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Evaluating the impact of hydrometeorological conditions on *E. coli* concentration in farmed mussels and clams: experience in Central Italy

Valentina Colaiuda, Federica Di Giacinto, Annalina Lombardi, Carla Ippoliti, Carla Giansante, Mario Latini, Giuseppina Mascilongo, Ludovica Di Renzo, Miriam Berti, Annamaria Conte, Nicola Ferri, Marco Verdecchia and Barbara Tomassetti

ABSTRACT

Highly populated coastal environments receive large quantities of treated and untreated wastewater from human and industrial sources. Bivalve molluscs accumulate and retain contaminants, and their analysis provides evidence of past contamination. Rivers and precipitation are major routes of bacteriological pollution from surface or sub-surface runoff flowing into coastal areas. However, relationships between runoff, precipitation, and bacterial contamination are site-specific and dependent on the physiographical characteristics of each catchment. In this work, we evaluated the influence of precipitation and river discharge on molluscs' *Escherichia coli* concentrations at three sites in Central Italy, aiming at quantifying how hydrometeorological conditions affect bacteriological contamination of selected bivalve production areas. Rank-order correlation analysis indicated a stronger association between *E. coli* concentrations and the modelled Pescara River discharge maxima ($r = 0.69$) than between *E. coli* concentration and rainfall maxima ($r = 0.35$). Discharge peaks from the Pescara River caused an increase in *E. coli* concentration in bivalves in 87% of cases, provided that the runoff peak occurred 1–6 days prior to the sampling date. Precipitation in coastal area was linked to almost 60% of cases of *E. coli* high concentrations and may enhance bacterial transportation offshore, when associated with a larger-scale weather system, which causes overflow occurrence.

Key words | bivalve molluscs, discharge, *Escherichia coli*, food security, hydrological modelling, precipitation

HIGHLIGHTS

- Using a grid-distributed hydrological model to assess hydrometeorological conditions in the absence of direct measurements.
- Analysis of a previous uninvestigated area.
- New discharge threshold determining *Escherichia coli* concentration peaks found in Central Italy in order to allow the development of an early warning system for risk assessment.

Valentina Colaiuda (corresponding author)
Annalina Lombardi
Barbara Tomassetti
CETEMPS,
University of L'Aquila,
L'Aquila,
Italy
E-mail: *valentina.colaiuda@univaq.it*

Valentina Colaiuda
Marco Verdecchia
Department of Physical and Chemical Sciences,
University of L'Aquila,
L'Aquila,
Italy

Federica Di Giacinto
Carla Ippoliti
Giuseppina Mascilongo
Ludovica Di Renzo
Miriam Berti
Annamaria Conte
Nicola Ferri
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale",
Teramo,
Italy

Carla Giansante
Agenzia Regionale per la tutela dell'ambiente – ARTA Abruzzo,
Pescara,
Italy

Mario Latini
World Organization for Animal Health – OIE,
Paris,
France

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INTRODUCTION

Foodborne disease caused by enteric bacteria in bivalves, mainly due to sewage discharges into the sea, has been reported since the 19th century (Foote 1895). Many studies have investigated bacteriological contamination of different mollusc species, with the last few decades seeing increased attention on the contamination of edible species (Prieur *et al.* 1990). The recent scientific literature has mainly focused on identifying how physiological functions of bivalves may affect their ability to accumulate or eliminate microorganisms in their flesh or intravalvular liquid. Environmental influences on faecal indicator organism (FIO) contamination of coastal waters have been more extensively studied; water temperature, turbidity, and salinity are the most important abiotic factors that affect bacterial contamination of molluscs (Cabelli & Heffernan 1970). Perry & Bayliss (1936) were the first authors to propose using *Escherichia coli* as an indicator of significant faecal pollution in water, based on an experiment in which they found a fair correlation between faecal pollution in ambient water and the concentration of *E. coli* in oysters, though the strength of this correlation varied with the season and weather conditions.

FIO contamination analysis has become increasingly common over the past decades (Hunt 1977; Hood *et al.* 1983; Wyer *et al.* 1997), especially since the implementation of the Water Framework Directive in the European Union (EU) and the Clean Water Act in the United States of America (Garcia-Armisen & Servais 2007; Kay *et al.* 2007). Contamination analyses focus on both point sources of contamination (such as wastewater treatment plant effluents or illegal dumping) and non-point sources of contamination (such as surface runoff or soil leaching from urban or agricultural land). According to the World Health Organization (WHO 2010), one of the primary causes of faecal pollution in seawater is untreated wastewater. Many regions in the world have inadequate (or absent) sanitation systems for human faecal waste, and so untreated wastewater is often poured directly into local watersheds. This wastewater contains a wide range of bacteria, viruses, and protozoa that reflect the health and sanitation of the developed areas where the water originates. Paruch *et al.* (2019) found that rural wastewater contains high microbial diversity, whereas urban wastewater has lower microbial biodiversity but higher rates of faecal contamination.

Wastewater treatment facilities are able to reduce faecal pollution; however, the effectiveness of depuration practices may vary across different regions, and some wastewater treatment plants are bypassed when water flow rates are high. Moreover, livestock faeces in areas with intensive agricultural production may represent important non-point sources of faecal contamination. In this case, increased runoff due to storms can transport pathogens into estuaries and coastal environments. Wildlife is another possible source of faecal contamination; however, the influence of wildlife on water pollution and the extent to which faecal contaminants from wildlife are transported by runoff are highly uncertain. Even though coliform pollution is mainly associated with animal and human faeces, it can also be caused by non-faecal sources. For example, Bermúdez & Hazen (1988) found *E. coli* in water from tropical epiphytes.

The Shellfish Waters Directive (Directive 2006/113/EC) was implemented based on the recognition that the discharge of pollutants into the sea may have harmful consequences for shellfish populations, and that actions to protect freshwater ecosystems are therefore required to safeguard shellfish habitats, seafood security, and human health (Touchon *et al.* 2009; Balière *et al.* 2015; Lamon *et al.* 2020; La Rosa *et al.* 2021). Regulation 2004/854/EC, as amended and supplemented, defines common rules for official control programmes for microbiological classification and monitoring of bivalve molluscs. This regulation provides a framework for classifying mollusc production areas based on *E. coli* concentrations in mollusc flesh and intravalvular liquid. The same Directive 2004/854/EC also defines post-harvesting treatments for live bivalve molluscs depending on the microbiological classification of the production area where they were harvested. These treatments are often performed at the expense of the farmer (Giusti *et al.* 2020). It is therefore clear that microbiological contamination of bivalve molluscs may have important implications for public health and economic sustainability.

