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Evaluating *Escherichia coli* contamination in bivalve mollusks using the impedance method: a comparison with most probable number analyses and correlation with environmental parameters

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Abstract

The application of an electrochemical (impedance) tool for monitoring *Escherichia coli* contamination in shellfish was evaluated after 13 months of observation. The primary aim of the present study was to compare the standard most probable number (MPN) and μ-trac 4200 (log imped/100 g) for the assessment of *E. coli* contamination (log MPN/100 g) in non-depurated bivalve mollusks (BM) from five sampling areas of the Veneto-Emilian coast (Italy) (118 samples). The secondary aim was to evaluate the correlation between *E. coli* concentrations in BM and environmental factors on a large data set (690).

The methods showed a moderate, positive correlation (0.60 and 0.69 Pearson and Spearman coefficients, respectively; P<0.01) in *Ruditapes philippinarum.* The McNemar test indicated analogous sample classification between methods, and the impedance method overestimated the most contaminated class (P=0.03; >4,600 MPN/100 g). The results highlighted the suitability of the impedance method for a faster evaluation and routine use especially in clams, while in *Mytilus* it seemed less effective. Different models built by multivariate permutational variance analysis and multinomial logistic regression selected the suitable environmental features able to predict the *E. coli* load. Overall, salinity and season affected the *E. coli* contamination, whereas locally it was mainly influenced by hydrometry and salinity. The application of the impedance method coupled with environmental data analysis could help purification phase management to adhere to legal limits and could represent an advantage for local control authorities to define actions, considering extreme meteorological events' effects as a proactive reaction to climate change.

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Introduction

The fecal contamination of coastal waters and farmed or caught organisms is an increasingly serious problem, especially in areas with a high anthropogenic impact. Numerous microbial targets have been proposed as fecal indicators [fecal indicator bacteria (FIB)]. Currently, *Escherichia coli* and fecal coliforms are the FIB investigated worldwide as markers for hazard identification and regulatory compliance to evaluate the microbiological quality of water and products collected from natural or farmed areas (Holcomb and Stewart, 2020; Saingam *et al*., 2020). For more than a decade, European legislation has consolidated the classification of relaying and production areas of live bivalve mollusks [(BM) filter-feeding lamellibranch mollusk] based on the level of FIB contamination (European Commission, 2004, 2005); indeed, European legislation established *E. coli* as the best FIB for classification of the production and harvesting areas of BM farming (European Commission, 2019). According to the fecal contamination level, the competent health authority classifies the production and harvesting areas and establishes the consequential procedures required to meet the health standard. In particular, BM collected in areas classified as A (230 MPN/100 g) can be directly commercialized for human consumption; whereas, BM from area classes B (≤4,600 MPN/100 g) and C (≤46,000 MPN/100 g) must be sent to a purification center and a relaying area, respectively. In the recent past (1980-2017), 39% of all notifications from the European Union alert system for food and feed (RASFF) related to high levels of *E. coli* were recorded in shellfish (Pigłowski, 2019). The main notifications were attributed to Italy (period 1/1/2010-25/1/2023 - 49%), where 18 and 6 notifications for *Mytilus galloprovincialis* and *Ruditapes philippinarum*, respectively, were recorded (data collected from RASFF by the authors). The economic relevance and the RASFF notifications in BM indicate that fecal contamination is a major concern for the Italian shellfish industry.

The degree of contamination in non-depurated BM results mainly from interactions with the environmental microbiological state (water and sediments); however, the kinetics or clearance accumulation largely depends on the species considered. Moreover, sources of contamination can be widespread or point-like, mainly linked to various human activities such as the contribution of urban or livestock wastewater or linked to wild animal populations (Campos *et al.*, 2013). To improve shellfish management and the level of safety for human health, a deeper (associated with environmental factors) and faster (in real-time) understanding of the potential degree of shellfish fecal contamination is desirable.

