Mok et al. Fisheries and Aquatic Sciences (2018) 21:36
https://doi.org/10.1186/s41240-018-0113-0

Fisheries and Aquatic Sciences

RESEARCH ARTICLE

Open Access

Bacterial quality evaluation on the shellfish-producing area along the south coast of Korea and suitability for the consumption of shellfish products therein

Jong Soo Mok[1], Kil Bo Shim[1*], Ji Young Kwon[2] and Poong Ho Kim[1]

Abstract

Background: To confirm whether shellfish are suitable for consumption, the quality of seawater and shellfish in shellfish-producing areas must be assessed regularly. This study was conducted to evaluate the bacterial quality on the Changseon area, containing a designated shellfish-producing area, in Korea during 2011–2013.

Result: Even though many inland pollutants near the area were identified, they showed no significant impact on the designated area and the shellfish therein. The concentrations of fecal bacteria in all the seawater and mussel samples from the designated area during the harvesting season were within the standards of various countries. Pathogenic bacteria were not detected in any of the mussel samples. In our previous study, the hazardous metal levels in all the mussels from the same area were also within the limits of different countries.

Conclusion: The mussel products in this area are suitable for consumption based on fecal pollution, pathogenic bacteria, and also heavy metals.

Keywords: Fecal-indicator bacteria, Mussel, Pollutant, Changseon area, Shellfish-producing area

Background

Shellfish are a commercially important seafood species all around the world. A variety of shellfish, such as oyster, mussel, and clam, are cultured extensively along the coastal regions of Korea (Ministry of Oceans and Fisheries (MOF) 2015b; Mok et al. 2015a). Especially, seven shellfish-producing areas were designated along the Korean coast based on the regulation standard of fecal coliform (MOF 2015b; Mok et al. 2016a). The Changseon area is one of shellfish-producing areas designated by the Korean government. In 2009, the Korean authority designated the Changseon area as the sixth designated shellfish-producing area, which is significant mussel production region (MOF 2015a, b). The Changseon area is a semi-enclosed coastal region located among Goseong-gun, Sacheon City, Changseon Island, and Saryang Island on the south coast of Korea (Fig. 1). Mussel products are both consumed domestically and exported, mainly to the European Union (EU) (Mok et al. 2014). In Korea, the production amount of mussels ranked second in the shellfish, accounting for 51,463 tons of meat/shell in 2014 (Statistics Korea 2014).

A large amount of inland wastewater is discharged into near-shore waters, delivering fecal-associated microorganisms such as fecal coliforms, *Salmonella* spp., and viruses (Biancani et al. 2012; Mok et al. 2016a; Park et al. 2016). Bivalves are filter-feeding animals that concentrate fecal-associated pathogenic bacteria from the surrounding water in their body, so that consumption of bivalves from polluted waters presents a risk to human health (Azalea et al. 2010; Mok et al. 2016b; Park et al. 2016). Each year, consumption of contaminated seafood including bivalves is implicated in food-poisoning outbreaks caused by pathogenic microorganisms in Korea (Korea Ministry of Food and Drug Safety (KMFDS) 2016). Fecal coliforms, including *Escherichia coli*, are

* Correspondence: kilbo1221@korea.kr
[1]Food Safety and Processing Research Division, National Institute of Fisheries Science, 216, Gijanghaean-ro, Gijang-eup, Gijang-gun, Busan 46083, Republic of Korea
Full list of author information is available at the end of the article

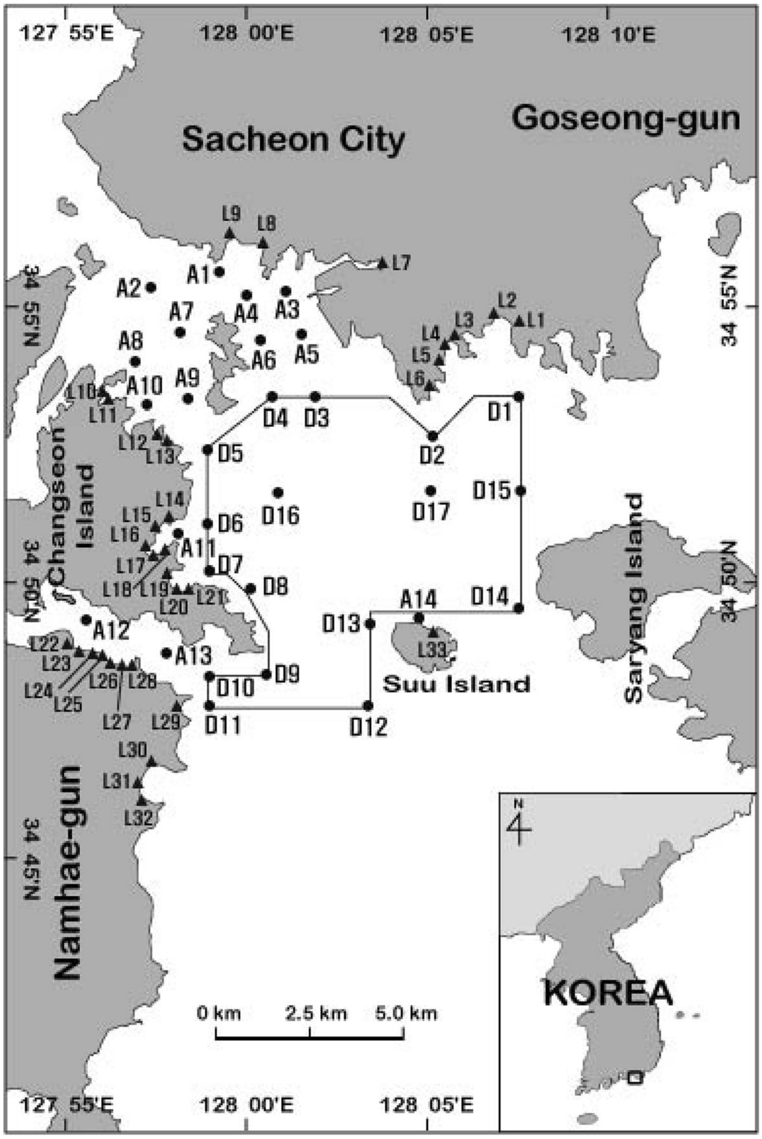

Fig. 1 Sampling sites of inland pollution sources (▲), seawater (●), and mussels from the Changseon area of the Korean coast. A black closed line represents the boundary line of the designated shellfish-producing area