In this context, European authorities have recommended mapping possible human and animal sources of faecal contaminants and have clearly indicated that hydrological context and numerical modelling are integral parts of the sanitary survey process (CEFAS 2017). The relationship between mollusc or seawater *E. coli* concentrations and weather conditions has often been investigated using statistical regression techniques. More recently, deterministic models have also been used to analyse the role of local precipitation or river discharge as predictors of *E. coli* contamination (Muirhead *et al.* 2004; Kay *et al.* 2008; Lin *et al.* 2008; De Brauwere *et al.* 2014; Campos *et al.* 2017; de Souza *et al.* 2018; Zimmer-Faust *et al.* 2018; Kaifeng *et al.* 2019). Modelling systems are also a valuable tool for implementing early prediction systems for bacterial contaminants (Mälzer *et al.* 2015).

However, the results obtained from these models can vary significantly depending on the geographic region being investigated. In some cases, rainfall over coastal areas is the main environmental factor driving increased FIO concentrations in molluscs (Brock *et al.* 1985; Ferguson *et al.* 1996). These events often have a specific 'lag time,' defined as the time between the precipitation event and the peak faecal bacterial concentration in molluscs or seawater. Precipitation is also the primary cause of bacterial transportation into the sea (Campos *et al.* 2013), especially after storms, which also enhances influxes of zoonotic pathogens downstream (Gywali & Hewitt 2020). However, the minimum amount of rainfall required to produce a significant increase in mollusc *E. coli* concentrations is site-specific (Brock *et al.* 1985; Ackerman & Weisberg 2003; Couliette 2009; Campos *et al.* 2011). This amount has been shown to vary with catchment size, land use in the watershed, distance of sampling from the coast, and climatic conditions due to seasonal variability (Campos & Cachola 2007; Bougeard *et al.* 2011; Bazzardi *et al.* 2014; Huang *et al.* 2015; Ciccarelli *et al.* 2017). Moreover, septic systems (Verhougstraete *et al.* 2015), severe meteorological events (Campos *et al.* 2016), and the observation network being used (e.g., the position of rainfall gauges within the catchment) can also affect estimates of how precipitation affects *E. coli* concentrations (Dwiwedi *et al.* 2013).

Hydrological conditions prior to peak river discharges are also important for determining the presence of *E. coli* in seawater, as weak precipitation events may cause significant increases of concentrations when they follow a dry period, whereas higher amounts of precipitation are needed to produce the same increase of faecal coliforms in wet conditions (Kashefipour 2002; Iqbal & Hofstra 2019; Leonardi *et al.* 2020). The combined effects of precipitation and river discharge also depend on the physiographical characteristics of the catchment and the representativeness of the data chosen for the analysis (Baudart *et al.* 2000; Lipp *et al.* 2001; Ackerman & Weisberg 2003; Crowther *et al.* 2003; Campos *et al.* 2011). As mentioned above, the lag time between a large precipitation or discharge event and peak *E. coli* concentrations is influenced by the bacterial lag time in water and molluscs, which is determined by environmental conditions (Campos *et al.* 2011). Lipp *et al.* (2001) and Campos *et al.* (2011) estimated that *E. coli* can persist in both shellfish water and flesh for up to 6 days after a significant rainfall event, while other authors have reported persistence times of less than 3 days (Kelsey *et al.* 2004; Coulliette *et al.* 2009).

In this study, we investigated the influence of the Pescara River on the *E. coli* concentration in molluscs harvested from areas that are located at various distances from the river mouth. The links between river discharge, precipitation in the catchment area, and mollusc *E. coli* concentrations were explored using a correlation analysis. Due to the lack of directly measured discharge data, we predicted river discharge rates using a hydrological simulation performed with the Cetemps Hydrological Model (CHyM; http://cetemps.aquila.infn.it/chymop), which has widely used since 2002 for hydrological predictions in Central Italy. The CHyM is a grid-distributed and physical-based hydrological model, meaning that the variables characterizing the hydrological cycle are defined on an equally spaced grid. The model was originally developed at the CETEMPS, Centre of Excellence (University of L'Aquila) for predicting floods as part of civil protection programmes (Tomassetti *et al.* 2005; Taraglio *et al.* 2019; Colaiuda *et al.* 2020). In the last few years, the model has been used for climatological studies and was calibrated for different geographical regions (Coppola *et al.* 2014; Sangelantoni *et al.* 2019). Lombardi *et al.* (2021) and Ferretti *et al.* (2020) specifically assessed the responsiveness of the CHyM discharge simulation to precipitation patterns in Central Italy and the Abruzzo Region.

For our analysis, the CHyM was forced using observed precipitation data from a network of rain gauges. Using precipitation measurements from rain gauges (or other instruments) as an input for hydrological models avoids the issue of not having time series of hydrological data available (e.g., Berenguer *et al.* 2005; Nerini *et al.* 2015). This advantage is particularly important because hydrological data are not always available from hydrographic services or may not have recently calibrated through updated rating curves. Moreover, hydrological models can then be used to investigate areas that are not commonly observed with stream gauges (e.g., seasonal streams). Because the CHyM is also used for hydrological forecasts when coupled with a meteorological model (Colaiuda *et al.* 2020), our study provides the basis for implementing a deterministic early warning system for sanitary risks associated with *E. coli* pollution areas, existing marine uses (Ippoliti *et al.* 2018) and dispersion models (Ippoliti *et al.* 2020).

In the following sections, an overview of the *E. coli* concentration and hydrometeorological data sources is given, as well as a description of the CHyM (Verdecchia *et al.* 2009) and experimental design. A physiographical characterization of the Pescara basin is also given, with particular emphasis on the land-use cover. The 'Results and discussion' section provides evidences and results of correlation analysis between *E. coli* concentrations, rainfall and runoff, as well as a deeper examination of three particular case studies, chosen among the sampling period. The case studies focus on how three different hydrometeorological dynamics may affect *E. coli* concentrations in molluscs. We conclude by addressing the concomitant signals of precipitation and discharge as precursors of bacterial concentrations in mollusc production areas.