The most probable number (MPN) method is the official international reference method to evaluate *E. coli* contamination in bivalve shellfish (ISO, 2015); however, this approach requires two days to obtain the outcome, making it unsuitable when rapid results are essential. The impedance method, already used in some Italian and French laboratories for the enumeration of *E. coli* in bivalves, represents a faster alternative (Walker *et al*., 2018). In this context, the application of impedance analyses allows a faster enumeration (5-11 h) of FIB (*E. coli*) than the classical standard methods employing MPN. Impedance methods are based on real-time monitoring of conductivity variation in growth medium due to the microorganism's growth and the release of charged ions and molecules (Dupont *et al*., 2004). This approach allows the simultaneous analysis of a large number of samples and measurement of the growth and proportional change in the number of *E. coli* in culture by estimating the detection time (DT) correlated with the target log number (Dupont *et al*., 2004). Currently, few studies are performed using the impedance method to enumerate *E. coli* in BM (Dupont *et al*., 2004; Dupont *et al.*, 2010; Ifremer, 2014), and although this method performed similarly to MPN, few European companies are applying these devices to *E. coli* monitoring (Walker *et al*., 2018); indeed, the equipment costs and skilled personnel required for output interpretation discourage the widespread use of the impedance technique in the shellfishery industry (Walker *et al*., 2018). The overall aim of the present paper is to monitor BM contamination by *E. coli* for 13 months via the impedance method, providing suitable information about the on-field application. First, the performances of impedance were compared with those of the MPN procedure, considering different BM species typical of production in northeastern Italy. Second, an in-depth analysis of the environmental factors affecting *E. coli* by impedance analysis was conducted to define the best environmental features suitable as predictors of these FIB loads in BM.

Materials and Methods

Shellfish sampling and sample preparation

A total of 690 pools of BM at commercial size were collected in 13 months (from September 3 2018 to September 30 2019) from five different production and harvesting areas of the Veneto-Emilian coast (Po di Volano and Sacca di Goro, Sacca di Scardovari, Vallona and Marinetta). The BM sampling plan was performed considering two samples per week, two weeks per month, and regarded three species *Chamelea gallina* (common clams, n=59), *R. philippinarum* (philippine clams, n=390) and *M. galloprovincialis* (mussels, n=241).

The BM were washed under potable running water and then valves were opened with a sterile shucking knife, and pooled samples comprising a minimum of 10 individual subjects (flesh and intravalvar liquid) were processed as defined by EC Reg. 2073/2005 s.a.a. (European Commission, 2005). In detail, to 100 g of product, 200 mL of Tryptone broth (NaCl 8.5 g/L, tryptone 1.0 g/L) was added and homogenized with a stomacher and then analyzed using the MPN and impedance analyses.

Most probable number analyses

Live BM were analyzed with the MPN procedure (reference method) in agreement with ISO (2015) before purification treatment (n. tested samples=118). The limit of detection (LOD) for the MPN procedure was <18 MPN/100 g.

Impedance analysis

7.5 mL of bacterial suspension was added to 7.5 mL of culture broth *E. coli* selective (BiMedia 155A, SY-LAB, Neupurkersdorf, Austria), and analyzed using the μ-trac 4200 (SY-LAB geräte gmbh, Neupurkersdorf, Austria), with the temperature set at 44°C according to the optimum temperature for *E. coli.* Every 10 min, the μ-trac 4200 measured the variation of the impedance of the potassium hydroxide solution at 0.2%, which takes the generated CO_2 from the broth inoculated with the strain/target under study.

The reference procedure used for impedance analysis performed in a purification center site located in Ferrara (Italy) was based on the protocol released by Ifremer (2014). The calibration curve was previously defined by the Generon company (www.generon.it) via the application of in-house datasets. Data collected at the end of the incubation period were exported using Bac-Eval software (Sy-Lab) and used to obtain the impedance values, detection time (orig. DT), and the estimated Log number of *E. coli* contamination after an incubation time of ~11 hours.