useful as fecal contamination indicators to assess the bacterial quality of bivalve-producing area and the bivalves therein (Mok et al. 2016a; Kang et al. 2017; Kim et al. 2017). To protect public health, various countries, including Korea, the United States (US), EU, and New Zealand, have established regulatory criteria and monitoring programs using fecal indicators for bivalves and their growing areas (European Commission (EC) 2005; New Zealand Food Safety Authority (NZFSA) 2006; US Food and Drug Administration (US FDA) 2015; MOF 2015a; KMFDS 2016). Hence, the levels of fecal coliforms in bivalves or their growing areas must be monitored regularly to determine whether shellfish are safe for consumption. Also, bacterial quality evaluation of shellfish-producing areas and the impact level of pollution sources on them are considerably needed to inform better shellfish management and protect public health.

In the present study, the fecal coliform levels were monitored in inland pollutants around the Changseon area, including a designated shellfish-producing area, along the Korean coast and their impact on the shellfish therein. In addition, bacteriological quality was assessed based on the levels of indicators of fecal contamination, including fecal coliform or *E. coli*, in seawater and mussel samples regularly collected from the study area during 2011–2013. While levels of these microorganisms in seawater and bivalves in other sea areas have been studied, this is the first work that we are aware of that evaluates the levels of fecal indicators using a full sanitary survey (e.g., inland pollution sources, seawater, and mussels) in Changseon area, Korea.

Materials and methods

Sample collection

An on-site shoreline survey is to identify and evaluate various inland pollutants (actual and potential), which may affect the water quality of shellfish-producing area (US FDA 2015; MOF 2015a). In the present study, the on-site shoreline survey was conducted during dry periods between October and November 2013 within the mussel-harvesting season in Korea to minimize non-point pollution source (NPS) inputs to the inland pollution sources, caused by precipitation. During this survey near the Changseon area, we identified 164 direct and indirect sources in the drainage area. Among them, 33 sources occurred with the flow of discharged water; however, the other sources were not flowing during the study period. The sources with discharging water were selected as sampling sites to assess the impact of these sources on the water quality of the shellfish-producing area, the Changseon area along the south coast of Korea (Fig. 1). The inland water samples were collected in sterilized wide-mouth bottles (250 mL) to analyze fecal coliform bacteria.

Seawater sampling locations were selected based on different geographical situations and potential fecal pollutants in or near the Changseon area, including a designated shellfish-producing area (Fig. 1). The bacteriological water quality of the shellfish-producing area was evaluated with seawater samples collected over 3 years according to the criteria set by the Korean Shellfish Sanitation Program (KSSP; MOF 2015a) and National Shellfish Sanitation Program (NSSP; US FDA 2015). Seawater samples were collected once a month from 2011 to 2013 at 31 sites in the study area. The 612 of seawater samples were collected from 17 sites in the designated area, and other 504 samples were collected from 14 sites in the adjacent area. Samples of mussel (*Mytilus galloprovincialis*) were also collected at five sites during the seawater sampling periods (Fig. 1). In the designated area, 108 mussel samples were collected from sites D6, D7, and D8, and in the adjacent area, 55 samples were collected from sites A13 and A14.

All collected samples for the analysis of fecal coliform bacteria including *E. coli* were maintained below 10 °C. Water temperature and salinity were measured during the seawater sampling periods using a YSI 556 Multiprobe System (YSI, Yellow Springs, OH, USA).

Analysis of fecal-indicator bacteria

The levels of fecal coliform and *E. coli* in the samples were enumerated by the most probable number (MPN) method. The MPN method used was a five-tube test using three 10-fold serial dilutions. The recommended procedures for the examination of seawater and shellfish according to American Public Health Association (APHA) were applied for fecal coliform enumeration (APHA 1970). In addition, the ISO/TS 16649-3 method (ISO 2015) was used for *E. coli* enumeration. Results are expressed as MPN/100 mL for seawater and MPN/100 g for shellfish tissue. The limits of detection of these methods were 1.8 MPN/100 mL of seawater and 18 MPN/100 g of tissue for fecal coliform, and 20 MPN/100 g of tissues for *E. coli*.

Evaluation of inland pollution sources and seawater quality

The pollution sources were evaluated using the method suggested by the US FDA (Mok et al. 2016a; Jung et al. 2017). The flow rates of the discharged water were calculated with the velocity–area method based on values measured using a hydrometer (Flo-Mate 2000, Marsh McBirney, Loveland, CO, USA). According to US FDA guidelines (US FDA 2015), the degree of pollution was calculated as the amount of water required to dilute the fecal coliform density to less than the standard level of 14 MPN/100 mL in seawater samples.

The seawater quality in the Changseon area was evaluated according to the sanitation standard operating procedure for shellfish-producing areas suggested by the KSSP (MOF 2015a) and NSSP (US FDA 2015). The seawater quality was evaluated based on the geometric mean and estimated 90th percentile of fecal coliforms in seawater samples collected from each station more than 30 times over 3 years.

Statistical analysis

The statistical test was conducted using analysis of variance with SAS software for Windows (SAS ver. 9.2, SAS Institute, Cary, NC, USA). Duncan's multiple-range tests were used to compare differences between bacteria numbers.