METHODS

Geographical framework

We analysed the Aterno-Pescara catchment, which represents a natural transect of the Italian region of Abruzzo. The catchment encompasses the inner part of the region, bordered by the Apennine Mountains to the west and the Adriatic slope to the east. The Aterno-Pescara watershed drains an area of 3,147.77 km^2. The basin is characterized by a complex orography, with altitudes spanning from zero to almost 3,000 m.a.s.l across a horizontal distance of only 150 km (Figure 1). The Aterno-Pescara River system originates in the northern part of the Abruzzo region. The first section of the river flows parallel to the Apennine ridge, between the Gran Sasso d'Italia Massif to the east, which includes the highest peaks in the Apennines (including Corno Grande at 2,912 m.a.s.l.), and the Velino-Sirente Mountains to the west. This tract is referred to as the 'Aterno' or 'upper flow' and is characterized by a torrential flow regime, with several ephemeral rivers and high infiltration rates.

In the 'middle flow' section, the river turns to the northeast and receives further water inputs from other springs. In the 'lower flow' section, the Aterno and Pescara Rivers join. The Pescara River is short but contributes significantly to the total discharge of the Aterno-Pescara basin, making the lower flow of the catchment perennial. Table 1 shows hydrological data for the Santa Teresa hydrometric station (42.424°N and 14.163°E), which is located about 9 km upstream from the mouth of the river (Russo 2003).

The catchment is exposed to weather fronts from both the west and the east. Western fronts, which originate from the Atlantic Ocean and primarily contain humid air masses, mainly cause precipitation in the inner part of the region, whereas unstable weather associated with an eastern front causes major precipitation along the coastal area. The most common land-use classifications along the upper and middle flow sections, which cover the highest elevations, include forests and semi-natural areas (Figure 2). Documentation provided by the Abruzzo Region Water Protection Program (Pescara *et al.* 2018) indicates that the northwestern side of the upper flow has sufficient or low water quality, as defined by the criteria provided by 2000/60/CE, as amended and supplemented, and the Italian Legislative Decree 152/2006. In particular, high levels of *E. coli* were reported due to malfunctioning purifiers. The same reports also noted a substantial amount of sewage discharge that contributed significantly to the total river discharge in low-flow conditions (Caputi *et al.* 2008; Primavera *et al.* 2016).

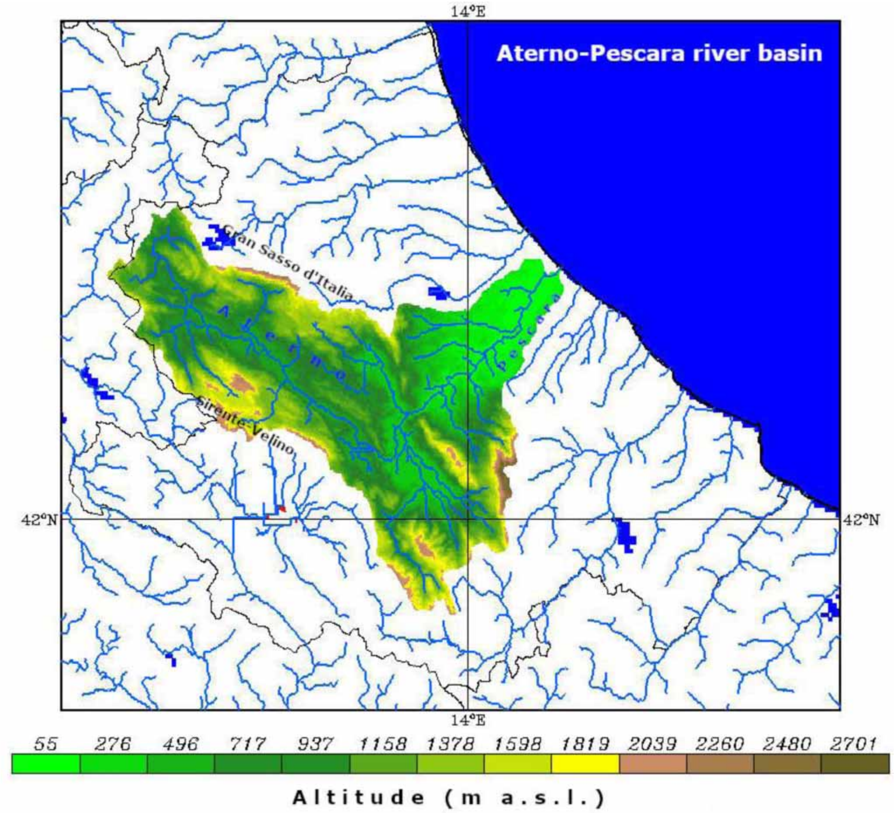

Figure 1 | The Abruzzo region drainage network (blue lines), as extracted by the CHyM. The shaded area delimits the Aterno-Pescara catchment and shows the elevation of the catchment in metres above sea level. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wh.2021.203.

Table 1 | Hydrological data from the hydrological annals of Santa Teresa hydrometer, located 9 km upstream of the mouth of the Pescara River

Minimum instantaneous historical proportions	**15** m^3/s
Historical average daily flow rate	50 m^3/s
Annual maximum daily flow rate	380 m^3/s
Historical maximum instantaneous flow rate	1,100 m^3/s

Land closest to the middle section of the Aterno channel is mainly devoted to agriculture and farming. In these territories, urban areas and areas with artificial surfaces are small and sparsely distributed, with a total of 14 urban agglomerations that comprise 36% of the total urban load in the Abruzzo region, as defined in Directive 91/271/CEE. The lower flow section of the catchment hosts six urban agglomerations, mainly located along the last 10 km of the river's path. These areas are more heavily urbanized and comprise the remaining 64% of the total urban load in the basin (Caputi *et al.* 2008). The Pescara River has been estimated to export over 40,000 m^3 of solid material per year (Barile *et al.* 2008), with peaks of up to 80,000–100,000 tons of dry mud per day during flood events (Damiani 2013). The estimated organic load due to zootechnical activities is about 5,600 tons/year. Most of this load is concentrated in the upper and lower flow regions of the basin, with the upper and lower flows accounting for 69 and 64% of the organic load, respectively.

