability of MPN and colony-forming unit (CFU) estimates of enteric bacteria in the same sample are a consequence of the probabilistic basis for calculating the MPN rather than human error or laboratory procedure variability. The characteristics of the enumeration media are also important in determining the accuracy, specificity, selectivity, precision and recovery efficiency of the methods (Catalao Dionisio and Borrego, 1995).

Uptake of several species of bacteria by the common mussel *Mytilus edulis* and the subsequent fate (clearance or degradation) of some polymers of the bacteria were investigated by Birkbeck and

McHenery (1982). Radiolabelled and unlabelled bacteria were added to 1.5-litre experimental jars containing a final concentration of $l0^7$ $m1^{-1}$. Samples were removed at 0, 1, 2, 4, 6 and 24h. Clearance of bacteria from the water in the jars by *M. edulis* was measured by viable colony counting and clearance of radiolabelled bacteria was measured by scintillation counting. Bacteria were cleared at similar, exponential rates, giving an overall mean clearance coefficient (time required to reduce the bacterial concentration by 90%; $C_{90}$) for three species of bacteria (*M. luteus*, *M. roseus* and *B. cereus*) of 1.93±0.12h (SEM, n=63). All three species of bacteria were rapidly removed from sea water by *M. edulis* and, for the first 4–6h, clearance was at an exponential rate. Between 6 and 24h, the bacterial counts continued to fall in the case of *E. coli*. Those bacteria with cell walls which were sensitive to *M. edulis* lysozyme were rapidly degraded by the mussel and Lysozyme-resistant bacteria *(M. roseus* and *S. aureus*) were cleared from suspension by *M. edulis* but most were rejected intact. Similarly, the $C_{90}$ for *E. coli, S. aureus* and Pseudomonad 1-1-1 were not significantly different (mean $C_{90}$=2.4±0.34h, (SEM, n=13) That selected bacteria can be degraded by *M. edulis* and selected polymers retained and presumably utilised is evident from this work. The study concluded 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.

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 flow-through tanks containing seawater (capacity = 6,000 litres) and to which 1,000 litres of effluent were dosed over 48h (the maximum concentration, achieved in tank water at the beginning of the clearance phase = 397 CFU $100ml^{-1}$). Results for the Pacific oyster indicated that accumulation of faecal coliforms in oyster flesh reaches the “equilibrium” stage within 20h of active filtration and the time required to reach this “equilibrium” was strongly influenced by season and varied between individuals of the same species. Similar individual variation was detected during the clearance phase. The most active species were cockles and mussels and the less active species were venus clams and native oysters. Figure 3 shows that cockles and mussels accumulated faster and to a greater level than the other four species/groups of species tested.

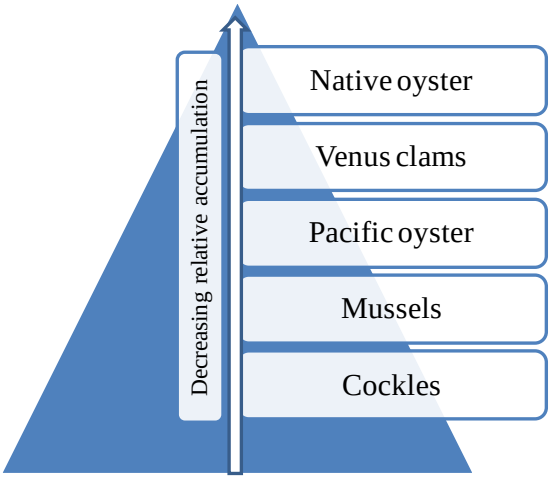


**Figure 3. Inter-species pattern of faecal coliform contamination during the accumulation phase obtained by Beucher (1993).**

Higher accumulation factors in cockles and mussels are consistent with higher filtration rates in these species reported in the literature (Table 4).

**Table 4. 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.*

Bernard (1989) contested the direct association between accumulation factors and uptake efficiencies. This author proposed that the apparent high accumulation efficiency of shellfish is probably attributable to the breaking up of bacterial loci by digestive processes, rather than to any actual change in bacterial absolute numbers. Bernard found that filtered oyster and mussel gastric

liquid significantly increased plate and MPN counts of *E. coli* when added to prepared tubes or plates and argued that the digestive process of shellfish is responsible for breaking up clumps of bacteria, leading to increased MPN counts. However, differences in inhalant feeding currents may also result in different intake of microbial contaminants.

Bean *et al*. (2006) carried out bioaccumulation experiments in depurated American cupped oyster (*Crassostrea virginica*) and Suminoe oyster (*C. ariakensis*) by inoculating six tanks with *E. coli* K12 strain, to achieve final concentrations of $10^3$–$10^4$ cells/100ml in the water. Oysters were sampled at time 0 and 4h after inoculation and *E. coli* and *Vibrio* sp. were enumerated. *C. ariakensis* had 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*. During the accumulation phase, *E. coli* levels in the water decreased whereas levels of the indicator in shellfish flesh increased where there was a three-fold decrease in bacterial levels over the 4h time period. Rates of bacterial uptake were statistically significantly different between species suggesting that there is difference in the response of *C. ariakensis* and *C. virginica* to bacterial contamination.

Kay *et al.* (2007) reports laboratory microcosm experiments carried out by Cefas to examine the effects of sewage effluent contamination on *E. coli* levels in shellfish (mussels and Pacific oysters) and relationships with concentrations in the overlying water. This work simulated a 12h contamination exposure of shellfish to dilute sewage (target seawater:sewage ratio=50:1) and provided empirical data to inform policy development in relation to intermittent sewage discharges. The main conclusions from the study were:

- *E. coli* concentrations in shellfish flesh and water increased rapidly in response to the addition of sewage, to levels above 46,000 $100g^{-1}$ and were consistently higher in shellfish than those in the overlying water. Concentrations of the indicator then decreased to a constant level.
- First order exponential decay functions were fitted to the observed phase of *E. coli* attenuation, following contamination. Mussels showed higher *E. coli* concentrations following contamination and more rapid decay then oysters.

Statistically significant ($p$<0.05) regression models were developed to predict *E. coli* concentrations in shellfish flesh from that in the overlying water. The resulting slope coefficient for mussels was almost twice that observed for oysters.

