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Time trends in the prevalence of *Escherichia coli* and enterococci in bivalves harvested in Norway during 2007–2012

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ABSTRACT

The objectives of this study were to describe time trends in the prevalence of *Escherichia coli* and enterococci in cultured blue mussels (*Mytilus edulis*) harvested from 152 localities along the coast of Norway during the six-year period from 2007 to 2012. Based on the available data, possible co-occurrence of these two indicator organisms of faecal contamination was assessed. Several localities for bivalve cultivation in Norway showed single high counts of *E. coli*, without any previous history of *E. coli* detection. For other localities, the pattern of *E. coli* detection was recurring, however low values were found, with some sporadic findings of higher values. There was a weak positive correlation between the detection of enterococci and *E. coli*, and a weak positive correlation between counts of *E. coli*/enterococci and rainfall. Sampling intervals should take into account knowledge of the occurring variation for bacterial faecal indicators, local knowledge on possible exposure to faecal material from livestock or humans, rainfall seasons, topography of the location, as well as tidal and water current patterns.

1. Introduction

Escherichia coli and bacteria in the genus *Enterococcus* are found in the faeces of warm-blooded animals, including humans, in high and relatively stable concentrations. The density of *E. coli* in human faeces normally varies from 10^6 to 10^7 cells g^{-1} (Forsythe, 2010). The corresponding numbers of *Enterococcus faecalis*, which are quantitatively the most important enterococci in humans, are reported to be 10^5 to 10^6 cells g^{-1} (Forsythe, 2010). However, the enterococci are known to survive better in the environment and may therefore be an indicator for older faecal contamination (Noble, Lee, & Schiff, 2004). Both *E. coli* and enterococci are frequently used as indicator organisms of faecal contamination of potable and recreational water, as well as foods.

Bivalves such as blue mussels (*Mytilus edulis*) are suspension feeders and may retain particles at 4 μm with 100% efficiency (Møhlenberg & Riisgård, 1978), yet seasonal variations in retention efficiency have been shown (Strohmeier, Strand, Alunno-Bruscia, Duinker, & Cranford, 2012). Suspension feeders may ingest viruses and bacteria bound to particles. Depending on numerous factors, including size, habitat and availability of feed organisms, an adult blue mussel (60 mm in shell length) may filter between 12 and 240 (mean 72) litre of water per day (Cranford, Ward, & Shumway, 2011), and have been proposed as bio-samplers for assessment of faecal contamination in recreational waters (Roslev, Bukh, Iversen, Sonderbo, & Iversen, 2010).

According to the current EU regulations (854/2004/EC, 2004), farm localities for cultivation of bivalves have to be classified according to their suitability in terms of microbiological and chemical water quality. Concerning the microbiological conditions, all localities should be affiliated as Class A, B or C areas, depending on the content of *E. coli* in the soft parts and mantle water of the harvested bivalves. The upper limit for a Class A area is that the concentration of *E. coli* should be 230 $\leq$ 100 g^{-1}sample material. The upper limit for a Class B area is 4600 *E. coli* 100 g^{-1}, and bivalves from localities with this classification must be purified by re-suspension at a Class A area, or heat-treated before distribution. The *E. coli* limit at a Class C area is 46 000 *E. coli* 100 g^{-1}, and to be able to distribute these bivalves, re-suspension at a Class A area over a long period of time, or sufficient heat treatment by approved procedure, is needed. With reference to these regulations, the microbiological conditions of farm localities for bivalve cultivation

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in Norway have been documented through regular monitoring. Several times throughout the year, bivalve samples are collected from a number of farms along the coast to be examined microbiologically for *E. coli* and enterococci, and occasionally *Salmonella*.

Enterococci are not included in the current EU-regulations on assessment of farming localities for bivalve cultivation, despite that they are known to survive better in the environment than bacteria in the Enterobacteriaceae family (Noble et al., 2004). Faeces from persons infected by foodborne viral agents, could contain viral particles in diarrhoeal stools and vomit of up to 10^{11} g^{-1} (Forsythe, 2010; Gerba, Kitajima, & Iker, 2013). These particles are only partially removed by sewage treatment (Gerba et al., 2013) and may retain infectivity for a longer time than bacteria in the Enterobacteriaceae family, such as *E. coli* (Cook & Richards, 2013). Of the over 9 million annually reported cases of foodborne illness, Norovirus accounts for 58% of which bivalve shellfish are a major food vehicle (Woods & Burkhardt III, 2013). Thus, organism groups able to indicate viral contamination in bivalve shellfish is highly required.