E. coli data

E. coli concentrations in the molluscs used in this work were measured during the execution of the official microbiological monitoring programme of three live bivalve mollusc production areas, already classified, in the Pescara Province (Italy). The EU reference method for determining the

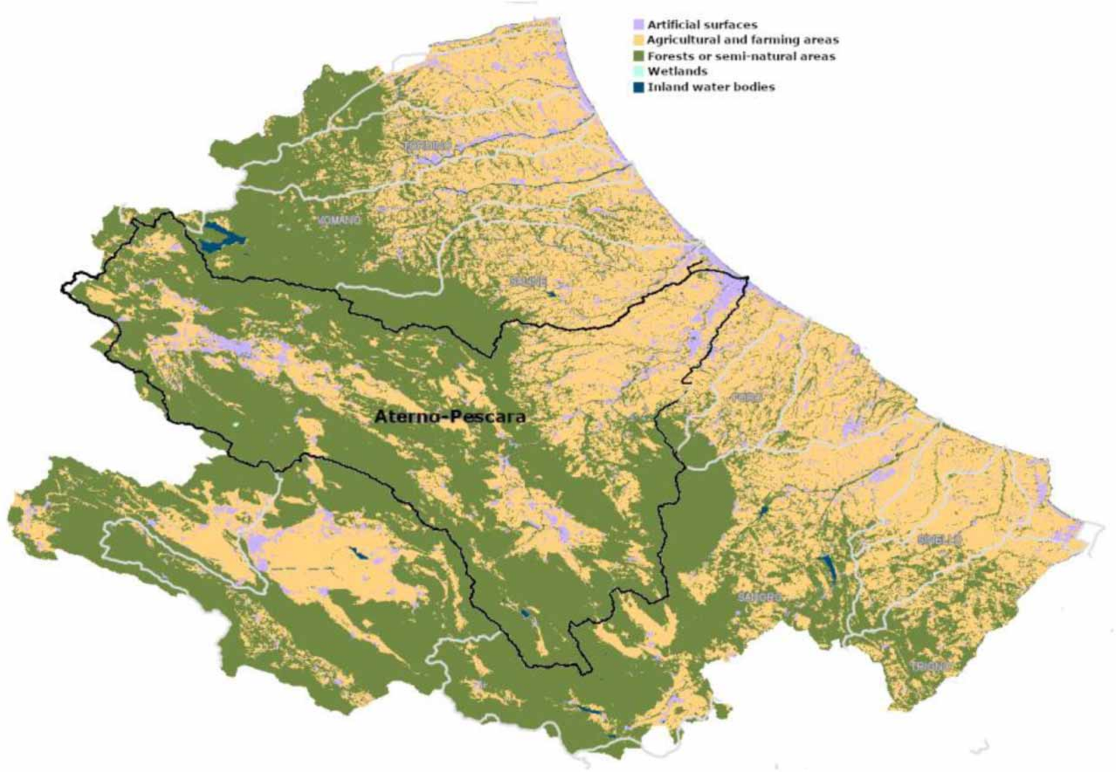

Figure 2 | Land cover distribution for the Abruzzo Region, updated in 2018. *Source*: Abruzzo Region Public Services (http://geoportale.regione.abruzzo.it/Cartanet).

concentration of *E. coli* in bivalve molluscan shellfish is ISO6 16649-3 (2015).

In the framework of the CAPS2 project (www.caps2.eu), the data of microbial monitoring were uploaded in the project database.

A web-based geographical information system (WebGIS) application was developed in the frame of the project to support the management of data, both geographical and analytical, and to make these data available to final users, policy makers, and risk assessors. The WebGIS platform is equipped with tools and functionalities to analyse and visualize laboratory analyses at two different levels, national and supranational, with public or restricted access. Tora *et al.* (2017) fully described the CAPS2 WebGIS system, its architecture, the data collection, the data flow, and its usefulness. The system is accessible at http://www.caps2.eu/caps2/.

Competent authorities, i.e. authorized users, access to non-public sections of the WebGIS, draw, and modify the geographic areas of production zones through dedicated tools. The geolocation of three sampling areas around the Pescara River mouth and the related sample data have been extracted from the CAPS2 WebGIS, as shown in Figure 3:

(1) *Clam harvesting area 'Pescara 1' (P1)*: A polygon 500–750 m from the coast, with a centroid at 42.47498471°N and 14.22677966°E (pink shape in Figure 3).
(2) *Clam harvesting area 'Pescara 2' (P2)*: A polygon 750–1,000 m from the coast, with a centroid at 42.47772568°N and 14.23073753°E (green shape in Figure 3).
(3) *Mussel farm 'Posidonia/Mitilmare' (PM)*: A polygon approximately 5,000 m from the coast, with a centroid at 42.46600575°N and 14.31908674°E (purple square in Figure 3).

The enumeration of *E. coli* in molluscs, expressed as the most probable number (MPN) per 100 g of molluscs, was