Environmental data collection

The environmental data on pH, percent dissolved oxygen in the water (mg/L), water temperature (°C), and salinity (‰) were collected by stations monitored by the Regional Agency for Environmental Protection of Veneto and Emilia-Romagna. Moreover, the pluviometry (mm) data were obtained by monitoring stations installed in the Ca' Venier (Veneto region) and in the Goro (Emilia-Romagna region) areas, which are under the control of the Po Delta Consortium (https://www.bonificadeltadelpo.it). Two different hydrometric stations (Po di Ficarolo and port of Barricata of Veneto region) were selected for monitoring the hydrometric levels (m) of the Po Rivers.

Datasets and statistical analyses

Statistical analyses were performed i) to evaluate the agreement between methods (impedance [log number/100 g] and MPN [log MPN/100 g]) in *E. coli* contamination detection and ii) to correlate *E. coli* contamination levels with intrinsic (species) and extrinsic factors (production and harvesting areas and chemical-physical environmental parameters).

The agreement between methods was performed on two different datasets using two specific statistical tests. In particular, a sub-set (regression-set, n=54;) was composed of samples with *E. coli* contamination ≥18 MPN/100g and analyzed using a linear regression relaying MPN (log MPN/100 g) and impedance (DT and log number) results (IBM SPSS Statistics, version 26). Then, a second sub-set (class set, n=118) involved also the samples lower both LODs (<18 MPN/100 g and <140 imped/100 g) (Ifremer, 2014; Walker *et al*., 2018). According to Regulation (EC) No 2015/2285 (European Commission, 2015) and Regulation (EU) 2019/627, (European Commission, 2019) the class-set samples were clustered in four categories based on MPN/100 g contamination levels (<230, 230-700, 701-4600, >4600) and then analyzed with the McNemar test using the Marginal Homogeneity program (v. 1.2) (http://www.john-uebersax.com/stat/mh.htm). The relationship between methods was evaluated by Pearson's correlation coefficient (r) and Spearman's rank correlation coefficient (rho), according to each species considered in the regression model, in the regression set and, in the class set.

Moreover, an environmental set (n=462), which included data from environmental probes, was considered to evaluate the effect of extrinsic parameters on *E. coli* contamination with the impedance method (DT and log number). In detail, the nonparametric combination test (NPC test) methodology was applied to define the statistical differences between species and extrinsic factors (production and harvesting areas and environmental parameters) in terms of *E. coli* contamination. A two-way permutational multivariate analysis of variance (PERMANOVA) was performed to highlight the effects of fixed factors, such as the season and the origin of production and harvesting areas, as well as their interactions; when the interaction was significant, pairwise comparisons were performed. The Gower distance was applied to create a dissimilarity matrix. As initial exploratory multivariate analyses, the distance-based redundancy analysis (dbRDA) and distance-based multivariate analysis (DISTLM) approaches were applied to the DT and log number (μ-trac outcomes) to perform forward selection of the environmental variables linked to the *E. coli* trend (PRIMER-e software https://www.primer-e.com/). Ordinal regression models were applied to select suitable environmental variables able to predict *E. coli* contamination to discover relationships between predictors and levels of *E. coli* contamination as ordinal variables (IBM SPSS Statistics version 26).

Results and Discussion

Being able to quickly know the level of microbial contamination of the BM is useful parameter information for the purification centers. Identifying analytical methods that facilitate the evaluation and/or make it faster is important to improve the guarantees of food safety for both food business operators (FBO) operating the purification centers and control authorities. To increase the efficiency of purification, which is one of the critical control points (CCP) identified in this production process (IZS Umbria, 2023), impedance methods could support FBO action in a short time (impedance 11 hours *vs* 48 hours MPN). Moreover, it could represent further support to the competent authority in the monitoring of the production and harvesting areas (European Commission, 2019).

Agreement between impedance and most probable number analysis

The agreement between methods (MPN *vs* impedance method) was performed in naturally contaminated shellfish collected during a 13-month sampling period (~9 samples per month). 98.3% of the 118 analyzed samples originated from production and harvesting areas classified as B (≤4600 MPN/100 g). While, only 6% of samples showed an *E. coli* contamination ascribed to the class of 701-4600 MPN/100 g, and non-conforming samples were never detected. After purification, all the batches were again checked for *E. coli* contamination and all the aliquots agreed with the European regulation requirements. These results highlighted that the fecal contamination in collected BM was very limited, where 52% of samples were lower than the MPN LOD.