Bacteria, parasites and viruses from animals and humans are transported to the sea by land runoff, or via the sewage systems. In periods with high rainfall, especially following dry periods, it is expected that increased amounts of faecal material from land living animals will reach the sea. In addition, heavy rain or high amounts of melting snow may give an overload and possible leakage in drains and sewage systems.

The current publication presents information on the seasonal variation for *E. coli* and enterococci in blue mussels (*M. edulis*), from selected localities in Norway with a continuous sampling history. As the enterococci show a better survival in the environment compared to *E. coli*, a major aim of this study were to evaluate if they could replace or supplement *E. coli* as an indicator of viral faecal contamination in bivalves.

In addition, we report on the covariance among these groups of microorganisms and rainfall. Furthermore, results from occasional sampling and analysis of *Salmonella* in Norwegian bivalves in the period from 2007 to 2012 are presented. In addition, results are presented from analyses of other bivalves: oysters (*Ostrea edulis*), scallops (*Pecten maximus*), cockles (*Cerastoderma edule*) and horse mussels (*Modiolus modiolus*).

2. Materials and methods

2.1. Sampling

From 2007 to 2012, a total of 2296 samples of bivalve molluscs were taken along the Norwegian coast. The examined material comprise mainly bivalves, including 2055 samples of blue mussels (*M. edulis*) from 152 localities (Fig. 1.), 106 samples of oysters (*O. edulis*), 100 samples of scallops (*P. maximus*), 21 samples of cockles (*C. edule*) and 14 samples of horse mussels (*M. modiolus*). Each sample comprised at least 10 individuals. Samples were transported under chilled conditions to the laboratory and the analyses were initiated within 24 h after sampling.

2.2. Microbiological analysis

All samples were examined quantitatively for *E. coli*, whereas a subset of 1902 and 352 of the samples were also examined quantitatively for enterococci and qualitatively for *Salmonella*, respectively.

The sample material, consisting of flesh and intravalvular fluid (mantle water), were pre-treated in accordance with ISO 6887-3 (ISO, 2003), and examined for *E. coli* by a three times five tube Most Probable Number (MPN) method using Oxoid Minerals Modified Glutamate Broth (MMGB) and the chromogenic medium Oxoid Tryptone Bile 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide agar (TBX), in accordance with the EU reference method ISO 16649-3 (ISO, 2005). In this method, 50 g of sample material were homogenised in 450 ml Peptone water (Difco Bacto peptone 1 g, NaCl 9 g, distilled water to 1L) giving an initial 1:10 dilution. Appropriate amounts from this homogenate were transferred to the tubes resulting in 1 g, 0.1 g and 0.01 g of the original sample material in the tubes at each dilution series, respectively. In the first five tubes, 10 ml of the 1:10 homogenate describes above were added, giving a final dilution factor of 1:1 of the original sample. This procedure makes it possible to quantify *E. coli* in lower concentrations compared to methods based on plating on agar. The MMGB tubes were incubated at 37 ± 1 °C for 24 ± 2 h and inspected for colour change. From each positive tube, material were transferred by a loop to the surface of TBX agar, followed by examination of the *E. coli* specific β-glucuronidase activity (Rice, Allen, & Edberg, 1990) after aerobic incubation at 44 ± 1 °C for 22 ± 2 h. The results were given as the number of *E. coli* 100 g^{-1}. The MPNs were read from tables in The National standard method, F16 Issue 4.2 from the Health Protection Agency (HPA), UK, as described in HPA (2004), and recommended by Donovan et al. (1998). The lowest detectable concentration of *E. coli* when applying this method was 20 *E. coli* 100 g^{-1}.

The quantification of enterococci was conducted by plating on *Enterococcus* agar (Slanetz & Bartley), in accordance with NMKL method No. 68, 5th Ed. (NMKL, 2011). A tenfold dilution was made by homogenising 10 g of sample material, consisting of flesh and intravalvular fluid, in 90 ml Peptone water for 30 s using a laboratory Stomacher as described in the NMKL method 91, 5th Ed. (NMKL, 2010). Aliquots of 0.1 ml of the homogenate were plated on the agar surface using a sterile L-rod, giving a detection limit of 100 colony forming units (CFU) per gram. Plates were incubated at 44.0 ± 0.2 °C for 48 ± 4 h before reading dark red colonies. Some enterococci may give weak colour when incubated on *Enterococcus* agar as described here, and when in doubt such colonies were tested with respect to esculin hydrolysis as described in the current NMKL method.