### *4.2 Effect of temperature*

The acute effects of temperature on pumping rates were studied by Jørgensen *et al*. (1990) in the blue mussel (*Mytilus edulis*) kept at constant temperatures between 6°C to 17°C, and at abruptly changing temperatures, where the temperature was controlled by means of cooling with ice packs or heating with an electrical heater. It was found that pumping rates increased with temperature and were correlated with the temperature-determined decrease in viscosity of the water. The variation in pumping rate with temperature thus corresponded to the varying viscous resistance to water flow in the canal system of the mussel pump.

McHenery and Birkbeck (1985) maintained shellfish in 750ml experimental jars containing filtered-sterilised seawater and exposed them to [$^3$H]thymidine-labelled *E. coli* and found that uptake rates in the common cockle (*C. edule*) was significantly slower at 15°C than at 10°C.

Rowse and Fleet (1984) compared *E. coli* clearance from overlying water by the Sydney cupped oyster *Crassostrea commercialis* harvested from Georges River (NSW, Australia) at three water temperature ranges. Oysters were artificially contaminated using dilutions of Nutrient Broth cultured *E. coli* previously isolated from local oysters and added to the water in the laboratory. Results indicated more complete and consistent clearance when oysters were exposed to water temperature ranges of 18–22°C and 24–27°C than that at water temperatures of 13–17°C. The spread of differences in *E. coli* concentrations in oyster flesh between the temperature ranges at the end of the clearance period exceeded 1Log$_{10}$ (Figure 4).

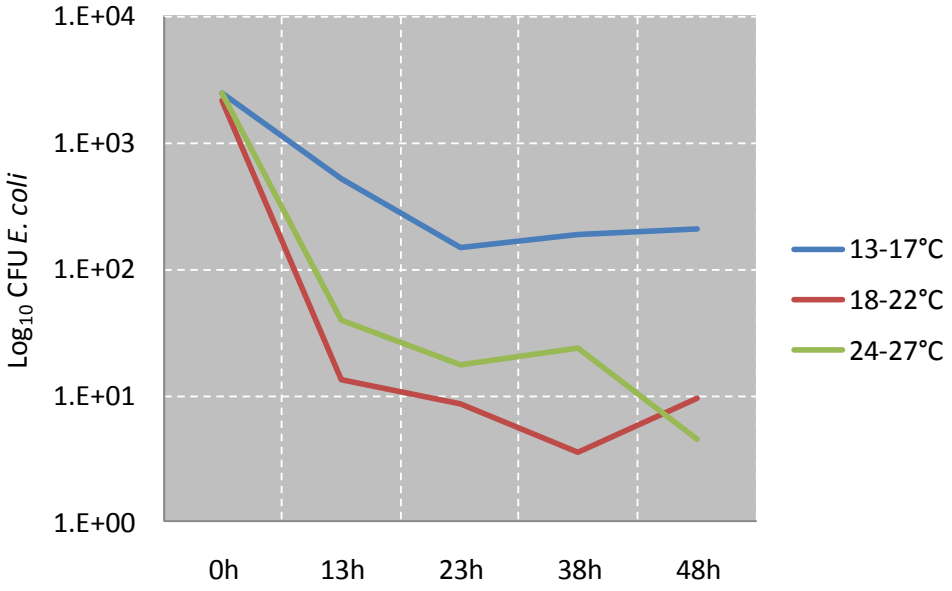

**Figure 4. Levels of *E. coli* in the Sydney cupped oyster (*C. commercialis*) at three temperature ranges. Data from Rowse and Fleet (1984).**

Šolić *et al*. (1999) collected Mediterranean mussels (*M. galloprovincialis*) and native oysters (*O. edulis*) from shellfish production areas in Split (Croatia) and maintained them in the laboratory in 30-litre fibreglass trays containing 15-litre of seawater to study the effect of temperature on faecal coliform accumulation in shellfish. Each tray was aerated and thermostated at experimental temperature. Ambient temperatures were changed to experimental ones at the rate of 0.5°C per day. After the experimental temperatures were reached, the bivalves were allowed to acclimate for at least 2 days. After this acclimation period, treatments under different conditions were run. The temperatures tested were 12°C (mean winter temperature), 24°C (mean summer temperature) and 18°C (temperature between two extremes). The faecal coliform concentrations in the trays were 10–$10^3$, $10^3$–$10^5$ and $10^5$–$10^7$ faecal coliforms $L^{-1}$. Results indicated that both temperature and the concentration of faecal coliforms in the seawater influenced faecal coliform accumulation rates and the slope of its decrease during the experiments. In mussels, as the concentration of faecal coliforms increased, the rates of concentration decreased more rapidly at the higher temperature. However, at the "turning point" concentration[3] in mussels, the rates of faecal coliform concentrations in shellfish were similar at all temperatures. As concentrations of the indicator in shellfish increased, concentration rates became inversely proportional to the temperature. Therefore, the lower the temperature the higher the maximal faecal coliform concentration in shellfish and more time required to reach that concentration. A similar pattern was observed in oysters, in which the highest concentration rate was obtained at 18°C (Table 5).

**Table 5. Time required for mussels and oysters to achieve maximum faecal coliform concentrations 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 |

[3] Concentration of faecal coliforms in bivalves at which positive correlation between concentration rate and temperature turns into negative correlation.

| | | | |
|---|---|---|---|
| | $10^5$–$10^7$ | 0.71 | 1.64 |

In general, filtration rate increases then stays constant at a level dependent on temperature (Šolić *et al*., 1999). Many bivalves may tolerate temperatures of 25°C or more. Evidence from field and laboratory experiments indicates that, in the above circumstances, temperatures above 20°C can be stressful and can result in mortalities, even after the animals have been transferred to ideal conditions (Laing and Spencer, 2006). Oysters and clams are tolerant of low winter temperatures down to around 3-5°C, below which deaths may occur.

The minimum temperatures for survival and growth of *O. edulis* are 4°C and 9°C, respectively (Table 6). Rödström and Jonsson (2000) found that feeding activity in this species along the Swedish west coast, as measured by the production of faecal material, is significantly lower when oysters are exposed to temperatures of 5°C compared to 10°C.