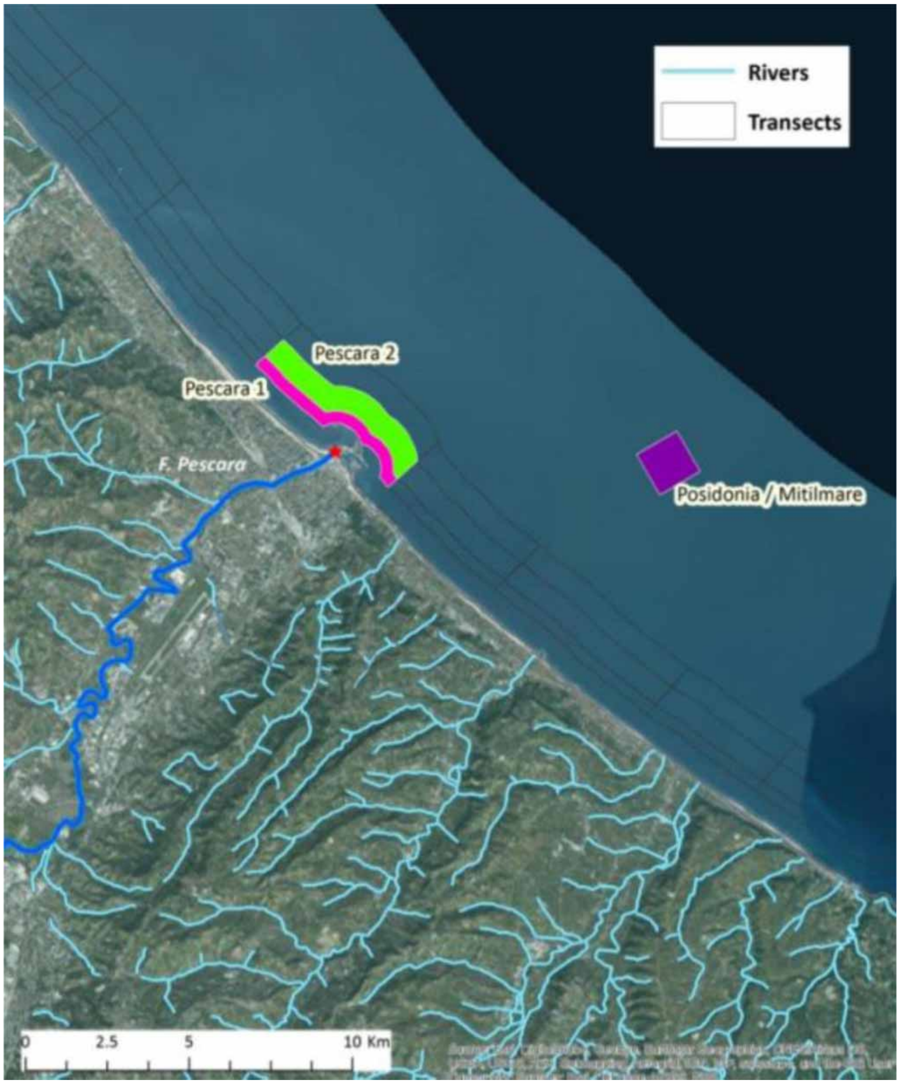

Figure 3 | Three pilot areas and *E. coli* sampling locations. The red star indicates the Pescara River outlet. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wh.2021.203.

used as the bacteriological data for our analysis. Samples were collected every 15 days for 12 months, from 1 August 2015 to 31 July 2016, with slight modifications to the sampling schedule depending on sea conditions. No data were collected if weather conditions prevented the instrumented boat from departing the harbour.

The Cetemps Hydrological Model

Hydrological conditions during the sampling period were determined using a long and continuous hydrological simulation performed using the CHyM for the Abruzzo region, where the Aterno-Pescara catchment is located. The model is written in Fortran code and can be run on any UNIX environment. One of the main features of CHyM is its wide geographic applicability: the CHyM can be used to simulate the hydrologic cycle at any geographical location and at any spatial resolution less than or equal to the resolution of the built-in digital elevation model (DEM). The current version of CHyM has a maximum spatial resolution of 90 m. The model extracts the local drainage network from the DEM matrix using a recurrent algorithm based on the cellular automata technique (Coppola *et al.* 2007; Shiffman 2012). A complete description of CHyM, which is beyond the main aim of this work, is provided by Coppola *et al.* (2014), Verdecchia *et al.* (2008), and Tomassetti *et al.* (2005). Here, we only describe the surface runoff scheme, as this calculation is relevant for the applications discussed in this article.

The CHyM implements an explicit parametrization of all the physical processes that contribute to the hydrologic cycle, including surface runoff, as explained below, evapotranspiration (Thornthwaite & Mather 1957), melting (Pellicciotti *et al.* 2005), and infiltration and percolation (Overtone 1964; Singh & Yu 1990). Surface routing is calculated using the kinematic wave approximation for shallow water (Lighthill & Whitham 1955). The continuity equation is applied in its simplified form, where the lateral inflow is balanced with the discharge variation across the channel length and the variation of the cross-sectional area over time:

$$\frac{\partial Q}{\partial x}+\frac{\partial A}{\partial t}=q$$

where A is the cross-sectional wet area (m^2), Q is the flow rate of water discharge, expressed in m^3/s, q is the rate of lateral inflow (which is given per unit of length and, therefore, expressed as m^2·s^{-1}) due to all the physical processes contributing to the hydrologic cycle, t is the time (s), and x is the location along the path of the river (m).

The de Saint-Venant momentum equation is replaced with the rating curve equation for a cylindrical riverbed. This equation defines a diagnostic relationship between flow discharge (Q), wet area (A), and the proportional constant (α):

$$Q=\alpha A^m$$

The value of m (dimensionless) is ~1 for cylindrical riverbeds, while the α coefficient has the dimensions of a speed (m·s^{-1}) and it represents the average flow velocity in the specific point, calculated through the following equation (Manning 1891):

$$\alpha=\frac{\sqrt{S}\sqrt[3]{R^2}}{n}$$

where S is the slope of the longitudinal bed of the flow element (dimensionless), n is Manning's roughness coefficient (expressed as $\mathrm{s\cdot m^{-1/3}}$) and varies with land use, and R_{H} is the hydraulic radius (m), which is calculated as a linear function of the drained area D_{A} (expressed in m^2):

$$R_{\mathrm{H}}=\beta+\gamma D_{\mathrm{A}}^{\delta}$$

where B (dimensionless), γ (m^{-1}), and δ (dimensionless) are empirical constants that are tuned during model calibration. The quantity D_{A} represents the area upstream of the flow element.

Hydrologic conditions for the Aterno-Pescara basin during our sampling period were determined using a hydrologic simulation. The simulation was forced with temperature and precipitation measurements taken by the Civil Protection Department official station network and provided through the Dewetra platform (Italian Civil Protection, CIMA Research Foundation 2014). The locations of the rain gauges that were used for the rainfall field spatialization in the CHyM of our study area are shown in Figure 4. The total precipitation was calculated from the rain gauges surrounding the river outlet, which are highlighted in red in Figure 4.

The hydrologic simulation covered our entire sampling period (1 August 2015 to 31 July 2016). The high spatial and temporal resolution of the observations used to feed the simulation ensured a realistic estimation of precipitation regimes. The horizontal spatial resolution of the CHyM was set to 300 m, and the simulated region encompassed the Aterno-Pescara catchment and the rest of Abruzzo region, as shown in Figure 1.