The impedance features (DT and log number) have already been compared with the MPN method by linear regression on BM samples (Dupont, *et al*. 2004; Ifremer, 2014). However, the aforementioned studies compared the methods on partially artificially contaminated oysters, cockles, and mussels. A high coefficient of determination (R^2 ranging from 0.84 to 0.90) was reported in the work of Dupont *et al*. (2004), demonstrating the suitability of *E. coli* estimation from DT. However, a comparison of the present study with that of Dupont *et al*. (2004) is difficult due to the lower *E. coli* load linked to the natural contamination of samples from the Po area considered in the present study. In particular, the contamination level ranged between 1.7×10^2 and 4.5×10^5 and between 1.8×10^2 and 2.4×10^3 of *E. coli*/100 g in the study by Dupont *et al*. (2004) and the present study, respectively.

In the present study, using the impedance method, the proportion of BM obtained with *E. coli* contamination higher than the impedance quantification limit (140 *E. coli*/100 g) (Ifremer, 2014; Walker *et al*., 2018) was 10% of the samples. This lower level of contamination and low sample number considered may have affected the comparison of the methods. As described in the Materials and Methods section, linear regression was applied only for samples with *E coli* contamination ≥MPN LOD.

On the other hand, according to the species, the best linear regression (log impedance×log MPN) was obtained in clams (*R. philippinarum*), which showed a significant positive correlation (r=0.60; P=0.01), suggesting a moderate relationship between methods (Table 1). The R^2 (0.36) observed in the present study was lower than those reported in the study of Dupont *et al*. (2004), R^2=0.84-0.90, probably due to the higher number considered per species (oyster, n=66-77; cockles, n=56-66; mussels, n=55-62) or

Table 1. Linear regression parameters and correlation coefficients between impedance features and log most probable number values (54 observations).

Species	Trait	Intercept	Slope	SE	r	R^2	P	PCC	Rho
R. philippinarum	Log number	0.51	0.59	0.41	0.60	0.36	0.003	0.60**	0.69**
	DT	2.39	-0.05	0.52	0.07	0.01	NS	-0.07	-0.30
M. galloprovincialis	Log number	1.51	0.19	0.65	0.31	0.1	NS	0.32	0.27
	DT	2.82	-0.12	0.65	0.27	0.07	NS	-0.27	-0.35

SE, standard error; r, coefficient of correlation; R^2, coefficient of determination PCC, Pearson correlation coefficient; Rho, Spearman correlation coefficient; DT, detection time; **P<0.01; NS, no significance P>0.05.

to the origin of contamination (partially artificial) and growth media (Malthus coliform broth with different NaCl concentrations) considered in their study. To overcome this bias, the McNemar test was applied to evaluate the agreement between methods considering four classes of contamination (<230, 230-700, 701-4600, >4600) (Table 2). According to the sample classification, the agreement between methods improved, showing a similar classification for almost all *E. coli* contamination categories; indeed, the impedance method overrated the higher levels of contamination (>4600 *E. coli*/100 g) concerning the MPN method (P=0.03). It is important to report that, in the case of class >4,600 MPN, the McNemar test X^2 is not well approximated by the chi-squared distribution. In this specific case, due to the low case number, a two-tailed exact test was performed (Table 2). In general, only this class of contamination was never reported by MPN analyses, moreover, also the class 701-4600 MPN/100 g showed a small number of samples. Despite this limitation, the agreement was performed in a real dataset composed of the observed variability of *E. coli* contamination along all the seasons and environmental conditions.

Generally, the McNemar test of overall bias showed that impedance analyses overestimated *E. coli* contamination (P=0.004). However, this trend should be confirmed by additional samples covering the *E. coli* contamination in more than one year of observation, to increase the representativeness of each contamination class.