**Table 6. Summary of literature on effects of temperature on feeding activity in bivalve molluscs.**

| Species | -4 | -3 | -2 | -1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *T. decussatus* | | | | | | | | | | | | | Preferred range | | | | | | | | | | | | | | | | | | | | | | | Laing and Spencer (2006) |
| *Mytilus* spp. | | | | | | Pumping rate increases with increasing temperature | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Jørgensen (1990) |
| *Mytilus* spp. | | | | | | | | | Valve opening retarded | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Jørgensen (1990) |
| *Mytilus* spp. | | Minimum† | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Laing and Spencer (2006) |
| *E. arcuatus* | | | | | | | | | | | | | | | Optimum* | | | | | | | | | | | | | | | | | | | | | | Younger et al. (1999) |
| *O. edulis* | | | | | | | | | Minimum† | | | | Optimum for growth | | | | | | | | | | | | | | | | | | | | | | | Laing and Spencer (2006) |
| *C. gigas* | | | | | | | | | | Minimum† | | | Optimum for growth | | | | | | | | | | | | | | | | | | | | | | | Laing and Spencer (2006) |
| *Pecten* spp. | | | | | | | | Minimum† | | | | | Optimum for growth | | | | | | | | | | | | | | | | | | | | | | | Laing and Spencer (2006) |

** for depuration*

*† minimum for survival*

Mussels, on the other hand, are very resistant to low temperatures and are able to ingest food particles and show some growth even during the winter. Other factors may influence the ability of bivalves to tolerate low temperatures. For example, scallops can survive temperatures as low as 3°C at salinities above 30 practical salinity units (psu), but they may die at temperatures below 5°C if salinity falls to less than 26 psu. This illustrates the point that two or more environmental factors acting together may induce a stress in bivalves that is much greater than that of any one factor acting alone (Laing and Spencer, 2006).

Bernard (1989) studied the kinetics of accumulation and clearance in the Pacific oyster (*C. gigas*), blue mussel (*M. edulis*), the Littleneck clam (*Protothaca staminea*) and in the Soft shell clam (*Mya arenaria*). Shellfish were maintained in 7000-litre tanks containing seawater and dosed with crude sewage (6.6–20.2 x $10^6$ faecal coliforms $100ml^{-1}$). Accumulation experiments were undertaken after an acclimation period of 6h and undertaken at 7, 12 and 17°C. The outdoor tanks were covered with black plastic to reduce bacterial die-off due to UV radiation. Patterns of faecal coliform accumulation varied between species, but were all positively correlated with temperature. The shapes of the temperature specific curves and the maximum accumulation levels were different. Interestingly, the lowest accumulation level in mussels was at 12°C, whereas in the other three species it was maximal at 12°C and significantly less at 17°C. Table 7 shows the time required for shellfish to accumulate 300 faecal coliforms on the basis of the equations obtained by Bernard.

**Table 7. Relationships between levels of faecal coliform bacteria and time at three different temperatures and time required to accumulate 300 FC $100g^{-1}$ in three species of bivalve molluscs**

| Species | Temp. (°C) | Equation | $R^2$ | Time (hh:mm) required to accumulate 300 FC $100g^{-1}$ |
|---|---|---|---|---|
| *Mya arenaria* | 17 | $Log_{10}$ MPN FC $100g^{-1}$=-275.27+432.17*time (h) | 0.88 | 01:20 |
| | 12 | $Log_{10}$ MPN FC $100g^{-1}$=-599.13+615.94*time (h) | 0.99 | 01:27 |
| | 7 | $Log_{10}$ MPN FC $100g^{-1}$=-191.33+167.57*time (h) | 0.99 | 02:56 |
| *M. edulis* | 17 | $Log_{10}$ MPN FC $100g^{-1}$=-2453+4311.86*time (h) | 0.87 | 00:38 |
| | 12 | $Log_{10}$ MPN FC $100g^{-1}$=-892.73+1062.97*time (h) | 0.84 | 01:07 |
| | 7 | $Log_{10}$ MPN FC $100g^{-1}$=-204.13+1113.51*time (h) | 0.88 | 00:27 |
| *C. gigas* | 17 | $Log_{10}$ MPN FC $100g^{-1}$=-512.33+641.57*time (h) | 0.99 | 01:16 |
| | 12 | $Log_{10}$ MPN FC $100g^{-1}$=-82.90+551.80*time (h) | 0.96 | 00:41 |
| | 7 | $Log_{10}$ MPN FC $100g^{-1}$=-182.13+153.50*time (h) | 0.96 | 03:08 |

*Equations adapted from Bernard (1989).*

It should be noted that these estimates are based on the author's linear regression equations. However the study implied that overall the best bit for those relationships is exponential.

Burkhardt III *et al.* (1992) examined the effects of temperature and season on the ability of the American hard clam to filter and retain several indicator microorganisms in Naragansett Bay (USA). The experiment consisted of exposing shellfish to ambient seawater to which a constant amount of raw wastewater was added to keep indicator levels in overlying water relatively constant. The ability of the clams to concentrate contaminants was considerably reduced when ambient seawater temperatures were below 7°C. This was attributed to their diminished physiological activity. When water temperatures fell below 4.5°C, bioaccumulation was interrupted. During the spring, when temperatures increased from 4.5 to 11.5°C, the threshold temperatures between which animal activity was significantly influenced correlated with a marked increase in the accumulation of FC and *E. coli*. Reduced metabolic rate following contaminant uptake in shellfish is considered to be one of the main causes for the periodic deterioration of the microbial quality of oysters from the outer Tomales Bay (USA), particularly during the winter when oysters require more time for cleansing microbial contamination (California Regional Water Quality Control Board, 2001).