Salmonella was detected qualitatively by the Enzyme Linked Fluorescent Assay (ELFA) performed by an automated immunoassay system (miniVidas) in accordance with the protocols provided by the supplier (bioMèrieux, Marcy l'Etoile, France). Subsequently, miniVidas-positive samples were cultured and characterised, applying the NMKL method No. 71, 5th Ed. (NMKL, 1999) and biochemical characterisation by the REMEL Micro-ID® Enterobacteriaceae system (Thermo Fisher Scientific, USA). The material examined for *Salmonella* comprised 279 samples of blue mussels, 54 samples of scallops, 10 samples of oysters and nine samples of horse mussel.

2.3. Precipitation data

For the selected stations, data on rainfall in mm per day were collected from the Norwegian Meteorological Institute (http://eklima.met.no) for nearby monitoring stations, and calculated as sliding average per 30 days.

2.4. Statistical analysis

Statistica version 12 (Statsoft Inc, USA) was used for preparation of the graphs.

3. Results

Of the 2296 samples examined for *E. coli* during the study period, 508 (22.1%) had MPN values < 20 *E. coli* 100 g^{-1}, 1966

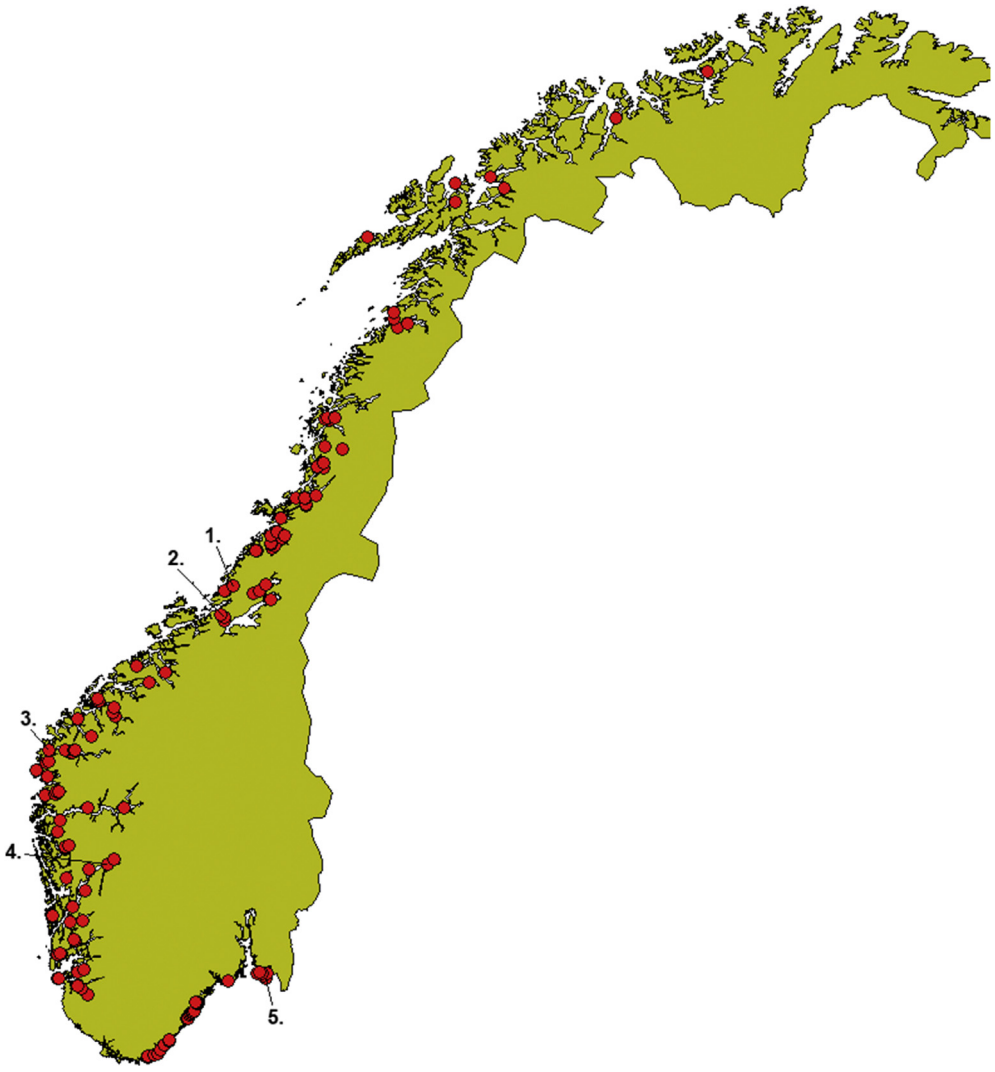

Fig. 1. In the six-year period from 2007 to 2012, a total of 2055 samples of blue mussels (*Mytilus edulis*) from 152 locations along the Norwegian were sampled and examined. Red points marked 1. to 5. represents the stations described in more detail in the text. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(85.6%) had values between 20 and 230 *E. coli* 100 g^{-1} (Class A areas), 300 (13.1%) had values between 230 and 4600 *E. coli* 100 g^{-1} (Class B areas) and 20 (0.9%) had values > 4600 *E. coli* 100 g^{-1} (Class C areas). The highest detected number was 1.8×10^4 *E. coli* 100 g^{-1}, found in one sample of scallops.