Thus, according to these preliminary results, the impedance method not substantially differed from the standard method; therefore, its application can support the FBO of purification centers in making faster decisions about the duration of the purification process and defining corrective actions, for example, during episodes of massive contamination of BM. Moreover, after harvesting, the detectability of the *E. coli* contamination level in a short time by the impedance method could be considered as a further guarantee in the preventive assessment to reduce the cost of the purification process. In addition, this device offers the possibility of identifying contamination fluctuations resulting from extraordinary events of pollution. It is necessary to underline that to be able to consider the impedance method, as an alternative method to be used in the official control, it will be necessary to validate it against the reference method (MPN) as indicated in the Commission implementing regulation 2019/627 (European Commission, 2019).

Correlations between environmental parameters and impedance features

Bivalve mollusks, as filter-feeding animals, reflect the general hygienic quality of seawater by accumulating *E. coli*. Several studies showed a relationship between *E. coli* contamination and environmental parameters (Tabanelli *et al*., 2017; Colaiuda *et al*., 2021). Indeed, seasonal and environmental parameters (salinity, pH, dissolved oxygen, temperature, freshwater outflow, rainfall, and river input) affect *E. coli* contamination in BM due to differences in filtering activity and anthropogenic activities. In general, the association of hydrometeorological parameters and the *E. coli* contamination of BM should be defined specifically for each relaying and production area, considering local interactions between microbial contamination and environmental parameters (Colaiuda *et al*., 2021).

Italy is the main European clam producer (*R. philippinarum;* 27,160 tons in 2019), and production is mainly concentrated on the Veneto-Emilia coasts along the Po delta (MIPAAF, 2014; EUFOMA, 2021). The present paper provides a general assessment of the main factors affecting *E. coli* contamination in BM harvested in areas close to the mouth of the Po river, the main river of the northeastern Adriatic Sea. Different multivariate approaches were considered to describe the relationship between environmental parameters and the features estimated by impedance analyses (DT and log number). The NPC test showed that the log number differed (P<0.05) between the opposite extremes of the coast considered; in detail, Po di Volano (below the Po River) showed the lowest value [0.82±1.33 m, mean±standard deviation (SD)], and the highest was observed above to the Po River in Marinetta (1.54±1.33 m, mean±SD). This divergence could probably be explained by the different sea currents.

Considering the 13 sampling months, pH, dissolved oxygen, and salinity differed significantly among harvesting areas (P<0.001). In particular, the main divergences for pH were observed between the Scardovari and Goro areas (pH=8.46 and 8.11, respectively), dissolved oxygen between the Po di Volano and Marinetta areas (103.4 and 67.15 mg/L, respectively), and salinity between the Scardovari and Vallona areas (28.05 and 20.51 ‰, respectively). The species effect on *E. coli* contamination was investigated exclusively in Po di Volano and in Scardovari production and harvesting areas in which both clams and mussels were harvested and collected. A significant difference (P<0.05) was observed between clams (n=52) and mussels (n=56); in particular, clams (1.24±1.80 log number) showed an 85% greater log number than mussels (0.67±1.15 log number) for *E. coli* contamination. The divergence between species could be affected by their different positions in the water column in the harvesting area.

PERMANOVA analyses indicated that *E. coli* impedance features (DT and log number) were affected by the harvesting area (P=0.04), season (P<0.001), and BM species (P=0.001), whereas the interactions among factors were not significant. Thus, pairwise comparisons were performed exclusively on significant factors. As for the NPC test, according to the five harvesting areas, *E. coli* impedance features in the Po di Volano were significantly lower than those observed in the BM collected from the Vallona and Marinetta harvesting areas (P<0.01). Considering the whole dataset (n. 690), 98.5% of samples were collected from production and harvesting areas classified as B. The differences observed in *E. coli* load could be related to the hydrodynamic, bathymetric, and anthropogenic features of each lagoon. The harvesting areas of Sacca di Goro, Sacca

Table 2. Agreement results between most probable number (MPN) and impedance methods considering 4 classes of *Escherichia coli* contamination (<230, 230-700, 701-4600, >4600 MPN/100 g) on 118 samples.