### *4.3 Effect of salinity*

Changes in salinity do not affect the growth of bivalves as much as variation in temperature (Laing and Spencer, 2006). However, most bivalves are very sensitive to dilutions of seawater and, generally, valve opening is progressively delayed with decreasing salinity (Motwani, 1956). This author found that in mussels (*M. edulis*) there is a considerable retardation in the time of valve opening in waters with salinity levels lower than 17.4‰ and that this critical threshold in relation to valve opening roughly corresponds to the lower limit for survival typical in UK coasts. Therefore, mussels will usually only feed at higher salinities (20–35psu) (Laing and Spencer, 2006). Flat oysters and clams prefer these higher salinities. Scallops are very intolerant of salinities lower than 30psu, so sites with a high inflow of fresh water are not suitable for the cultivation of these species. Mussels grow well above 20psu and Pacific oysters prefer salinity levels nearer to 25psu (Laing and Spencer, 2006).

Oyster species can be subdivided further according to their distribution in water of different salinities and temperature from shallow areas to the open sea. Generally, *Ostrea* species are distributed in areas with full strength salinities. Feeding rates in *O. edulis* began to decline at 28‰ and cease at 16‰ (Rödström and Jonsson, 2000). Those belonging to the genus *Crassostrea* tend to be more euryhaline and they are found in shallow water with fluctuating salinity levels. Generally,

oyster species belonging to the genera *Ostrea* are found below low water in the sublittoral zone and *Crassostrea* species in the intertidal zone (Park *et al*. 1988).

Salinity affects both pumping rates and filter-feeding processes in shellfish were also studied by Rowse and Fleet (1984) (for further detail, see section 4.2 and Table 11 below). Samples of 20 individuals were removed from tanks following exposure of shellfish contaminated with *E. coli* to water salinities of 16-20‰, 32-36‰ and 43-47‰[4] at specified times during the clearance period Results indicated slow and inconsistent clearance in oysters exposed to waters in the lower salinity range (Figure 5). At the end of the clearance period, the differences in *E. coli* levels in oyster flesh exposed to the different salinity levels exceeded 1$\log_{10}$. In one of the trials at 16–20‰, high numbers of bacteria (4,300) persisted after the 48h clearance period. In addition, low salinities induced oyster mortality.

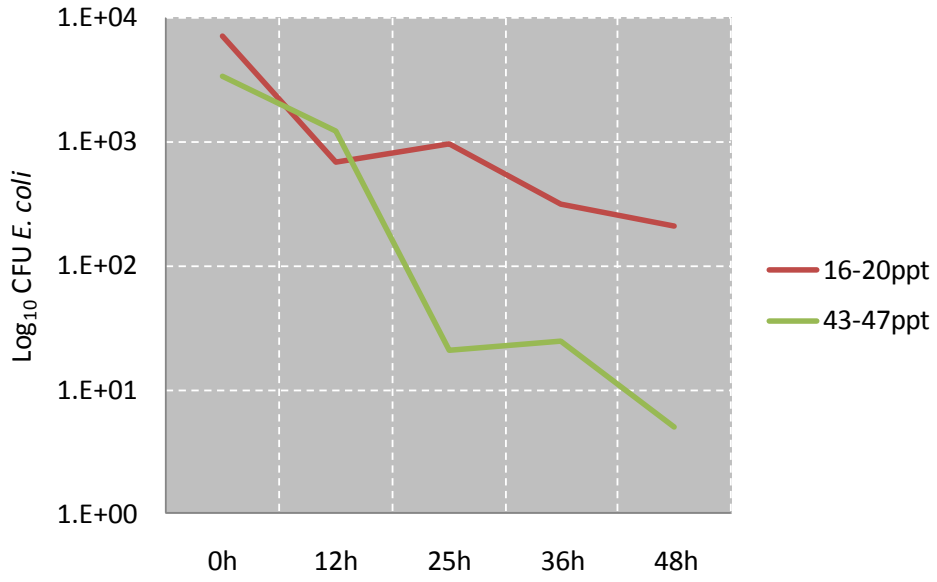


**Figure 5. Levels of *E. coli* in the Sydney cupped oyster (*C. commercialis*) at three salinity ranges. Data from Rowse and Fleet (1984).**

Razor clams (*Ensis* spp.) are commonly associated with 'fully' saline waters. Younger *et al*. (1999) undertook purification trials in *E. arcuatus* at four salinity levels (20, 25, 30 and 35‰) at an average temperature of 10.75°C. On the basis of dissolved oxygen consumption rates, Younger *et al.* concluded that the level of activity is greater at 35‰ than that at the other three salinities. Visual inspection confirmed these results, suggesting that shellfish had very little activity at 20‰. In a separate experiment using *E. siliqua*, the pattern of filtration activity at 50% dissolved oxygen and

[4] The recommended minimum salinity for depuration of Pacific oysters in the UK is 20.5‰.

35‰ salinity appeared to be similar to that obtained at 100% dissolved oxygen and 20‰ salinity suggesting that the shellfish do not filter-feed well at 50% dissolved oxygen despite being in fully saline waters.

Cabelli and Heffernan (1970) studied factors significant to the uptake and elimination of faecal coliform bacteria by the soft shell clam (*Mya arenaria)*. Results indicated similar uptake kinetics to that in American hard clams summarised above (see section 4.1). In contrast to the American clams, most of the soft shell clams accumulated microorganisms very shortly after being placed in the environmental water. The lower limits on the temperature, salinity and flow rate of the environmental water for acceptable elimination (depuration) were about 10°C, 20‰ and 7ml rain per animal, respectively.

Fanshawe (1995) investigated the effect of reduced salinity on the uptake and elimination of *E. coli* by mussels. Its experimental protocol consisted of maintaining mussels collected in Menai Strait (Wales) in 140-litre tanks in continuously circulating closed system containing UV disinfected water. Two sets of experiments were performed:

(a) visual inspection of mussel behaviour at low salinity by means of adding distilled water to tank water (initial salinity = 32‰) until 10‰ was reached;
(b) uptake and elimination of *E. coli* by mussels were tested at 32‰, 20‰ and 16‰.

During the first set of experiments, all mussels appeared to be fully closed when salinity reached 15‰ and reopened their valves again at 19‰. During the second set of experiments, levels of the indicator in mussel flesh increased consistently within 4h exposure when immersed in water at three different salinities (Table 8).