Results and discussion

Fecal coliform number and impact of inland pollution sources

Table 1 summarizes the fecal coliform numbers in the pollution sources collected from the 33 sites near the Changseon area and their impact on the sea area therein. According to the locations of the shoreline pollution sources, the drainage area consisted of four subregions: Hai-myeon in Goseong-gun, Sacheon City, and Changseon-myeon and Samdong-myeon in Namhae-gun. Of 164 potential pollution sources during this survey, 33 sites were actual pollution sources with discharged water, including six in Hai-myeon, three in Sacheon City, 12 in Changseon-myeon, and 12 in

Table 1 Concentration and impact of fecal coliforms in the water samples collected from inland pollution sources in the drainage area near the Changseon area, in Korea between October and November 2013

Station	TD [a]	FRD (L/min)	FC (MPN/100 mL)	AD (m)	DL (MPN/day)	DWR (m^3)	IA (m^2)	RHC (m)
Hai-myeon in Goseong-gun								
L1	DW	3	160,000	1	6.9×10^9	4.9×10^4	4.9×10^4	177
L2	SW	149	330	1	7.1×10^8	5.1×10^3	5.1×10^3	57
L3	SW	48	790	1	5.4×10^8	3.9×10^3	3.9×10^3	50
L4	LFFW	14,000	< 1.8	–	–	–	–	–
L5	SW	74	2400	1	2.6×10^9	1.8×10^4	1.8×10^4	108
L6	LFFW	16,000	< 1.8	–	–	–	–	–
Sacheon City								
L7	SW	500	33	1	2.4×10^8	1.7×10^3	1.7×10^3	33
L8	SW	600	790	1	6.8×10^9	4.9×10^4	4.9×10^4	176
L9	SW	900	49	1	6.4×10^8	4.5×10^3	4.5×10^3	54
Changseon-myeon in Namhae-gun								
L10	SW	78	4900	1	5.5×10^9	3.9×10^4	3.9×10^4	158
L11	SW	21	330	1	1.0×10^8	7.1×10^2	7.1×10^2	21
L12	LFFW	30,000	< 1.8	–	–	–	–	–
L13	LFFW	30,000	< 1.8	–	–	–	–	–
L14	SW	500	11,000	3	7.9×10^{10}	5.7×10^5	1.9×10^5	347
L15	SW	35	79	1	4.0×10^7	2.8×10^2	2.8×10^2	13
L16	DW	3	2400	1	1.0×10^8	7.4×10^2	7.4×10^2	22
L17	SW	510	2400	2	1.8×10^{10}	1.3×10^5	6.3×10^4	200
L18	SW	55	49	1	3.9×10^7	2.8×10^2	2.8×10^2	13
L19	SW	3	920	1	4.0×10^7	2.8×10^2	2.8×10^2	13
L20	DW	20	5400	1	1.6×10^9	1.1×10^4	1.1×10^4	84
L21	DW	10	28,000	1	4.0×10^9	2.9×10^4	2.9×10^4	135
Samdong-myeon in Namhae-gun								
L22	SW	10	24,000	1	3.5×10^9	2.5×10^4	2.5×10^4	125
L23	SW	60	240,000	3	2.1×10^{11}	1.5×10^6	4.9×10^5	561
L24	SW	90	130	1	1.7×10^8	1.2×10^3	1.2×10^3	28
L25	SW	3400	110	1	5.4×10^9	3.8×10^4	3.8×10^4	157
L26	SW	220	33	1	1.0×10^8	7.5×10^2	7.5×10^2	22
L27	SW	18	490	1	1.3×10^8	9.1×10^2	9.1×10^2	24
L28	SW	8	2	1	2.3×10^5	1.6×10^0	1.6×10^0	1
L29	SW	1000	79	1	1.1×10^9	8.1×10^3	8.1×10^3	72
L30	SW	55	7900	1	6.3×10^9	4.5×10^4	4.5×10^4	169
L31	SW	120	79	1	1.4×10^8	9.8×10^2	9.8×10^2	25
L32	SW	82	24,000	2	2.8×10^{10}	2.0×10^5	1.0×10^5	254
L33	DW	2	1300	1	3.7×10^7	2.7×10^2	2.7×10^2	13

TD type of discharge, *FRD* flow rate of discharge, *FC* fecal coliform, *AD* average depth; *DL* daily load = *FC* concentration × FRD; *DWR* dilution water required = DL/standard level, where FC standard level is 14 MPN/100 mL; *IA* impact area = DWR/AD; *RHC* radius of half-circle = square root (IA × 2 /3.14)
[a] *DW* domestic wastewater, *SW* stream water, *LFFW* land-based fish farm wastewater

Samdong-myeon (Fig. 1 and Table 1). The other sources did not discharge during the survey period. The total flow rate of 33 sites was 118,573 L/min (range, 2–50,000 L/min), which included 8535 L/min of stream water, 38 L/min of domestic wastewater, and 110,000 L/min of land-based fish farm wastewater. No food-processing plant or industrial or livestock wastewater was included. The daily load of fecal coliforms in 33 discharges ranged from 2.3×10^5 to 2.1×10^{11}

MPN/day. Their affected radii into the sea varied from 1 to 561 m, among which radii > 200 m were identified for sites L14, L17, L23, and L32. These findings support that the pollutants appear to reach the boundary line of the designated area but they are at acceptable levels.

Two stream waters (L14 and L17) in Changseon-myeon of Namhae-gun had high flow rates and fecal coliform levels, with ranges of 500–510 and 2400–11,000 MPN/100 mL, respectively. Their impact radii ranged from 200 to 347 m due to a relatively densely populated residential area in the drainage area. The flow rates of two stream waters (L23 and L32) from Samdong-myeon, Namhae-gun, were relatively low, with the ranges of 60–82 L/min; however, their fecal coliform numbers were very high, in the ranges of 24,000–240,000 MPN/100 mL. The impact radii of these sources ranged from 254 to 561 m. The results show that the stream waters were very large contamination sources in the study area. The highest impact radius among the discharges was observed at site L23, a stream located in Samdong-myeon of Namhae-gun, because the stream flowed directly into the sea area through a relatively densely populated residential area without a wastewater treatment plant (WWTP).