Of the blue mussel samples (n = 2055), 1776 (86.4%) had a number of *E. coli* corresponding to a Class A area, 264 (12.9%) to a Class B area and 15 (0.7%) to a Class C area. I total 21 localities for blue mussels had only A samples during the sampling period covered in this study. One or more samples from 80 localities had *E. coli* numbers corresponding to Class B. Of these, 33 localities had Class B samples on three or more occasions. Samples corresponding to Class C where originating from 12 localities. The two localities having the highest number of B and C samples, showed a combined (B + C) percentage of 42 and 27, respectively.

A total of 1870 (98.3%) of the 1902 samples examined for enterococci had counts < 100 CFU g^{-1} (i.e. not detected) 27 samples (1.4%) had counts of 100 CFU g^{-1}, and five samples (0.3%) had counts above 100 CFU g^{-1}. The highest detected count of enterococci was 300 CFU g^{-1}, found in a sample of blue mussels.

Of the 352 samples examined for *Salmonella*, only one (0.3%) was positive. The *Salmonella* positive sample was from a horse mussel analysed in 2007, and the strain was identified as *Salmonella enterica* serovar Infantis, seroprofile: 6,7: r:1,5. The MPN of *E. coli* in this particular sample was 500 *E. coli* 100 g^{-1}, whereas the count of enterococci was <100 CFU g^{-1}.

During the six years of sampling, longer sampling series (12–60 samplings per locality) were established from 18 of the blue mussel localities. The *E. coli* data from mussels from these localities were compiled to assess possible long-term variations. These localities showed varying patterns. Two typical patterns can be seen in Fig. 2. At Askerholmen (Åfjord, locality 1., Fig. 1), there were repeated low-level detections and several samples had *E. coli* values corresponding to a class B area or higher. A second pattern was seen at Kaland (Inner Hardanger, locality 4., Fig. 1), where a single high value corresponding to a Class B area was found, against a background of a long series with levels mainly below the detection limit (<20 *E. coli* 100 g^{-1}).

A compilation of the *E. coli* data from the years 2007–2012, divided into sampling months, shows a generally lower counts in the period from February throughout May. In addition there are somewhat higher *E. coli* values in samples collected in January,

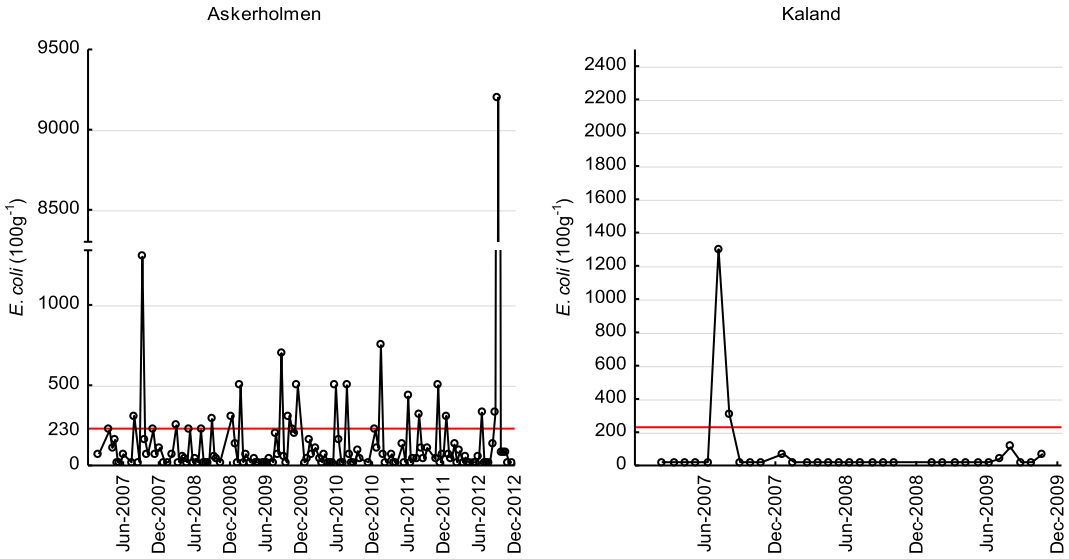

Fig. 2. Number of *E. coli* (100 g^{-1}) detected in blue mussels (*Mytilus edulis*) collected at Askerholmen (Åfjord, Station 1., Fig. 1) and Kaland (Inner Hardanger, Station 4., Fig. 1) between 2007 and 2012. Red horizontal line shows the limit for an A area of 230 *E. coli* 100 g^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