**Table 8. Accumulation and clearance of *E. coli* by mussels immersed in water at three salinities in experiments undertaken by Fanshawe (1995).**

| | Exposure time (h) | | | Clearance time (h) | | |
|---|---|---|---|---|---|---|
| Salinity (‰) | 1 | 4 | 6 | 10 | 13.5 | 24 |
| 32 | $7.2 \times 10^5$ | $7.1 \times 10^6$ | $1 \times 10^6$ | $6.7 \times 10^4$ | $1.2 \times 10^4$ | $9.6 \times 10^3$ |
| 20 | $6.4 \times 10^4$ | $1.5 \times 10^5$ | $7.9 \times 10^5$ | $8.5 \times 10^4$ | $3.8 \times 10^4$ | $1.1 \times 10^4$ |
| 16 | $1.1 \times 10^4$ | $7 \times 10^4$ | $1.3 \times 10^5$ | $7.3 \times 10^4$ | $5.8 \times 10^4$ | $7.4 \times 10^3$ |

The author considered that these are representative of the low extreme at which one might expect to elicit a response (16‰), a concentration known to cause mussels to respond to their surroundings (20‰) and a concentration typical of normal seawater (32‰).

Statistically significant (Tukey's pairwise comparison) differences were found between the accumulation in mussels immersed in water at 32‰ and those immersed at 20‰ and 16‰. At the end of the uptake phase, the *E. coli* content in mussels decreased in mussels kept at 32‰ and increased in mussels kept at 20‰ and 16‰. Within the four hours clearance period, the *E. coli* content in mussels decreased by at least $1\log_{10}$. At the end of the clearance period, the *E. coli* content in mussels kept at salinities 32‰, 20‰ and 16‰ decreased $3\log_{10}$, $1\log_{10}$ and $2\log_{10}$ relative to that at of the start of the clearance period, respectively. The optimal salinity ranges, and key thresholds relating to salinity in commercially important bivalve mollusc species reported in the literature, are summarised in Table 9 below.

**Table 9. Summary of literature on effects of salinity on feeding activity in bivalve molluscs.**

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *T. decussatus* | | | | | | | | | | | | | | | | | | | | | | | | | Preferred range | | | | | | | | | | | | Laing and Spencer (2006) |
| *E. arcuatus* | | | | | | | | | | | | | | | | | | | | Activity increases with increasing salinity | | | | | | | | | | | | | | | | | | Younger et al. (1999) |
| *M. arenaria* | 20=limit for clearance | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Cabelli and Heffernan (1970) |
| *Mytilus* spp. | Valve opening retarded with decreasing salinity | | | | | | | | | | | | | | | | | | | | | | | | Usual feeding range | | | | | | | | | | | | Motwani (1956) |
| *Mytilus* spp. | | | | | | | | | | | | | | | | | | | | | Mussels grow well >20psu | | | | | | | | | | | | | | | | Laing and Spencer (2006) |
| *C. gigas* | | | | | | | | | | | | | | | | | | | | Preferred salinity 25psu | | | | | | | | | | | | | | | | | Laing and Spencer (2006) |
| *O. edulis* | | | | | | | | | | | | | | | | | | | | | | | | | Optimal for growth | | | | | | | | | | | | Laing and Spencer (2006) |
| *O. edulis* | Feeding rate ceases at 16psu | | | | | | | | | | | | | | | | Feeding rate declines <28psu | | | | | | | | | | | | | | | | | | | | | Rödström and Jonsson (2000) |
| *Pecten* spp. | Intolerant | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Tolerant | | | | | | Laing and Spencer (2006) |

# 5. FIO accumulation in shellfish: evidence from environmental data

Significant differences in relative levels of FIOs accumulation between different species of shellfish have been reported in environmental studies. Levels of *E. coli* in common cockles (*C. edule*) commercially harvested in France have been shown to be approximately 3 times higher than those in Pacific oysters from the same waters (Amouroux and Soudant, 2011). 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). The average *E. coli* accumulation in mussels (*Mytilus* spp.) commercially harvested in England and Wales is 1-2 times greater than that in Pacific oysters (*Crassostrea gigas*) (Table 10). Using 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.

**Table 10.**

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

| Shellfish species | Native oyster (*O. edulis*) | Pacific oyster (*C. gigas*) | Common cockle (*C. edule*) | Manila clam (*T. philippinarum*) | American hard clam (*M. mercenaria*) |
|---|---|---|---|---|---|
| Mussels (*Mytilus* spp.) | 1.5 (n=596) | 1–2 (n=1837) | [0.8] (n=113) | =[1.4] (n=64) | No data |
| Native oyster (*O. edulis*) | | = (n=227) | =[0.2] †* (n=11) | =[0.5]?* (n=11) | 2.4 (n=153) |
| Pacific oyster (*C. gigas*) | | | 1–0.1 (n=145) | 0.3–0.1 (n=253) | No data |
| Common cockle (*C. edule*) | | | | =[0.9] (n=148) | No data |
| Manila clam (*T. philippinarum*) | | | | | No data |

*Adapted from Younger and Reese (2011).*

** denotes inconclusive data. n denotes number of paired samples analysed. Square [] denotes non-statistically significant ratio observed in the study.*

*† denotes non-statistically significant ratio likely due to low number of paired samples.*

Whilst 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 are subject to different contamination effects than those grown on the riverbed.

Similarly, for shellfish grown in ropes or lantern nets, there may be a difference in contamination influence with depth in the water column (Younger *et al*. 2003).

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) found accumulation factors for faecal coliforms and enterococci in mussels from Concarneau (France), an area directly impacted by sewage discharges, of 13.2 and 250 (arithmetic MPN per 100ml), respectively. These variations were assumed to be associated with seasonal effects and/or differential accumulation dynamics between indicators/groups of indicators (Plusquellec *et al.,* 1990; Prieur *et al*., 1990).

# 6. Relationship between FIO threshold levels in water and shellfish contamination

### *6.1 Balance between level of exposure and time of exposure*

Timoney and Abston (1984) studied contamination and subsequent elimination of *E. coli* and *Salmonella typhimurium* in *Mercenaria mercenaria* held in aquaria and transferred to depuration tanks containing water sterilised by UV radiation. The authors found that both species of bacteria were accumulated to a similar degree and estimated that, after 15 minutes of exposure, each clam would accumulate 1 x $10^6$–1 x $10^7$ CFU of *E. coli*, what corresponded to a contamination rate of about 1 x $10^8$–2 x $10^8$ CFU $100g^{-1}$ clam tissue. Assuming a pumping rate of 100 ml $minute^{-1}$ at 20°C, the authors estimated that each clam would pump *circa* 7.5 x $10^7$ organisms, of which roughly one-tenth would be retained in their tissues.