The results indicate that four discharges, containing sites L14, L17, L23, and L32 with the large impact radii, were clearly confirmed as substantial polluters. Although the discharged waters are highly contaminated with different pollutants from populated residential areas, they were diluted sufficiently in the sea area, such that they had no significant impact on the designated shellfish-producing area. However, we recommend that the local government authority in Namhae-gun should construct new WWTPs in regions without WWTPs to better protect the shellfish-producing area and to secure the safety of shellfish produced in the area.

Seasonal variation in environmental factors

Figure 2a shows the monthly mean values in water temperature and salinity in the Changseon area during 2011–2013. The mean water temperature varied from 7.3 °C in January to 25.8 °C in August, exhibiting large seasonal variation. The mean salinity was in the ranges of 30.86–33.92 practical salinity units (psu). The lowest salinity (28.78 psu) was measured in September 2012 due to heavy rainfall prior to sampling. These results mean that the water temperature was relatively high in summer and low in winter; however, the salinity was

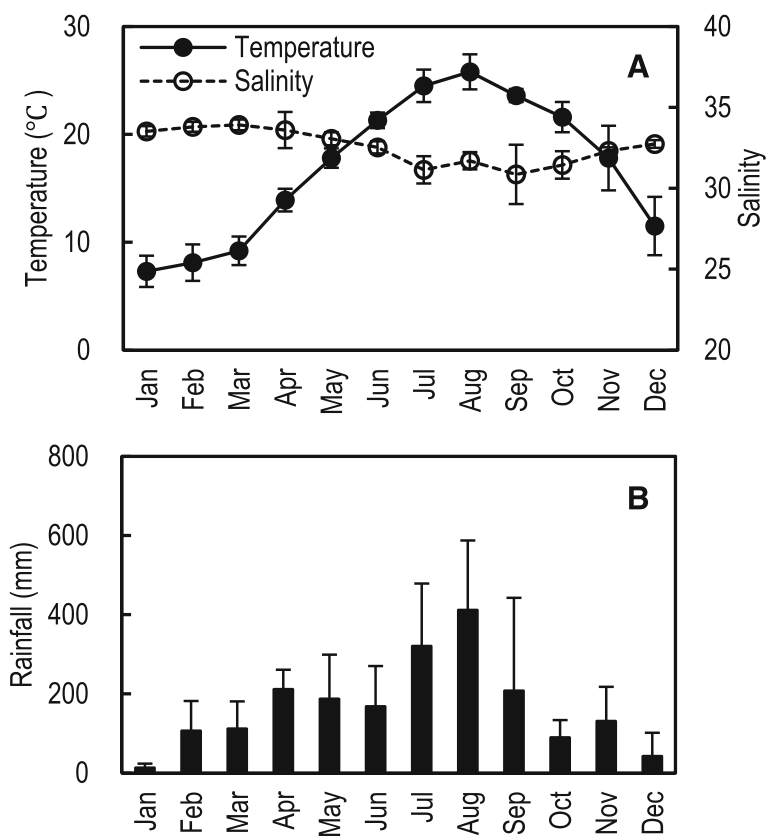

Fig. 2 Seasonal variation in mean water temperature and salinity (**a**), and mean rainfall (**b**) in the Changseon area, Korea during 2011–2013. The scale bars represent standard deviations. Rainfall data were obtained from the meteorological observatory of Namhae-gun in Korea

relatively high in winter and low in summer. Figure 2b shows the monthly mean variations in rainfall throughout the survey period. The mean rainfall was relatively high between July and August within the Korean wet season. The present findings show that the variation patterns in the water temperature and salinity and rainfall in the Changseon area are similar to those on the southern coast of Korea reported by Mok et al. (2016a).

Spatial distribution of fecal coliform concentrations in seawater

Figure 3 shows the geometric mean and estimated 90th percentiles of fecal coliforms in 1116 seawater samples from 31 monitoring stations, including the designated (17 stations) and adjacent (14 stations) areas in the Changseon area during 2011–2013. In the designated area, the geometric mean and estimated 90th percentile levels of fecal coliforms at each station ranged from < 1.8 to 2.1 and from 1.8 to 8.9 MPN/100 mL, respectively, with a maximum observed at station D13. In the adjacent area, their values at each site were with the ranges of 1.9–4.1 and 3.9–39.6 MPN/100 mL, respectively. The highest values were observed at station A3. We assume that high concentrations of fecal coliforms (240–17,000 MPN/100 mL) in the samples collected at sites A1 and A3 after heavy rainfall between June and July 2011 (range, 47.0–52.2 mm) were due to waste discharge from the densely populated residential area of Sacheon City (Fig. 1). Our results demonstrated that the fecal coliform levels in the designated area were significantly higher ($P < 0.05$) than those in the adjacent area.

Seawater stations A1–A7 and D3–D5 were established to evaluate the impacts of major pollution sources in Sacheon City, located to the north of the Changseon area, on the water quality of the shellfish-producing area (Fig. 1). Among these stations, A1 and A3 were closest to Sacheon City, including a densely populated residential area. Station A1 was located at the mouth of the