	Frequency		Proportion (Base Rate)		
E. coli /100g class	MPN	MicroTrac	MPN	µ-trac	P
<230	99	91	0.84	0.77	0.06
230-700	12	17	0.10	0.14	0.16
701-4600	7	4	0.06	0.03	0.36
>4600	0	6	0	0.05	0.03#

MPN, most probable number; *#exact test.

degli Scardovari, and Po di Volano are affected by the inputs of fresh water from the southern mouths of the Po delta (Goro and Gnocca or Donzella). Marinetta and Vallona are mainly in contact with the Po di Venezia and Adige river mouths that collect water from other urban and rural sites in the Po valley. The five sites and lagoons largely differed in hydrodynamics exchange and renewal time of waters, whereas Scardovari showed poor hydrodynamics (Maicu *et al.*, 2018). This lagoon shows a complex hydrodynamic behavior with a reduction in water exchange in the northern sites of the lagoon. This can lead to problems in water quality, especially in warm months with several critical issues related to water temperature, algal blooms, or on the opposite problem of excessive reduction in water salinity due to the fresh water of Po river (Consorzio di Bonifica Delta del Po, 2014). Sacca degli Scardovari and Sacca di Goro are the biggest lagoons of the Po delta and the two most productive areas for shellfish production. Despite similar surfaces, the geographic position and orientation with respect to the dominant wind also affected water circulation. On the other hand, a rapid exchange with riverine flux can increase problems of water pollution, especially in the aquaculture systems. Moreover, certain lagoons, such as Vallona,