Figure 5 shows that mussels and cockles are more efficient in clearing *E. coli* than oysters and scallops.

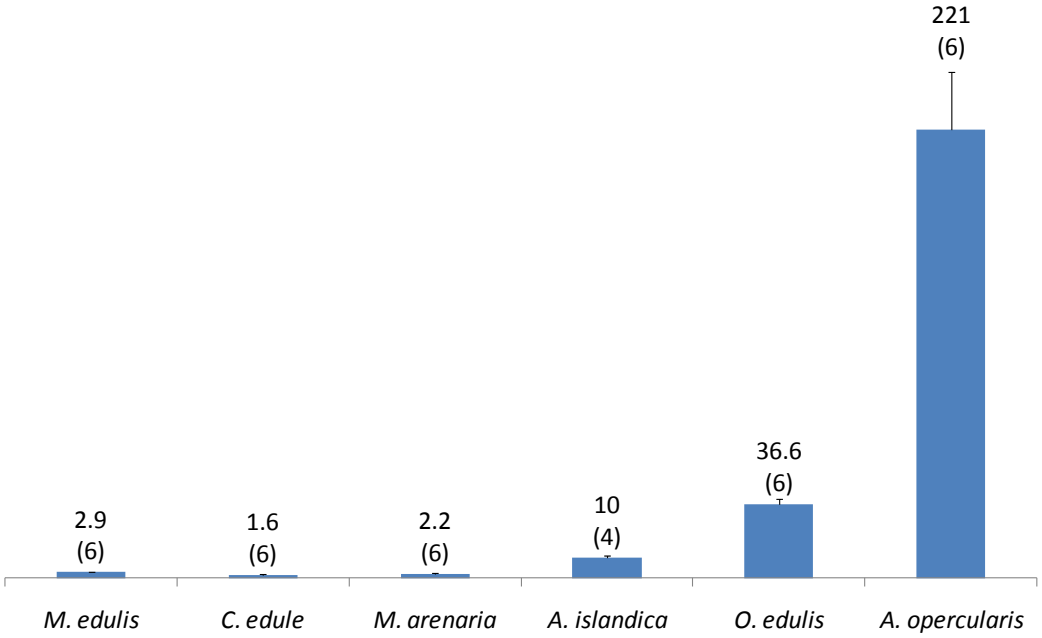


**Figure 5. Time (hours + standard error of the mean followed by the number of experiments in parenthesis) to clear 90% of [$^3$H]TdR-radiolabelled *E. coli* by six species of bivalves. Data from McHenery and Birkbeck (1985). *M. edulis*=blue mussel; C. edule=common cockle; *M. arenaria*=soft-shell clam; *A. islandica*=Icelandic cyprine (not commercially harvested in England and Wales); *O. edulis*=native oyster; *A. opercularis*=queen scallop.**

Using radiotracer methods, Charles *et al.* (1992, 1992a) found that the Mediterranean mussel (*Mytilus galloprovincialis*) takes approximately half of the time required by venus clams (*Venus verrucosa*) to assimilate a given quantity of *E. coli* suspension in the water. In this study, this difference was corroborated by the kinetic filtration coefficients obtained (0.120 $h^{-1}$ for clams versus 0.280 $h^{-1}$ for mussels).

In the study by Beucher (1993) (see section 4.1 above), more than 90% of the shellfish population were contaminated after 2-4h exposure to contaminated water. Three distinct phases were observed during the accumulation phase:

- 0-1h: the number of contaminated oysters increased quickly. However, significant variation was observed in the level of contamination between individuals. During this phase, the limiting factor was the number of active oysters and their levels of filtration activity.
- 1-20h: the number of contaminated oysters reached its maximum. The level of contamination progressed in relation to exposure time according to the linear model $\log_{10}$

CFU faecal coliforms = 2.9 + 0.05time (h). The levels of contamination in the oyster population tended to be more homogeneous.

- After 20h: the level of contamination in the oyster flesh reached its maximum.

Ho and Tam (2000) maintained UV-depurated green mussels (*Perna viridis*) in tanks (40 litres) exposed to natural light. The mussels initially contained less than 4 *E. coli* $100g^{-1}$ FIL when laid in sterile seawater inoculated with high concentrations of cultured *E. coli* ATCC 25922 (two spot concentrations: $2.6 \times 10^3$ and $2.9 \times 10^5$ $100ml^{-1}$). Levels of *E. coli* accumulated by mussels peaked within 3–5h exposure and reached 50–100 fold of the seawater concentration. Levels of the indicator subsequently declined, first sharply, then at a slower rate within the following 15–20h to approximately 0.2% of the peak level at the end of the experiment. The authors observed a slight uptake of *E. coli* at around 25–30h and 40–45h. *E. coli* levels in shellfish remained relatively constant thereafter. The concentration of *E. coli* in tank water also declined, initially sharply when mussels exhibited higher uptake rates, and then gradually declined to less than 1% of the initial concentration at the end of the experiment. An increase in *E. coli* levels in tank water was observed in all experiments just prior to the second uptake of bacteria by the mussels at around 25–30h.

Martins *et al.* (2006) maintained native clams (*Ruditapes decussatus*) in 20-litre aquaria containing seawater contaminated with *E. coli* ATCC 25922. Levels of the indicator in the water were $3 \times 10^8$ $100ml^{-1}$ and $9 \times 10^4$ $100ml^{-1}$ in the first and second experiments, respectively. Accumulation of *E. coli* by clams followed the same pattern during the experiments, but differed according to the initial concentration in the water. Levels of the microorganism in clams did not exceed those in the water during the first 6h of the first experiment, reaching a maximum concentration of $1.3 \times 10^9$ MPN $100g^{-1}$. In the second experiment, levels of *E. coli* exceeded those of the water within 30 minutes of exposure.