June, July, October and December (Fig.3). The co-variation of sliding averages of daily rainfall and *E. coli* in blue mussels is illustrated by the two localities Hvaler (Outer Oslofjord, locality 5., Fig. 1) and Vemmelsvik (Gangsøyfjord, locality 3., Fig. 1) (Fig. 4). Here, the *E. coli* peaks followed episodes of heavy rainfall, except the highest peak of 3500 *E. coli* 100 g^{-1}. Factors such as land and sea topography, will also strongly affect the land runoff into cultivation areas (Hernroth, Conden-Hansson, Rehnstam-Holm, Girones, & Allard, 2002). One additional complicating factor is the precipitation in the form of snow, which will only bring land borne faecal material into the sea after thawing. This may be why the *E. coli* peak in Vemmelsvik occurred in April while the precipitation peaked in February.

In the present material, a weak co-occurrence was seen between detection of enterococci and *E. coli*, where detections of enterococci were made in samples with high concentrations of *E. coli*, but in several cases enterococci and *E. coli* occurred independently in the samples (Fig. 5).

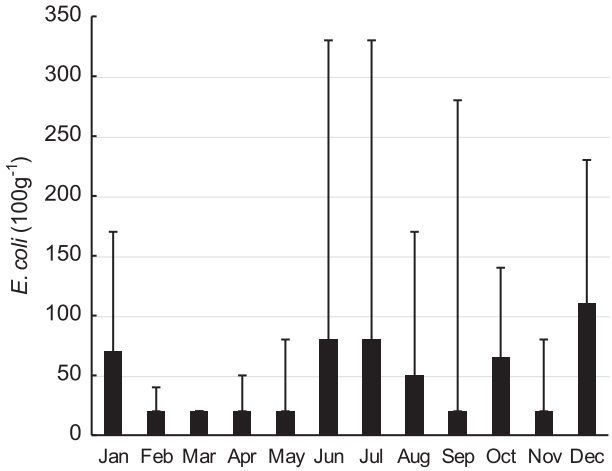

Fig. 3. Number of *E. coli* (100 g^{-1}) detected in blue mussels (*Mytilus edulis*) according to season, during the shellfish monitoring programme 2007–2012. Bar showing the 75th percentile.

4. Discussion

In the current study, *E. coli* and enterococci have been included as indicators of faecal contamination of marine bivalves. In 261 of 320 samples (81.6%) included in our sample material, the concentration of *E. coli* was above 230 *E. coli* 100 g^{-1}, whereas the corresponding concentration of enterococci were below the limit of detection of 100 CFU g^{-1}. On the other hand, 17 of 1976 samples (0.9%) with *E. coli* values < 230 *E. coli* 100 g^{-1} had detectable concentrations of enterococci. The less frequent detections of enterococci, as compared to *E. coli*, can at least partially be explained by the common concentrations of these organisms in faecal material from homoeothermic animals. In a study by Havelaar, Furuse, and Hogeboom (1986), the concentration of thermotolerant coliforms and faecal streptococci in animals and humans were compared. For the faecal material from pig, chicken, dog, cow, horse, sheep and calf, these authors found the gross average of thermotolerant coliforms to be 6.5×10^7 g^{-1} and faecal streptococci to be 2.0×10^6 g^{-1}, giving an approximate ratio of 30 to 1. The corresponding numbers from human faeces were reported to be 1.9×10^8 g^{-1} for thermotolerant coliforms and 3.7×10^5 g^{-1} for faecal streptococci, resulting in a ratio of approximately 500 to 1. According to Litsky, Rosenbaum, and France (1953), raw sewage contains 13 times higher concentrations of coliforms, as compared to enterococci. In addition, the enterococci have the ability to enter a viable but not culturable (VBNC) state when stressed (Lleo, Bonato, Benedetti, & Canepari, 2005), and this might also explain the poor growth on selective agar plates. Improvements of the method concerning the detection limit is needed to analyse samples that might hold such low numbers of enterococci. A standardised MPN method should be considered developed, where cultivation in broth will increase the resuscitation of the VBNC cells (Lleo et al., 2001).