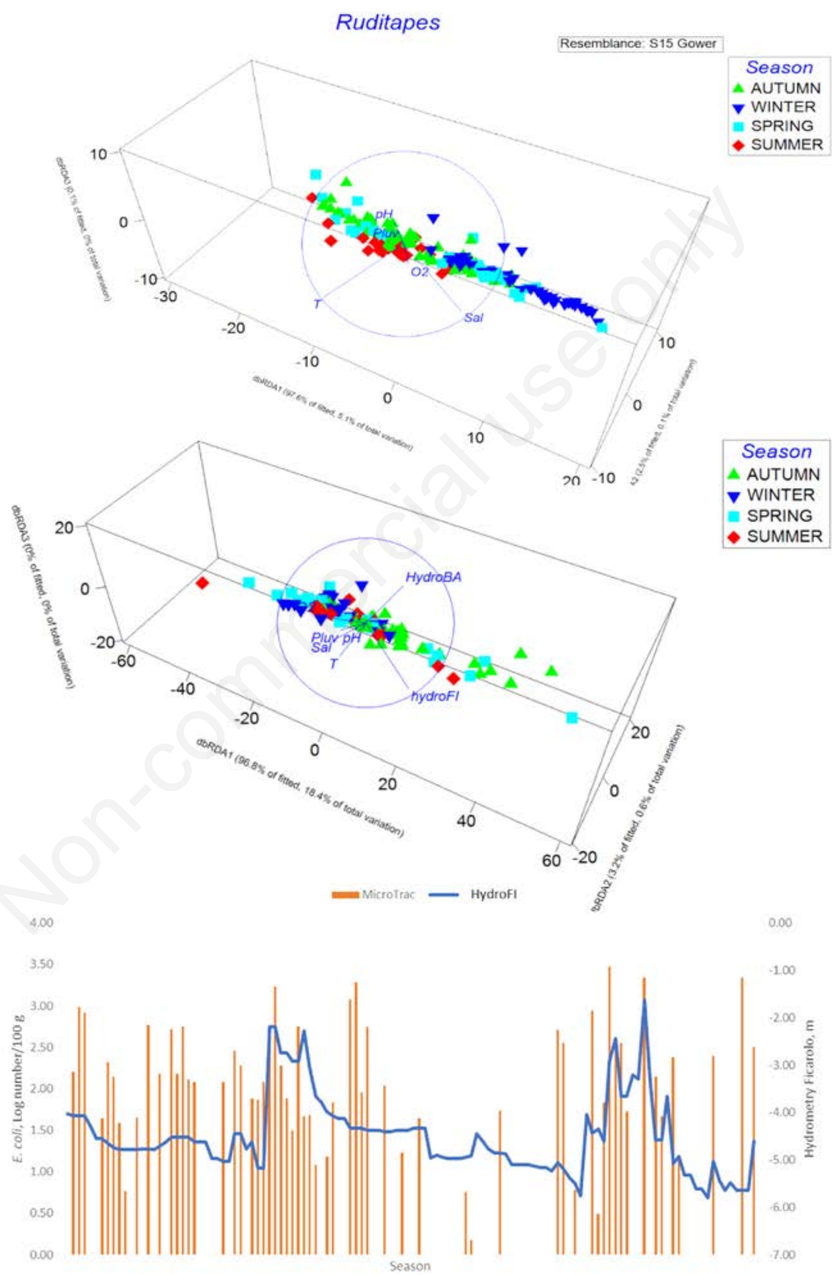

Figure 1. A) Distance-based redundancy analysis performed on *E. coli* evaluation (detection time and log number) according to environmental factors (pH, pluviometry, salinity, O_2, temperature) in *R. philippinarum*; B) Distance-based redundancy analysis performed on *E. coli* evaluation (detection time and log number) according to hydrometric parameters (hydrometry level of Barricata and Ficarolo) and environmental factors (pH, pluviometry, salinity, O_2, temperature) in *R. philippinarum*; C) Hydrometric trend recorded by the Ficarolo hydrometric station (m; blue line) and *E. coli* contamination level (log number; red bars) variations among seasons of *R. philippinarum* sampling.

Table 3. Results of distance-based multivariate analysis stepwise for the selection of environmental features (n=120); the variables were tested by marginal test and then fitted sequentially.

	Environmental traits	Pseudo F	P	Var %	Var. Cum. %
Marginal test	Hydro-Barricata	5.25	*	0.04	-
	Hydro-Ficarolo	17.5	***	0.13	-
	pH	1.75	NS	0.01	-
	Temperature	0.82	NS	0.07	-
	Dissolved O_2	2.53	NS	0.02	-
	Salinity	8.35	**	0.07	-
	Pluviometry	0.26	NS	0.02	-
Sequential test	Hydro-Ficarolo	17.53	***	0.13	0.13
	Temperature	3.71	*	0.03	0.16
	Pluviometry	1.94	NS	0.02	0.17
	Hydro-Barricata	1.07	NS	0.08	0.18
	Salinity	1.05	NS	0.07	0.19
	Dissolved O_2	0.62	NS	0.04	0.19
	pH	0.08	NS	0.06	0.19

***, P<0.001; **, P<0.01; *, P<0.05; NS, no significance P>0.05; Var %, variance; Var Cum%, variance cumulative.

showed low salinity due to the segregation of freshwater inputs in agreement with our observations (Maicu *et al*., 2018). All these lagoon features can affect the observed differences in the *E. coli* load. Seasons affected the *E. coli* impedance features; in particular, the values detected in BM analyzed in autumn were significantly higher than those collected in winter and spring (P<0.005). Similar divergences were observed and reported in a study by Tabanelli *et al*. (2017), which reported the highest concentration in autumn and the lowest in spring and summer. However, in the present study, the average log impedance concentration was similar between summer and autumn (1.58 and 1.66 log impedance, respectively); indeed, these similarities between seasons can be explained by the higher contamination related to the riverine network features and also by the higher tourism density of the coastal area during the summer and autumn seasons. The environmental variables reselected by DISTLM stepwise explained a very limited fraction of variability (2.8%), implying a minimal influence of environmental factors on *E. coli* contamination. Indeed, according to the individual effect among environmental parameters (using the marginal test), temperature (P<0.001), dissolved oxygen (P=0.03), and salinity (P<0.001) were the main factors; however, among parameters tested jointly (forward test), salinity was the only significant factor affecting *E. coli* contamination. In this context, the dbRDA highlighted sample clusterization related to salinity and seasonality (Figure 1A).