Plusquellec *et al*. (1990) studied the mechanisms of uptake, retention and elimination of bacteria in mussels (*M. edulis*) during laboratory microcosm experiments. Depurated mussels were maintained in 100L aquaria aerated and stabilised at 19°C and inoculated with *E. coli*, *Streptococcus faecalis* and *Salmonella anatum* cultivated in the laboratory and added to a suspension of sterilised domestic sewage. Mussels exposed to sudden bacterial input attained maximum concentrations in the flesh within 30 minutes of the experiment (i.e. confirming the results obtained in clams by Martins *et al.* (2006). After the initial accumulation phase, contamination in mussel flesh remained higher than that in the overlying water. After the uptake phase, the accumulation factor remained relatively

constant at about 10. To determine the effect of bacterial loading from the tank water to the shellfish flesh, the authors tested different *E. coli* densities ranging from 4 x $10^1$ $ml^{-1}$ to 3 x $10^7$ $ml^{-1}$. Results from the experiment with the lowest *E. coli* concentration in tank water showed a contamination curve in mussels different from that described previously: i.e. after the accumulation stage in mussel flesh, *E. coli* levels in tank water exceeded those in the flesh at approximately 125 minutes of exposure and remained higher than those in mussel flesh thereafter. In contrast, the contamination pattern found in mussels for tank water *E. coli* 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. Taken together, these results indicate that there is a minimum level of contamination in the water above which the enrichment in mussel flesh is not influenced by contamination in the water.

### *6.2 Dynamics of FIO clearance*

Removal of *E. coli* in *M. mercenaria* was studied by Timoney and Abston (1984) as referred above. A group of clams was taken from the exposure tank and placed in the elimination tank containing sterilised water. *E. coli* elimination was rapidly cleared from shellfish, with the greatest clearance occurring over the first 8h of the experiment and then less evident in the following hours. By 24h exposure to clean water, *E. coli* counts had fallen by factors of $10^4$. Bacterial removal was studied both in a small group of clams collected from the exposure tank and in the group that was placed in the clearance tank. Bacterial counts in the water from the holding tank in which the clams were placed were much lower than counts in clam homogenates for the first 8h of the experiment. By then, counts in clam homogenates approximated those in the water. By 24h, they were higher than in homogenates, in which they continued to decline. The greatest concentration of *E. coli* was found in faeces and pseudofaeces, in which they appeared to be closely bound to the particulates. Only a small proportion of bacteria were free in the water, suggesting that their association with faecal material was stable. By 2h exposure, each clam had eliminated about 1.4 x $10^7$ CFU *E. coli* and continued to eliminate bacteria over the next 4h.

The study of uptake and elimination of coliform bacteria by Bernard (1989) (see section 4.1) suggested that, independently of temperature variations, mussels are more efficient than Pacific oysters and littleneck clams in clearing FIOs. This author argued that there is no relationship between clearance rate and ventilation volume and suggested that the initial level of accumulation has no influence on length of time to clearance.

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 pure running seawater. Progressive bacterial elimination was observed with a four day period necessary to achieve complete elimination of *E. coli* from the mussels. Clearance rates of approximately 100 times the initial concentrations were observed in shellfish flesh within 24h exposure to clean waters.

In the study by Beucher (1993) (see section 4.1 above), more than 90% of the shellfish population had completely cleared this contamination within 30min-4h exposure to clean waters. It took a maximum of 4h for more than 95% of the oyster population to evidence filtration activity during the clearance phase. Two different phases were identified during this clearance stage as follows:

- 0–10h: the level of contamination in the oysters decreased quickly in relation to exposure time according to the linear model $\log_{10}$ CFU faecal coliforms = 2.8 - 0.7$\log_{10}$ time (h). Some oysters reach maximum clearance levels within 1h of clearance phase. Some variability was observed in terms of the number of oysters showing active filtration; however, most of the oyster population showed filtration activity within 3h clearance phase. The limiting factor during this phase was thus the difference in filtration activity between individual animals.
- 10–30h: all oysters reached minimum levels of faecal contamination. Any differences in the levels of contamination between individual oysters could be equivalent to the limits of sensitivity of different enumeration methods.

# 7. Conclusions

7.1 The relationships between FIOs in shellfish and those in the overlying water in environmental studies are usually weaker than those in controlled microcosms. These differences are usually attributed to:

i) the duration of the contaminating event(s);
ii) variations in the background quality of the overlying waters (both spatially and temporally), and, to a lesser extent;
iii) the natural habitat of wild farmed species and bivalve growing method for cultured species; and
iv) the physiology of each species.

7.2 Evidence from experiments on the physiology of water processing by shellfish was summarised in this report to provide guidance on the fourth factor above. The concentration of particulate matter processed by filter-feeding shellfish is determined by the capacity of the pump and the concentration of food in the water. The animals tend to be fully open and process water at relatively constant rates under normal conditions of salinity and temperature for the species. However, undisturbed shellfish may also process water at lower rates than those expected under maximal capacity of the pump. Any deviations from these normal conditions and changes in the viscosity of the water may cause partial to complete closure of valves.

7.3 Exposure to a source of pollution increases the numbers of animals contaminated to the maximal concentration as determined by the physiology and concentration of food in the ambient water for optimal environmental conditions for each species. There is evidence in the literature that the concentrations of faecal indicators accumulated by shellfish may exceed those in the overlying waters within 30 minutes exposure to the pollution source. However, there are significant differences between species: cockles and mussels tend to accumulate faster and to a greater level than oysters.

7.4 Three distinct phases have been identified during the period of accumulation:

a) Quick accumulation during the first hour, characterised by significant variation between different individuals of the same population;

b) Followed by a period of less than 20h during which the levels of contamination in shellfish flesh tend to be more homogeneous.

c) A period during which all individuals of the population reach the maximum levels of contamination. The duration of this period is mainly determined by the physiology of the species, the levels of contamination in the ambient water and the concentration of food in the water.

7.5 Relatively few studies are published that evidence 'chronic' effects of FIO contamination and accumulation in bivalve molluscs, beyond 48hours exposure.

7.6 The sensitivity and tolerance towards the natural environmental conditions are reflected by the growth and survival characteristics of each species. Intertidal species are more subject to instability periods (e.g. depleted food or oxygen in the water, exposure during periods of low water) than subtidal species.