Correlation analysis

We calculated the correlation between *E. coli* concentrations in our three sampling areas (P1, P2, and PM) and each of the amount of rainfall affecting the coastal area (R) and the streamflow at the mouth of the Pescara River (D) using the Spearman's (or rank-order) correlation coefficient. Rank-order correlation has been found to be more robust than simple linear correlation for data characterized by outliers or non-Gaussian distributions

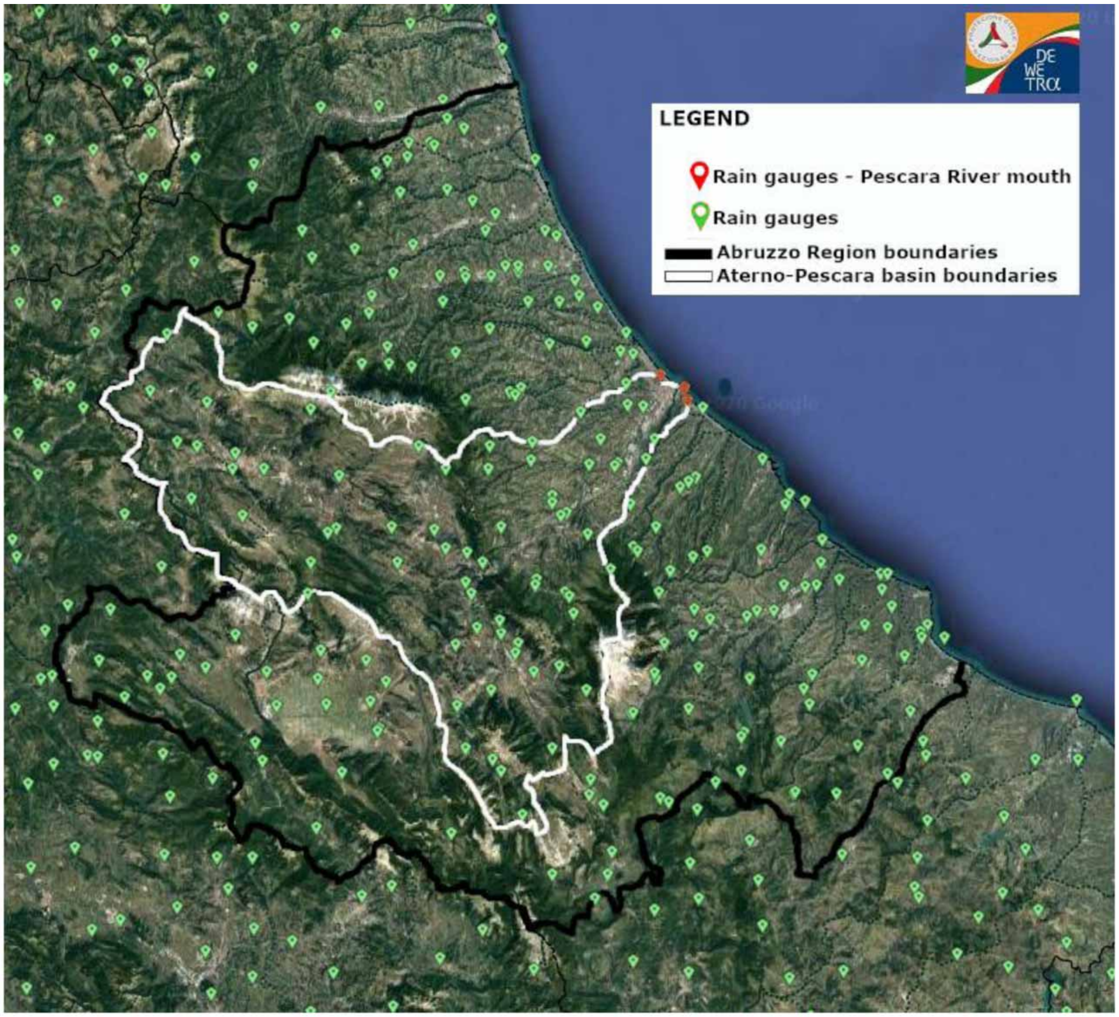

Figure 4 | Distribution of rain gauges across the Abruzzo region. Rain gauges are part of an official network operated by the Civil Protection Department. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wh.2021.203.

(Wilks 1995). The Spearman's coefficient is equal to Pearson's correlation coefficient (Kirk 2008) computed using the rank of the data of the x and y time series rather than the values themselves. Because data values are replaced with their ranks, the Spearman's coefficient r is given by:

$$r = 1 - \frac{6\sum_{i=1}^{n} \mathrm{Diff}_i^2}{n(n^2 - 1)}$$

where Diff is the rank difference between pairs of data (i), and n is the number of observations. P-values were calculated for both correlations to assess whether the data reject the null hypothesis.

We chose to consider a time window of 6 days prior to each sampling event. This time frame was chosen based on the results of Lipp *et al.* (2001) and Campos *et al.* (2011), who found that microbial contamination persists in shellfish flesh for 6 days after a rainfall event, though other authors have reported shorter lag times (<3 days) (Kelsey *et al.* 2004; Coulliette *et al.* 2009). Differences in the residence time for microbial contaminants in shellfish are often associated with the hydrogeology of the catchment and residence times in the receiving water (Campos *et al.* 2011).

Table 2 | Land cover distribution over the Aterno-Pescara basin

Land cover	Area (%)	Land cover	Area (%)
Forest	35	Water courses	0.1
Shrub and/or herbaceous vegetation association	8.5	Grassland	22.1
Wetlands	N	Beaches, dunes, sands	N
Archaeological areas	N	Sparsely vegetated areas	2.8
Annual crops	21,4	Mines, dumps, and construction sites	0.2
Vegetable garden	N	Industrial, commercial, and transport units	0.9
Vineyards, fruit trees and berry plantations, olive groves	6.6	Urban areas	2.4

Land-use classifications are from the Corine Landcover project (Büttner *et al*. 2017). 'N' indicates the negligible area (i.e., less than 5 ha).

We further defined rainfall and discharge peaks for each sampling data. Discharge peaks were defined as the local maxima for the streamflow time-series data within the 6 days preceding the sampling date. Maximum values were only considered discharge peaks if they exceeded the yearly average discharge of the Pescara River as calculated from historical time-series data from the Santa Teresa hydrometer, 6 km upstream of the outlet (i.e., values exceeding 50 m^3/s; see Table 1). Rainfall peaks were similarly defined as the local maxima in the R time series within the 6 days preceding the sampling date. Maximum values were only considered rainfall peaks if they exceeded a 2-mm threshold, according to Campos *et al.* (2011).