When the *E. coli* data for 2007 to 2012 were divided into sampling months, generally lower counts were seen in the period from February throughout May. In addition, there are somewhat higher *E. coli* values in samples collected in January, June, July, October and December. A possible explanation for seasonally high values may be periodical transport of manure from farm animals, or overload of the sewage system in periods with high rainfall. Rainfall can

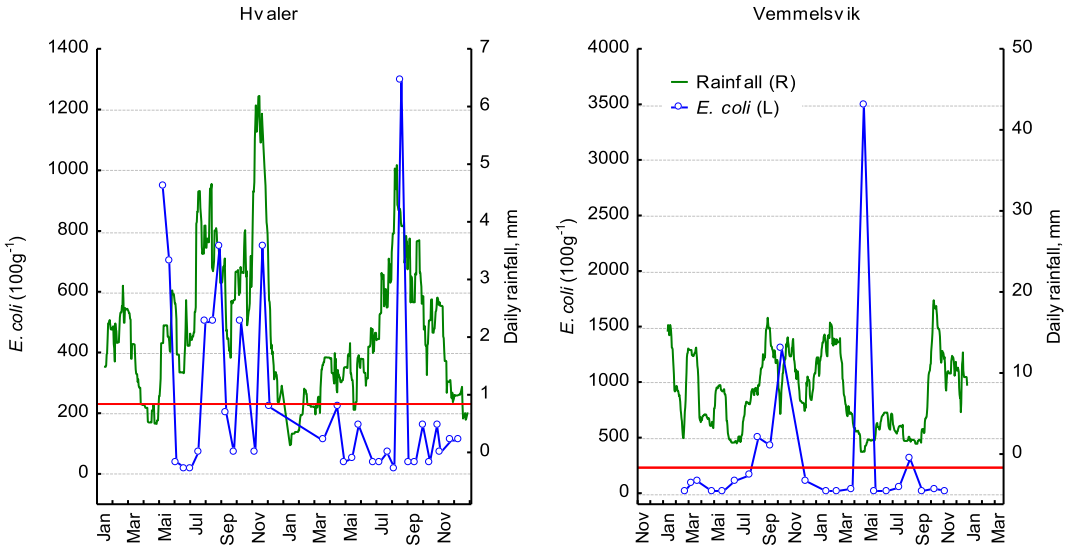

Fig. 4. Detection of *E. coli* (100 g^{-1}) in samples of blue mussels (*Mytilus edulis*) from Hvaler (Station 5., Fig. 1, during 2009 and 2010) and Vemmelsvik (Station 3., Fig. 1, during 2008 and 2009), and daily rainfall in millimetre (sliding average over 30 days). Red horizontal line shows the limit for an A area of 230 *E. coli* 100 g^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

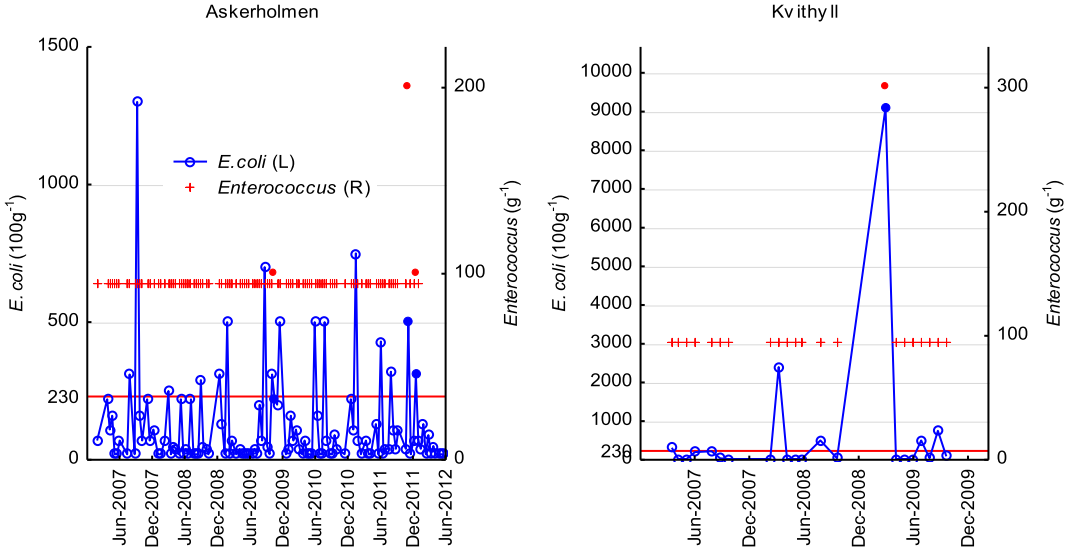

Fig. 5. Detection of *E. coli* (100 g^{-1}) and *Enterococcus* (g^{-1}) in samples of blue mussels (*Mytilus edulis*) from Askerholmen (Station 1., Fig. 1., between 2007 and 2012) and Kvithyll (Station 2., Fig. 1, between 2007 and 2009). For *Enterococcus*, detections above the detection limit are indicated with filled circles, and corresponding *E. coli* determinations are also indicated with filled circles. Red horizontal line shows the limit for an A area of 230 *E. coli* 100 g^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