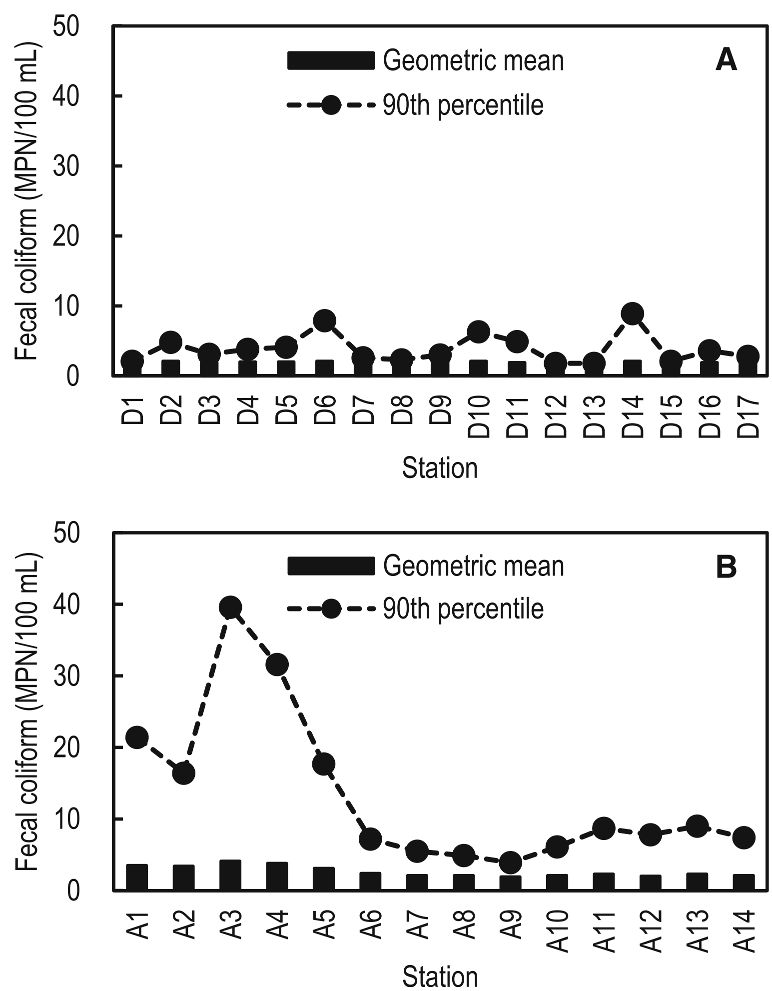

Fig. 3 Spatial variation of the geometric mean and estimated 90th percentile of fecal coliform levels in seawater samples from the designated shellfish-producing area (**a**) and adjacent area (**b**) of the Changseon area in Korea during 2011–2013

Samcheonpo Port in Sacheon City, and was affected by waste discharges from the Bongnam Stream (L8) and Samcheonpo Stream (L9), which drain into the sea after passing through the city. Station A3 was located ~ 2.0 km from Bonghyeon Stream (L7) and the Samcheonpo WWTP in Sacheon City. The Samcheonpo WWTP had a discharge flow rate of 19,210 L/min, with 7900 MPN/100 mL and 2.2×10^{12} MPN/day daily load of fecal coliform during the on-site shoreline survey in 2013. The estimated 90th percentiles of fecal coliforms over 3 years at sites A1 and A3 were 21.4 and 39.6 MPN/100 mL, respectively. At these stations, contamination increased rapidly due to heavy rainfall, as noted above. Moreover, station A4, which was ~ 2.0 km from both stations A1 and A3, had an estimated 90th percentile of 31.6 MPN/100 mL for fecal coliforms, which was ~ 1.5-fold higher than that of station A1 but 0.8-fold lower than that of station A3. The estimated 90th percentiles of fecal coliforms at stations A5 and A6, which were 2.0 and 2.5 km from station A3, were 17.7 and 7.2 MPN/100 mL, respectively. The estimated 90th percentiles of fecal coliforms at these stations, which were relatively closer to the designated area, decreased in the order of station A3 (39.6 MPN/100 mL) > station A4 (31.6 MPN/100 mL) > station A5 (17.7 MPN/100 mL) > station A6 (7.2 MPN/100 mL). Conversely, stations D3, D4, and D5, located on the boundary line of the designated area, were > 6.0 km from station A3. The estimated 90th percentiles of fecal coliforms at stations D3–D5 over the three study years ranged from 3.1 to 4.1 MPN/100 mL, showing good water quality. These results proved that, although many fecal contaminants including bacteria flowed into the sea area from inland area, they were diluted and reduced in passing through the buffer zone between the coastline and designated area in the Changseon area. The pollutant dilution pattern was similar to that in the Jaranman-Saryangdo area reported by Mok et al. (2016a). In summer, the fecal coliform levels at these stations were affected by rainfall. Other researchers have reported that reductions in fecal coliform concentrations within coastal water from wastewater discharges were attributed to dilution, removal, death, or sedimentation (Chigbu et al. 2005; Azalea et al. 2010; Park et al. 2016). After fecal coliform bacteria are transported into the sea area via runoff, their loss rates from the water column depend on a multitude of factors, including nutrient availability, temperature, salinity, turbidity, degree of water mixing, solar radiation, predation and competition (Chigbu et al. 2005).

In this study, the fecal coliform levels at all stations in the designated area were far below the regulation limit of the geometric mean and estimated 90th percentile (14 and 43 MPN/100 mL, respectively) for fecal coliforms set by Korea (MOF 2015a), the US (US FDA 2015) and New Zealand (NZFSA 2006) for approved areas. Moreover, no stations in the adjacent area exceeded the limit. These results support that the sanitary conditions of the Changseon area meet the criteria set by Korea, the US, and New Zealand, and shellfish, including mussels, produced in the area are suitable for raw consumption. By comparison, Chigbu et al. (2004) reported that only one of 11 years met the approved area classification criterion for shellfish-producing areas in the Mississippi Sound, USA, which was consistent with the current classification for the conditional approval of areas for shellfish harvest.

Seasonal variation of fecal coliform in seawater

Figure 4 shows the monthly variations of fecal coliforms in seawater samples from the Changseon area over 3 years. In the designated area, the monthly geometric mean and estimated 90th percentile levels of fecal coliforms ranged from < 1.8 to 2.7 and from < 1.8 to 14.7 MPN/100 mL, respectively, with the highest values observed in July (Fig. 4b). In particular, the monthly estimated 90th percentile level of fecal coliforms in July 2011 was 88.1 MPN/100 mL, which exceeded the fecal coliform criteria of 43 MPN/100 mL for the approved area set by the KSSP (MOF 2015a) and NSSP (US FDA, 2015). However, with the exception of samples collected immediately after a heavy rainfall (47.0 mm) in July 2011, the monthly bacteriological water quality showed favorable conditions in the designated area.