In addition, salinity was defined as a significant factor affecting the level of contamination exclusively in *R. philippinarum* (P<0.001) due to the wider variability of all sampled collection areas, whereas in *M. galloprovincialis*, the same factor showed no effect, probably because only two collection areas were sampled (Po di Volano and Scardovari).

According to the PLUM - ordinal regression stepwise (applied on class set), the most appropriate environmental factors to predict the trend in *E. coli* concentration using the impedance method were the variables season and salinity (P=0.024). In particular, the most contaminated class (701-4600 MPN; P=0.019) was mainly affected by the salinity factor (P=0.039), with an odds ratio (OR) of 0.95, suggesting lower *E. coli* contamination at high levels of salinity; this is also supported by the very low variability explained by the model (of Pseudo R-Square). Interestingly, a higher contamination load (2.88-3.56 log impedance) was mainly associated with a salinity range between 5 to <18 ‰ (27% of samples) and 18 to <30 ‰ (68% of samples). In the contamination class <230 impedance/100 g, this proportion shifts to 19% (range 5-18‰) of samples and 74% (range 18-30‰) respectively, with the 7% of samples harvested from waters with salinity over the 30‰ (European Commission, 2003). These observations suggested a trend that could be considered mainly by the FBO for a better definition of the purification step. The quickness of the impedance method could be employed in the CCP monitoring allowing frequent checks on the progress and effectiveness of the purification in case of low salinity levels (downpour, river flood, *etc*.).

A reduced dataset (120 observations) was applied to study the effect related to the hydrometry of the Po river only on the data related to *R. phylippinarum* harvested in the Scardovari, Marinetta, and Vallona areas. The hydrometric levels were collected from the online available data series of Ficarolo (~85 km from the mouth of the river) and Barricata (on the outlet of the river) hydrometers. From the stepwise DISTLM procedure, a 19% cumulative percentage of variance was explained by the model (Table 3). The marginal test defined hydrometric and salinity as significant features and sequential selection suggested that the most important variables were the hydrometric levels of Ficarolo and water temperature. All the environmental variables were selected to evaluate the *E. coli* features; however, in the PLUM analysis, only the hydrometric level of Ficarolo (P=0.001) and the season (P=0.04) were considered as predictive features, suggesting that at local level, seawater factors were less important in explaining the variation on the microbiological quality of the clams. Indeed, the Ficarolo hydrometry showed an OR of 1.67, and the dbRDA showed a positive multiple partial correlation (0.73) with the dbRDA1 coordinate axis (Figure 1B). These data suggested a positive correlation between hydrometry and *E. coli* loads; indeed, Figure 1B shows the trend in the variables, in which the highest loads of *E. coli* observed were related to the highest hydrometric levels. In particular, although from the dbRDA analysis, only autumnal months were associated with the highest levels of hydrometry (Figure 1B), two different peaks of hydrometry were observed in autumn and spring (Figure 1C). Thus, corrective actions to prolong the purification phase could be considered according to meteorological events.

Conclusions

Despite an overestimation of the higher level of contamination (>4600 *E. coli*/100 g), the *E. coli* load estimated by the impedance method agreed with the reference MPN analysis. The present results highlighted the feasibility of the μ-trac 4200 application for routinely use; however, the tool performance should be carefully evaluated according to the mollusk species and the specific contamination levels for each production and harvesting area. Moreover, the agreement between methods should be estimated in larger data sets considering different years of production and more samples from the different mollusk species.

The data reported in the present study, although they should be considered as preliminary results, suggest that at local level, *E. coli* contamination in clams could be predicted by data analyses of Po hydrometric levels. However, further studies will be necessary to enhance the knowledge required to interpret the relationships between environmental, species factors, and *E. coli* in bivalve mollusks. Thus, the impedance method should represent a further guarantee as a preventive and faster approach than MPN to plan and manage the purification phase, considering fluctuations of contamination due to seasons and other factors affecting the pollution. The application of the impedance method coupled with environmental data analysis represents a support for self-monitoring system according to hazard analysis critical control point principles, and could also be a strategy for local authorities to define more appropriate actions, especially after extreme meteorological events.

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