introduce large amounts of microbial contaminants, including viruses and bacteria of human health concern, to rivers and coastal areas (Campos, Kershaw, Lee, Morgan, & Hargin, 2011; Hata et al., 2014; Schernewski, Schippmann, & Walczykiewicz, 2014). As could be seen from the presented results (Hvaler, Fig. 4), there is for some localities a correlation between rainfall and following detection of *E. coli* in blue mussels. In other cases, there is no such obvious correlation (Vemmelsvik, Fig. 4) indicating that other factors, such as land and sea topography will also have an impact. In addition, precipitation in the form of snow, which will only bring land-borne faecal material into the sea after thawing, will affect the transport from land to the sea. In a study by Campos et al. (2011), on the correlation by rainfall and accumulation of *E. coli* in bivalves from the Dart Estuary in England, the authors show that the rainfall intensity and river flow, explained the spatial and temporal variation in *E. coli* accumulation significantly. In their study, a three to four days lag-phase between rainfall and increased bacterial accumulation in exposed bivalves could be seen. This could be explained by a combination of water travel time in the terrain, river and estuary, as well as the time of *E. coli* uptake by the bivalves.

Several other organisms, or groups of organisms, have been applied as indicators of faecal contamination, including the Enterobacteriaceae family, coliforms, faecal coliforms, *E. coli*, enterococci, bifidobacteria, clostridis and enteroviruses (Jay, Loessner, & Golden, 2005). Certain criteria apply for indicator organisms. They should be normally present in faecal material in high and stabile concentrations, they should be easy and rapid to detect at low numbers and have a survival in the environment comparable to relevant pathogens (Buttiaux & Mossel, 1961). As early as in the 1890s *E. coli* (formerly *Bacillus coli communis*) were proposed as an indicator

organism for examination of drinking water, since the prevalence in faecal material was high and the methods for detection were rapid (Jay et al., 2005; Smith, 1895). However, the survival of *E. coli* in the environment are shorter than for enterococci. The enterococci include two important species found in human and animal intestines, *E. faecalis* and *E. faecium*. The former is reported as mainly associated with the human intestine, whereas the latter is found in both humans and animals (Forsythe, 2010). In runoff exposed costal environments, enterococci concentrations are reported to show high temporal and spatial variability when sampling water from the same station repeatedly. Boehm (2007) reported a mean change in the concentration of enterococci of 60%, and a maximum change of 700%, within minutes, during consecutive samplings at the same station. However, filtering organisms will level out these fluctuations in the water concentration.

Viruses rank among the most important infective agents causing food- and water-borne gastrointestinal disease (Cook & Richards, 2013; Donaldson, Lindesmith, Lobue, & Baric, 2010). In particular, norovirus cause large outbreaks in all age groups. It has been estimated that the total number of norovirus infections may be more than 267 million cases annually, causing more than 200 000 fatalities, predominantly among previously weakened persons (Debbink, Lindesmith, Donaldson, & Baric, 2012; Donaldson et al., 2010). In temperate regions, the infection rate is particularly high during the cold months (Patel et al., 2008), whereas in tropical and sub-tropical regions the prevalence seems to be more evenly distributed throughout the year (Allen, Iturriza-Gomara, & Brown, 2013). The infective dose of noroviruses may be as low as 10 particles, and vomit and faeces from diseased persons may contain up to 10^{11} particles g^{-1} (Gerba et al., 2013). After recovery, patients my shed viruses in their faeces for up to 14 days (Cook & Richards, 2013). As infections with noroviruses are very common and high numbers of viruses are found in vomit and faeces, sewage is likely to contain viral particles. Gerba et al. (2013) reported 10^7 replicons/litre sewage by PCR techniques, and that common treatment techniques may reduce numbers but not remove noroviruses, if present.