In the adjacent area, the monthly geometric mean and estimated 90th percentile levels of fecal coliforms ranged from < 1.8 to 5.5 and from 1.8 to 100.8 MPN/100 mL, respectively, with maximum levels observed in July (Fig. 4b). In particular, the monthly estimated 90th percentile levels of fecal coliforms in the samples collected immediately after heavy rainfalls in June (52.2 mm), July (47.0 mm), and November (76.6 mm) 2011 and in June 2012 (37.0 mm), exceeded the regulation limit of fecal coliform for the approved area set by the KSSP (MOF 2015a) and NSSP (US FDA 2015). Similarly, Chigbu et al. (2004) reported that fecal coliform levels were positively correlated with rainfall in the Mississippi Sound.

In this study, rainfall information was useful for evaluating the potential effects on water quality and the classification of shellfish-producing waters to protect humans from consuming contaminated shellfish. According to the KSSP criteria based on the amount of rainfall for the conditionally approved area in the Changseon area, when 15–22 or > 22 mm of rain falls within 24 h, shellfish harvesting is prohibited for 48 or 168 h, respectively, after the rain stops (MOF 2015a). In this study, relatively high fecal coliform concentrations were observed in samples collected immediately after heavy rainfalls. However, based on the KSSP closure

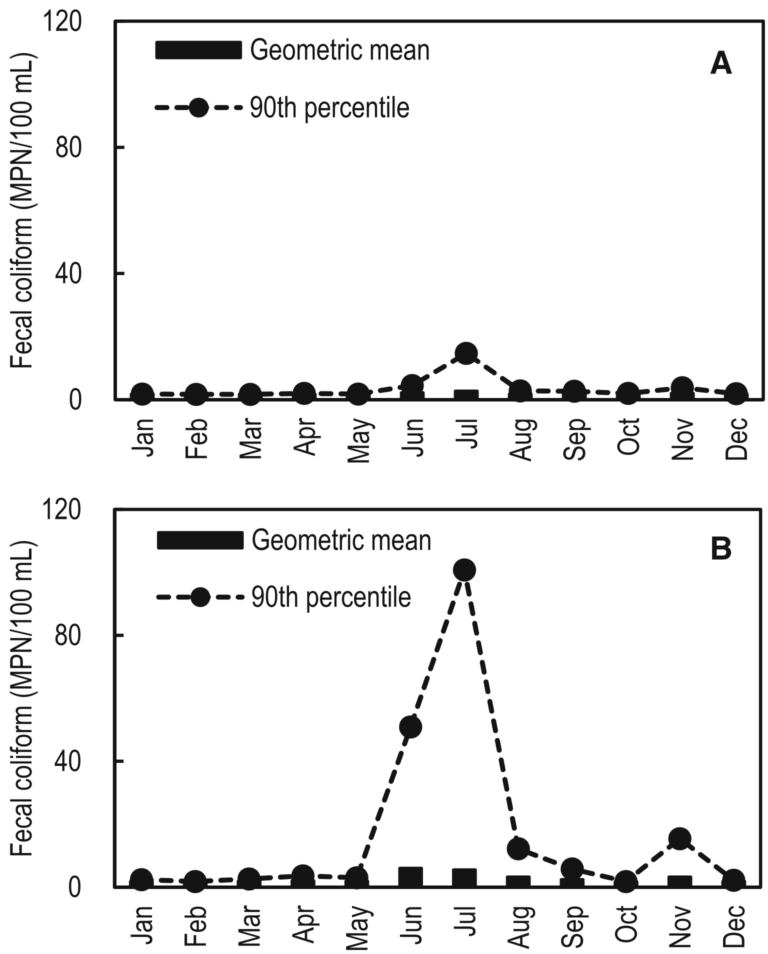

Fig. 4 Seasonal variation of the geometric mean and estimated 90th percentile of fecal coliform levels in seawater samples from the designated shellfish-producing area (**a**) and adjacent area (**b**) of the Changseon area in Korea during 2011–2013

Table 2 The concentration of fecal coliform or *Escherichia coli* in seawater and mussels, and bioaccumulation factors of fecal coliform in mussels

Station	Seawater		Mussel				BF[c]
	90th of FC [a] (MPN/100 mL)	No. of samples	90th of FC (MPN/100 g)	*E. coli* [b] (MPN/100 g)		No. of samples	
				Range	> 230 (No. of samples)		
Designated area							
D6	7.9	36	120.2	< 20–1300	1	36	15.2
D7	2.6	36	79.2	< 20–490	1	36	30.5
D8	2.3	36	62.7	< 20–220	0	36	27.3
Adjacent area							
A13	9.0	36	105.4	< 20–2400	1	36	11.7
A14	7.4	36	109.5	< 20–700	2	19	14.8

[a] *90th* the estimated 90th percentile; *FC* fecal coliform
[b] *E. coli Escherichia coli*
[c] The bioaccumulation factor (BF) was calculated as 90th value of fecal coliform in 100 g of oyster divided by that in 100 mL of seawater

criteria, shellfish harvesting in the designated area is prohibited during regular closure periods based on rainfall. Therefore, it is unnecessary to adjust both the existing boundary line and the current classification of the designated area.