7.7 FIO accumulation factors obtained in microcosm studies appear to be relatively similar with respect to the coliform group. Larger variations have been detected in environmental data. Data from shellfish safety monitoring programmes indicates higher average FIO accumulation rates in mussels and cockles than in Pacific oysters. Laboratory experiments also suggest that, whilst mussels may concentrate FIOs to a higher level, the time required for clearance of bacteria in oysters is longer. These inter-species differences are consistent with higher filtration rates in the former group of species.

7.8 Studies using the Pacific oyster indicate that this species could reach an "equilibrium" phase between the accumulation and clearance phases within 20h of active filtration. This "equilibrium" varies however between seasons and between individuals of the same species.

7.9 There is very little evidence on the relationship between the level of exposure and the time of exposure. Some accumulation experiments suggest that there is a threshold of FIO concentrations in the water above which bivalves are unable to accumulate more bacteria. This will vary for individual species according to variations in environmental factors (e.g. temperature, salinity, food availability and water exchange), from the species optimum.

7.10 When comparing FIO uptake in and clearance from shellfish at selected salinities, it is obvious that these animals accumulate the microbiological indicators more quickly in saline waters than at brackish waters. Interestingly, shellfish held in water at salinities close to the lower limit of normal functioning for valve closure, show considerable ability to accumulate FIOs in a short space of time.

7.11 FIOs are rapidly cleared from shellfish when these animals are exposed to clean waters. There is an initial phase of greatest clearance, which usually lasts less than 10h, followed by less evident clearance phase lasting 10–30h, during which levels in the population usually reach levels below the limit of detection of conventional enumeration methods. Clearance rates of approximately 100 times the initial concentrations have been observed in mussels and oysters within 24h exposure to clean waters.

A summary table of key information on FIO accumulation and clearance is shown in Table 11 below.

**Table 11. Summary data of faecal indicator organism accumulation and clearance in shellfish.**

| Shellfish species | Indicator | Faecal Indicator organism CFU/MPN $100ml^{-1}$ | | | Temp. (°C) | Salinity (ppt) | Bivalve acclimation period (d:hh:mm) | FIO contamination period (hh:mm) | Observations on clearance | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Water target | Water measured | Bivalve measured* | | | | | | |
| *C. commercialis* | *E. coli* | $1 \times 10^4$ | - | $2 \times 10^2$ | 13–17 | 32–36 | 00:40 | 01:30 | Inconsistent and incomplete | Rowse and Fleet (1984) |
| *C. commercialis* | *E. coli* | $1 \times 10^4$ | - | $1 \times 10^1$ | 18–22 | 32–36 | 00:40 | 01:30 | Rapid and uniform | Rowse and Fleet (1984) |
| *C. commercialis* | *E. coli* | $1 \times 10^4$ | - | $5 \times 10^0$ | 24–27 | 32–36 | 00:40 | 01:30 | Slower and more inconsistent than that at 18–22°C | Rowse and Fleet (1984) |
| *C. commercialis* | *E. coli* | $1 \times 10^4$ | - | $2 \times 10^2$ | 18–22 | 16–20 | 00:40 | 01:30 | Slow and inconsistent. Oyster mortalities exceeded 30% | Rowse and Fleet (1984) |
| *C. commercialis* | *E. coli* | $1 \times 10^4$ | - | $5 \times 10^0$ | 18–22 | 43–47 | 00:40 | 01:30 | No abnormal mortalities observed | Rowse and Fleet (1984) |
| *M. edulis* | Faecal coliforms | - | $4 \times 10^3$-$3 \times 10^9$ | <20 | 19 | | - | 22:00 | 4 days necessary to achieve clearance down to undetectable levels | Plusquellec *et al*. (1990) |
| *P. viridis* | *E. coli* | - | $2.6 \times 10^3$ | $\approx 4 \times 10^2$ | 17–20 | 30 | - | - | *E. coli* levels in mussel flesh and water were 0.2% and 1% of the peak levels at the end of the clearance period | Ho and Tam (2000) |
| *P. viridis* | *E. coli* | - | $2.9 \times 10^5$ | $\approx 2.9 \times 10^4$ | 17–20 | 30 | - | - | See above | Ho and Tam (2000) |
| *M. mercenaria* | *E. coli* | $5 \times 10^6$ | - | $<3 \times 10^3$ | 20 | 22 | - | 00:15 | - | Timoney and Abston (1984) |

**End of clearance period*

*All figures converted to be expressed as $100ml^{-1}/100g^{-1}$ as appropriate.*

# 8. Knowledge gaps

This literature review revealed three main obstacles to the development of numerical models predicting contaminant burden in commercially harvested shellfish. Firstly, there is an obvious paucity of data characterising prolonged exposure of shellfish to microbial pollution and this constrains our ability to adequately characterise the functional relationship between the length of exposure and level of contamination in shellfish flesh. In the vast majority of the studies appraised, exposure of shellfish populations to contaminated water rarely exceeded 48h. Furthermore, the sampling frequency used in many studies does not adequately allow characterisation of the variation of the pollutant concentration with time. These findings are perhaps unsurprising given the difficulties associated with maintaining shellfish in continuous flow-trough tank systems in the laboratory. Secondly, the technical challenges posed by targeting and maintaining low to moderate FIO concentrations in the receiving tank water, particularly those near the limit of detection of the enumeration methods was also apparent from this review. This gap in knowledge presents a limitation in our ability to adequately characterise the dynamics of FIO clearance in shellfish flesh during periods of good water quality. Thirdly, very little information exists outside the scope of commercial depuration experiments comparing a range of dosed FIO levels in more than one shellfish species. Future research in this respect would assist development of technical guidance on monitoring regimes to ensure protection of shellfish beds under their statutory obligations.

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**Head office**
**Centre for Environment, Fisheries & Aquaculture Science**
**Pakefield Road, Lowestoft,**
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**Tel +44 (0) 1502 56 2244**
**Fax +44 (0) 1502 51 3865**

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**Centre for Environment, Fisheries & Aquaculture Science**
**Barrack Road, The Nothe**
**Weymouth, DT4 8UB**

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