The following sections discuss how hydrometeorological conditions can influence *E. coli* concentrations in molluscs, review *E. coli* lag time estimations found in the scientific literature, and illustrate these effects using three case studies (CS). We specifically focus on the hydrometeorological conditions that determine *E. coli* concentration peaks to investigate the effect of two different precipitation patterns typical to this region of Central Italy. The first precipitation pattern (CS1) arises from perturbed fronts from the Atlantic, which are primarily responsible for precipitation in the inland part of the Abruzzo region. The second precipitation pattern (CS2) is associated with eastern fronts, with precipitation mainly affecting coastal areas. Unlike the first pattern, the second pattern does not always result in an increasing discharge, in the western side, with respect to the main Apennine's ridge (displaced in NW–SE direction). These first two case studies are characterized by different precipitation distributions (CS1 and CS2), whereas the third case study is related to dry conditions (Table 4).

Table 3 | List of selected case studies (CS), time windows for each study, and a brief description of the hydrometeorological conditions for each study

Codes	Dates	Hydrometeorological conditions
CS1	11–14 January 2016	Wet days, precipitation inland
CS2	15–20 January 2016	Wet days, precipitation over coastal areas
CS3	1–20 April 2016	Long, dry period with negligible precipitation

RESULTS AND DISCUSSION

We analysed 6 months of *E. coli* concentration data, collected from November 2015 to April 2016, to find links between precipitation over the coastal area, streamflow conditions in the Pescara River, and whether *E. coli* concentrations exceeded threshold values. Although our sampling period ran from August 2015 to July 2016, significant levels of *E. coli* contamination were only observed from November 2015 to May 2016, so we confined our analysis to this window. Data collected during this time frame are summarized in Table 4, where *E. coli* concentrations that exceed threshold concentrations are highlighted in red. According to published criteria for the microbiological classification of molluscs (EC 854/2004 and, by cross-reference, the Council Regulation on microbiological criteria for foodstuffs), the maximum acceptable *E. coli* concentration, or 'threshold value,' was set to 230 MPN of *E. coli* per 100 g of flesh and intravalvular liquid.

Overview from November 2015 to April 2016

We established two preliminary conclusions based on an overview of the sampling period we analysed: (i) *E. coli*

Table 4 | Summary of *E. coli* concentrations (MPN per 100 g) at the three pilot areas at each sampling date (dd/mm/yyyy)

Sampling date	*Pescara 1*	*Pescara 2*	*Posidonia/Mitilmare*
01/08/2015	-	-	-
05/08/2015	-	-	20
01/09/2015	17	20	-
02/09/2015	-	-	17
07/10/2015	-	-	17
28/10/2015	-	-	70
03/11/2015	330	490	-
09/11/2015	-	-	80
17/11/2015	230	20	-
03/12/2015	110	270	-
14/12/2015	-	-	330
17/12/2015	17	50	-
24/12/2015	-	-	790
08/01/2016	790	790	-
14/01/2016	-	-	490
20/01/2016	-	-	3500
21/01/2016	330	110	-
09/02/2016	-	-	17
11/02/2016	1400	1400	-
01/03/2016	1300	1300	-
08/03/2016	-	-	5400
21/03/2016	490	-	-
30/03/2016	-	-	17
06/04/2016	-	17	-
13/04/2016	-	-	17
22/04/2016	-	17	-
18/05/2016	-	-	130
26/05/2016	330	17	-
12/07/2016	17	17	-
31/07/2016	-	-	-

The sampling period highlighted in blue was analysed in this work. Dashes indicate that no samples were collected for a given date/location.

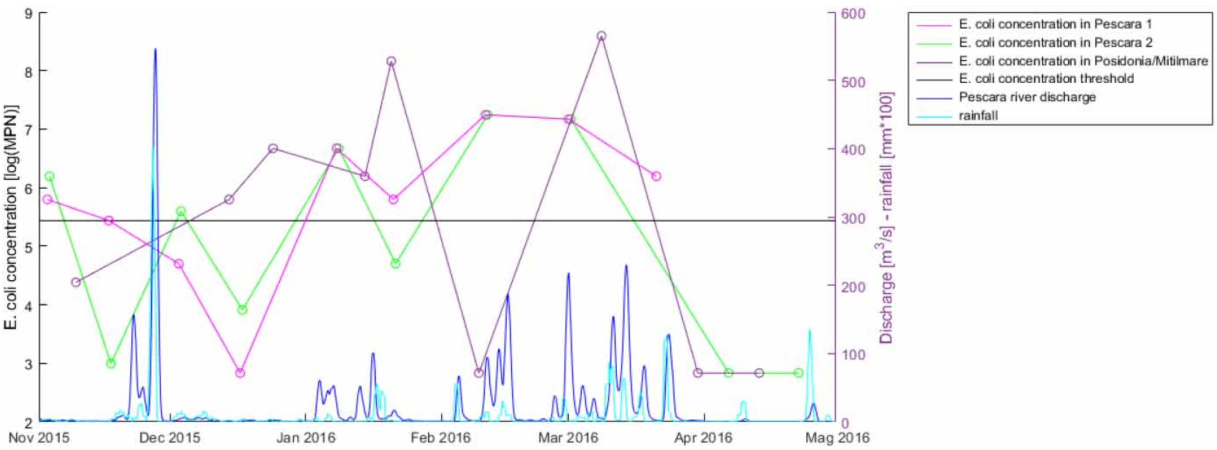

Figure 5 | Time series showing *E. coli* concentrations at P1, P2, and PM, the discharge at the mouth of the Pescara River, and the amount of precipitation at the same location from 1 November 2015 to 30 April 2016.

concentrations appear to be more closely linked to the Pescara River discharge peaks than to precipitation around the river mouth and (ii) most bacterial peaks exceeding the sanitary threshold occur 3–4 days following a discharge peak.