Levels and bioaccumulation of coliform bacteria in mussels

Table 2 shows the *E. coli* counts in 163 mussel samples collected from five stations in the sea area throughout the sampling period. The *E. coli* levels in all mussel samples varied from < 20 to 2400 MPN/100 g. Among them, the five samples, including two and three samples from the designated and adjacent areas, respectively, exceeded the regulation limit of *E. coli* 230 MPN/100 g for raw shellfish set by New Zealand (NZFSA 2006) and the EU (EC 2005). All samples exceeding the limit for *E. coli* were collected after heavy rainfall (47–51 mm) during the rainy season between June and July 2011, which was within the non-harvesting season for mussels in Korea. Therefore, these cases had no effect on the safety of shellfish in this area. Korean consumers eat only the boiled tissue of mussel; however, some consumers from other cultures occasionally eat raw mussels. No pathogenic bacteria, such as *Salmonella* spp. or *Shigella* spp., were detected in all the mussel samples collected from the study area (data not shown). According to Korea Centers for Disease Control & Prevention (KCDC 2018), only two infected patients were caused by both *Salmonella* spp. and *Shigella* spp. in Namhae-gun including the survey area during same period as this study, accounting for 0.17% of total infected patients associated with these pathogens in Korea. Therefore, it indicates that, although various fecal pollution sources were discharged into the sea area, they contained not only very few pathogenic bacteria due to very low rate of their infection around this survey area, but also were diluted sufficiently and reduced in the sea area, such that the pathogens were not detected in all the mussel samples.

In addition, we previously also reported that the concentration of eight heavy metals was determined in the mussels collected from the Changseon area along the southern coast of Korea from 2008 to 2013 including this study period (Mok et al. 2014). In all tested mussel samples, the concentrations of the three hazardous metals (cadmium, lead, and mercury) were below the limits set by Korea and other countries, and the hazard index for all tested metals was far less than 1.0. Therefore, these results confirm that the mussels produced in the Changseon area do not present an appreciable hazard to human health based on heavy metals as well as fecal pollution and pathogenic bacteria.

Microorganisms usually accumulate in bivalve to concentrations much higher than that in the surrounding seawater, and accumulation amounts vary according to their filter ability, from a few folds to above a hundred folds (Doré and Lees 1995; Burkhardt and Calci 2000). In this study, the accumulation factors of fecal coliforms in all mussel samples were in the ranges of 11.7–30.5 folds; the highest level was observed at site D7 in the designated area (Table 2). The bioaccumulation factor of fecal coliforms in mussels showed relatively high values at the sites with lower fecal coliform concentration in seawater and also slightly higher at sites in the designated area than at them in the adjacent area. It showed that the accumulation level of fecal coliforms in mussels was slightly higher than that in oysters from the southern coast of Korea, with the ranges of 6.9–13.4 folds (Mok et al. 2016a). But the accumulation pattern of fecal coliforms in mussels, accumulated relatively higher in cleaner seawater sites, was similar to that in oysters. We previously reported that the bioaccumulation levels of heavy metals in oysters (Mok et al. 2015b) and mussels (Mok et al. 2014) from the Korean coast varied from 1413 to 618,958 fold and from 429 to 74,794 fold, respectively. Furthermore, Burkhardt and Calci (2000) reported that F-specific coliphages were selectively accumulated up to 99 fold in oysters. These results signify that bivalves such as mussels and oysters accumulate fecal coliforms at relatively lower levels than they accumulate heavy metals and F-specific coliphages. Also, they accumulate a variety of pollutants at different levels relative to the surrounding water.

Conclusions

In the present study, we determined the fecal coliform concentrations in inland pollution sources near the Changseon area on the southern coast of Korea and evaluated their impact on the shellfish therein. In addition, the concentrations of fecal-indicator bacteria in seawater and mussels were determined to evaluate the bacteriological quality of the seawater and mussels. The 164 of potential pollution sources were identified during the survey, including 33 sources with discharged water. Four stream waters (sites L14, L17, L23, and L32) were confirmed as the major pollution sources, with impact radii > 200 m. Overall, many pollution sources were identified in this study, but the pollutants had no any of impact on the boundary line of the designated area because of an existing buffer zone in which bacteria were diluted, thereby reducing their concentrations in the designated area.

We confirmed that all seawater stations in the designated area from 2011 to 2013 had fecal coliforms concentrations far below the regulation limit set by a variety of countries. Of 108 mussel samples collected over 3 years in the designated area, only three samples had *E. coli* concentrations exceeding the regulation limit for raw shellfish set by New Zealand and the EU; however, these samples were collected after heavy rainfall (47–51 mm) during the non-harvesting season in Korea. Fecal coliform levels were higher in seawater samples collected immediately after heavy rainfall than in other samples. However, according to the KSSP closure criteria based on rainfall volume, shellfish harvesting in this area has been prohibited during regular closure periods. Therefore, it is unnecessary to adjust the existing boundary line of the designated area.

No bacterial pathogens, such as *Salmonella* spp. or *Shigella* spp., were detected in all the mussel samples. In our previous study, the concentrations of the three hazardous metals (cadmium, lead, and mercury) in all of the mussels collected from this study area, during 2008–2013 including this study period, were below the limits set by Korea and other countries. These results indicate that mussels produced in this area do not present an appreciable hazard to human health based on not only their bacterial quality including fecal bacteria and bacterial pathogens, but also heavy metals.

Abbreviations
E. coli: *Escherichia coli*; KSSP: Korean Shellfish Sanitation Program; MPN: Most probable number; NSSP: National Shellfish Sanitation Program; WWTP: Wastewater treatment plant

Acknowledgements
This work was supported by a grant from the National Institute of Fisheries Science in Korea (R2018056).

Availability of data and materials
Not applicable.

Authors' contributions
JSM and KBS designed this study, carried out the analysis of the fecal coliform, and drafted the manuscript. JYK and PHK carried out the sampling and analysis of the fecal coliform. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

Author details
[1]Food Safety and Processing Research Division, National Institute of Fisheries Science, 216, Gijanghaean-ro, Gijang-eup, Gijang-gun, Busan 46083, Republic of Korea. [2]Southeast Sea Fisheries Research Institute, National Institute of Fisheries Science, 397-68, Sanyangilju-ro, Sanyang-eup, Tongyeong 53085, Republic of Korea.

Received: 20 August 2018 Accepted: 14 November 2018
Published online: 10 December 2018

References
APHA (American Public Health Association). Recommended procedures for the examination of seawater and shellfish. Washington DC: American Public Health Association; 1970.