Marine filter-feeding organisms are particularly prone to viral accumulation, and detection of norovirus and hepatitis A virus in bivalves are frequent (Boxman et al., 2006; Mesquita et al., 2011; Myrmel, Berg, Rimstad, & Grinde, 2004; Pavoni et al., 2013; Pinto & Bosch, 2013; Roldan, Rodriguez, Garcia, & Navajas, 2013).

There are considerable uncertainty regarding how long viral particles retain their infectivity in the environment, including seawater. One reason for this is that the commonly applied methods detect the nucleic acids rather than the complete viral particle, giving the possibility for false positive results (Gerba et al., 2013). On the other hand, the concentration of viruses in contaminated seawater can be much higher than the number of commonly applied bacterial indicator organisms. Therefore, indicator organisms may be diluted to non-detectable concentrations, while the virus concentration are still high. *E. coli* has been reported to die of in the environment at rates similar to common intestinal pathogenic bacteria, but will not be as resistant as enterococci and intestinal virus (Jay et al., 2005). On several occasions, viruses of concern have been detected in shellfish harvested from areas with low concentrations of *E. coli* (Hernroth et al., 2002; Mesquita et al., 2011). Enterococci are rapidly acquired by and more slowly released from mussels and have a better survival in the marine environment compared to *E. coli*, and could represent a better indicator of viral contamination (Marino et al., 2005; Roslev et al., 2010; Roslev, Iversen, Sonderbo, Iversen, & Bastholm, 2009).

One of the 354 examined samples (horse mussel) was positive for *Salmonella*, and the strain was identified as *S. enterica* serovar Infantis, seroprofile: 6,7: r:1,5.

In Norway, *S.* Infantis represented the eleventh most common serovar, counting 17 cases of infections in 2013, of which one was acquired in Norway. The source of contamination of the positive horse mussel sample is unknown. The prevalence of *Salmonella* in Norwegian feed, food and livestock animals, as well as among humans, is considered low in a European perspective (Heier, Lange, Hauge, & Hofshagen, 2014). According to these authors, a total of 1364 cases of salmonellosis in humans were registered in 2013, of which 235 (17%) were domestically acquired. The overall most common serovar was *S.* Enteritidis, counting for 44% of the registered cases, followed by *S.* Typhimurium, with 19% of the registered cases. For this *Salmonella* contaminated sample, *E. coli* would have functioned as an indicator organism, since the count was 500 *E. coli* 100 g^{-1}. However, the concentration of enterococci in the same sample was <100 CFU g^{-1}. This finding is in line with Morinigo, Córnax, Muñoz, Romero, and Borrego (1990), who reported that the faecal coliforms had a better correlation with *Salmonella* in polluted natural waters, compared to faecal streptococci. Additionally, Efstratiou, Mavridou, and Richardson (2009) found that presence of *Salmonella* in seawater are adequately predicted by total coliforms or faecal coliforms, and that enterococci has less power to discriminate between presence and absence.

The current EU legislation on official control of products of animal origin intended for human consumption (854/2004/EC, 2004), describes in Annex II the regulations for bivalve molluscs. According to these regulations, the sampling plans for microbiological quality should take particular account for likely variations in faecal contamination of the cultivation area. The knowledge of the occurring variation for faecal indicators covered in this article, as well as the knowledge of the Local offices of the competent authority on possible exposure to material from livestock or humans, topography of the location, as well as tidal and water current patterns should be taken into account when designing the sampling plans.

Even though cocci of faecal origin are known to persist in the environment for a longer period than coliforms, the differences in the concentration in faeces may result in a more rapid dilution to undetectable concentrations, as compared to coliforms including *E. coli*, as described in our study. A revision of the method giving a lower limit of detection, would make enterococci suitable for indicating viral contamination in bivalves.

5. Conclusion

The objectives of this study were to describe time trends in the detection of two commonly applied indicator organisms of faecal contamination in commercially cultured blue mussels (*M. edulis*) harvested along the coast of Norway during the six-year period from 2007 to 2012. Possible covariance in the occurrence and concentration of *E. coli* and enterococci, and the effect of precipitation (rainfall or snow) in the period of examination, were assessed. Several localities for bivalve cultivation in Norway showed single high counts of *E. coli*, without a previous history of *E. coli* detection. One other pattern of *E. coli* detection was repeated low values above the limit and some higher values in between. There also seemed to be a weak positive correlation between detection of enterococci and *E. coli*, and a weak correlation between the number of *E. coli*/enterococci and rainfall. Due to the low numbers of enterococci present in the current sampling material, the detections and quantifications of enterococci in this study were uncertain. Hence, if enterococci are to be used as indicator organisms of faecal contamination in bivalves, an MPN method with lower dilutions should be considered.

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