Azalea AM, Katherine CG, William AM. Fecal coliform accumulation within a river subject to seasonally-disinfected wastewater discharges. Water Res. 2010;44: 4776–82.

Biancani PJ, Carmichael RH, Daskin JH, Burkhardt W, Calci KR. Seasonal and spatial effects of wastewater effluent on growth, survival, and accumulation of microbial contaminants by oysters in Mobile Bay, Alabama. Estuar Coasts. 2012;35:121–31.

Burkhardt W, Calci KR. Selective accumulation may account for shellfish-associated viral illness. Appl Environ Microbiol. 2000;66:1375–8.

Chigbu P, Gordon S, Strange T. Influence of inter-annual variations in climatic factors on fecal coliform levels in Mississippi Sound. Water Res. 2004;38:4341–52.

Chigbu P, Gordon S, Tchounwon PB. The seasonality of fecal coliform bacteria pollution and its influence on closures of shellfish harvesting areas in Mississippi Sound. Int J Environ Res Public Health. 2005;2:362–73.

Doré WJ, Lees DN. Behavior of *Escherichia coli* and male-specific bacteriophage in environmentally contaminated bivalve molluscs before and after depuration. Appl Environ Microbiol. 1995;61:2830–4.

EC (European Commission). Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. 2005. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2005R2073:20071227:EN:PDF. Accessed on 22 June 2016.

ISO (International Organization for Standardization). ISO 16649-3:2015, microbiology of the food chain – horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* – part 3: detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide. Geneva: International Organization for Standardization; 2015.

Jung YJ, Park YC, Lee KJ, Kim MS, Go KR, Park SG, Kwon SJ, Yang JH, Mok JS. Spatial and seasonal variation of pollution sources in proximity of the Jaranman-Saryangdo area in Korea. Mar Pollut Bull. 2017;115:369–75.

Kang CH, Oh SJ, Shin YJ, Kim YG, Oh EG, So JS. Impact of inland pollution sources on the bacteriological water quality of the Southern Ganghwado Bay Area, South Korea. Urban Water J. 2017;14:69–73.

KCDC (Korea Centers for Disease Control & Prevention). 2018. Infectious disease portal. http://www.cdc.go.kr/npt/biz/npp/nppMain.do. Accessed 29 Oct 2018.

Kim JH, Shim KB, Shin SB, Park K, Oh EG, Son KT, Yu HS, Lee HJ, Mok JS. Comparison of bioaccumulation and elimination of *Escherichia coli* and male-specific bacteriophages by ascidians and bivalves. Environ Sci Pollut Res. 2017;24:28268–76.

KMFDS (Korea Ministry of Food and Drug Safety). Korea food code. 2016. https://www.foodsafetykorea.go.kr/foodcode/index.jsp Accessed 22 June 2016.

MOF (Ministry of Oceans and Fisheries). Korean shellfish sanitation program (KSSP). Sejong: Ministry of Oceans and Fisheries; 2015a.

MOF (Ministry of Oceans and Fisheries). Korean shellfish sanitation program (KSSP) FY 2014 annual report. Sejong: Ministry of Oceans and Fisheries; 2015b.

Mok JS, Kwon JY, Son KT, Choi WS, Kim PH, Lee TS, Kim JH. Distribution of heavy metals in internal organs and tissues of Korean molluscan shellfish and potential risk to human health. J Envron Biol. 2015a;36:1161–7.

Mok JS, Lee KJ, Kim PH, Lee TS, Lee HJ, Jung YJ, Kim JH. Bacteriological quality evaluation of seawater and oysters from the Jaranman-Saryangdo area, a designated shellfish growing area in Korea: impact of inland pollution sources. Mar Pollut Bull. 2016a;108:147–54.

Mok JS, Lee TS, Kim PH, Lee HJ, Ha KS, Shim KB, Lee KJ, Jung YJ, Kim JH. Bacteriological quality evaluation of seawater and oysters from the Hansan-Geojeman area in Korea, 2011–2013: impact of inland pollution sources. Springerplus. 2016b;5:1412.

Mok JS, Yoo HD, Kim PH, Yoon HD, Park YC, Lee TS, Kwon JY, Son KT, Lee HJ, Ha KS, Shim KB, Jo MR, Kim JH. Bioaccumulation of heavy metals in mussel *Mytilus galloprovincialis* in the Changseon area, Korea, and assessment of potential risk to human health. Fish Aquat Sci. 2014;17:313–8.

Mok JS, Yoo HD, Kim PH, Yoon HD, Park YC, Lee TS, Kwon JY, Son KT, Lee HJ, Ha KS, Shim KB, Kim JH. Bioaccumulation of heavy metals in oysters from the

southern coast of Korea: assessment of potential risk to human health. Bull Environ Contam Toxicol. 2015b;94:749–55.
NZFSA (New Zealand Food Safety Authority). Animal products (specifications for bivalve molluscan shellfish). 2006. https://www.mpi.govt.nz/processing/seafood/seafood-processing/bivalve-molluscan-shellfish-bms/growers-and-harvesters-of-bivalve-molluscan-shellfish-bms. Accessed 22 June 2016.
Park YC, Kim PH, Jung YJ, Lee KJ, Kim MS, Go KR, Park SG, Kwon SJ, Yang JH, Mok JS. Seasonal variation of physicochemical factor and fecal pollution in the Hansan-Geojeman area, Korea. Fish Aquat Sci. 2016;19:17.
Statistics Korea. Korean statistical information service (KOSIS). 2014. http://kosis.kr/index/index.do. Accessed 22 June 2016.
U.S. FDA (Food and Drug Administration). National shellfish sanitation program (NSSP), Guide for the control of molluscan shellfish. 2015. http://www.fda.gov/Food/GuidanceRegulation/FederalStateFoodPrograms/ucm2006754.htm.. Accessed 20 June 2016.