The Aterno-Pescara basin is a natural transect of the Abruzzo region because it includes inland areas as well as the whole hill slope from the Apennines Mountains to the sea. This territory is heterogeneous and characterized by different land uses, including urban areas. Precipitation measured at the river outlet is therefore only weakly representative of precipitation over the entire river basin, especially when watershed physiography is complex. Tables 5 and 6 indicate whether the *E. coli* sanitary threshold was exceeded at P1/P2 (Table 5) and PM (Table 6) on each sampling date. The timing of the preceding discharge and rainfall peaks, as described in the Methods section, are also reported (if present) and are expressed as the lag time (in the number of days) prior to sample collection. During the 6-month investigation period, a total of 28 samplings were performed over the three pilot areas, 16 of which (58%) yielded *E. coli* concentrations above the sanitary threshold. Of these 16 concentration peaks, 14 were

Table 5 | Overview of whether *E. coli* concentrations exceeded maximum acceptable thresholds (yes or no) at the Pescara 1 and Pescara 2 sampling sites

Sampling date	Concentration threshold exceeded P1/P2	Discharge peak occurrence	Precipitation on coastal area
3 Nov 2015	Yes/yes	4 days before	5 days before
17 Nov 2015	No/no	NO	NO
3 Dec 2015	No/yes	6 days before	6 days before
17 Dec 2015	No/no	NO	NO
8 Jan 2016	Yes/yes	4 days before	NO
21 Jan 2016	Yes/no	5 days before	3 days before
11 Feb 2016	Yes/yes	Same day	1 day before
1 Mar 2016	Yes/yes	1 day before	2 days before
21 Mar 2016	Yes/–	3 days before	4 days before
6 Apr 2016	–/No	No	No
22 Apr 2016	–/No	No	No

Results for the two sites are separated by a slash. The table also shows hydrometeorological conditions for each sampling date.

Table 6 | Overview of whether *E. coli* concentrations exceed maximum acceptable thresholds (yes or no) at the Posidonia/Mitilmare sampling site

Sampling date	Concentration threshold exceeded?	Discharge Peak occurrence	Precipitation on coastal area
9 Nov 2015	No	No	No
14 Dec 2015	Yes	3 days before	No
24 Dec 2015	Yes	No	No
14 Jan 2016	Yes	2 days before	No
20 Jan 2016	Yes	4 days before	2 days before
9 Feb 2016	No	5 days before	5 days before
8 Mar 2016	Yes	6 days before	2 days before
30 Mar 2016	No	No	No
14 Apr 2016	No	No	No

Hydrometeorological conditions are also indicated.

preceded by a discharge maximum, and 10 were also preceded by precipitation at the Pescara River mouth. Only one bacterial maximum (24 December 2015) was not preceded by either a precipitation or a discharge peak.

In some cases, precipitation over coastal areas and a river discharge peak occurred simultaneously, and the contribution of the two effects therefore could not be discriminated. The rainfall effect may also include combined sewer overflows, direct land-runoff into the estuary, and re-suspension of contaminated sediments within the estuary itself, as reported by Ferguson *et al.* (1996). A combination of precipitation and discharge from synoptic-scale perturbations seems to increase the concentration of FIOs across distant sampling points by enhancing the bacterial contribution of non-point sources such as inland wash-off. The same mechanism was also reported by Iqbal & Hofstra (2019) and Campos *et al.* (2013). The time between precipitation or discharge peaks and high bacterial concentrations in our analysis was larger than in other studies performed in wider and more populous watersheds (e.g., Schilling *et al.* 2009); however, Campos *et al.* (2011) reported that microbial contamination can persist for up to 1 week after a rainfall event in a smaller watershed.

We must stress that the waters and molluscs in our study were monitored for *E. coli* contamination at fixed 15-day intervals, as dictated by local regulations. These regulations do not consider weather conditions, river flow rates, or other abiotic factors that may affect the concentration of FIOs, and our sampling intervals for detecting potential microbial contamination may therefore not be representative of changes in these conditions because they were planned independently from them. For this reason, we suspect that our data underestimate the strength of the correlations among bacterial concentration, rainfall, and river discharge.

Based on our correlation analysis, we found a significant association between *E. coli* concentrations and the magnitude of the antecedent discharge peak: Spearman's correlation coefficient r_D calculated for the two variables was 0.69, and the associated *p*-value was low ($\sim 4.5 \times 10^{-5}$), indicating that our hypothesis was not rejected. The correlation between rainfall maxima and *E. coli* concentrations resulted in a lower correlation coefficient ($r_R = 0.35$). The associated *p*-value was high (~0.065), indicating that our hypothesis was rejected.

CS1: 11–14 January 2016

Most of the precipitation that fell in the 5-day period from 11 January to 14 January 2016 was concentrated inland, with rainfall maxima (yellow shades in Figure 6) observed in the upper flow section of the Aterno River. During this same period, *E. coli* concentrations exceeded thresholds at the Posidonia/Mitilmare sampling site; no sampling was performed at the two sites closer to the Pescara River outlet. The weather station installed along the lower section of the Pescara River did not register any precipitation within the 5 days preceding the sampling date, whereas almost 40 mm of rain fell over those same 5 days in the upper part of the Aterno-Pescara catchment area. Observed precipitation maxima of approximately 60–70 mm were located outside the catchment area (Figure 6). This precipitation pattern resulted from a moist southwestern air mass that arose due to a large-scale perturbation originating from a wide trough over the western Mediterranean basin. The CHyM for the river outlet simulated a discharge maximum of 80 m^3/s on 12 January, almost 36 h before the sampling date. Hydrometeorological data and *E. coli* concentrations for the last 20 days of January 2016 are shown in Figure 7. This figure enables multiple comparisons between several variables:

- hourly discharge time series as simulated by the CHyM for the Pescara River mouth;
- observed precipitation measured by the rain gauges closest to the Pescara River mouth;
- *E. coli* concentrations for the three different sampling locations (P1, P2, and PM); and
- *E. coli* concentration threshold.

To present all the data within the same figure, we scaled the precipitation amount to 0.0, as indicated in the legend of each panel of Figures 7 and 9. *E. coli* concentrations and the *E. coli* threshold value are log-transformed in each figure.

Simulated streamflow conditions were associated with *E. coli* concentrations exceeding the threshold value at the Posidonia/Mitilmare sampling site, distant from the river mouth. We measured 490 MPN of *E. coli* per 100 g of mollusc flesh and intravalvular liquid.

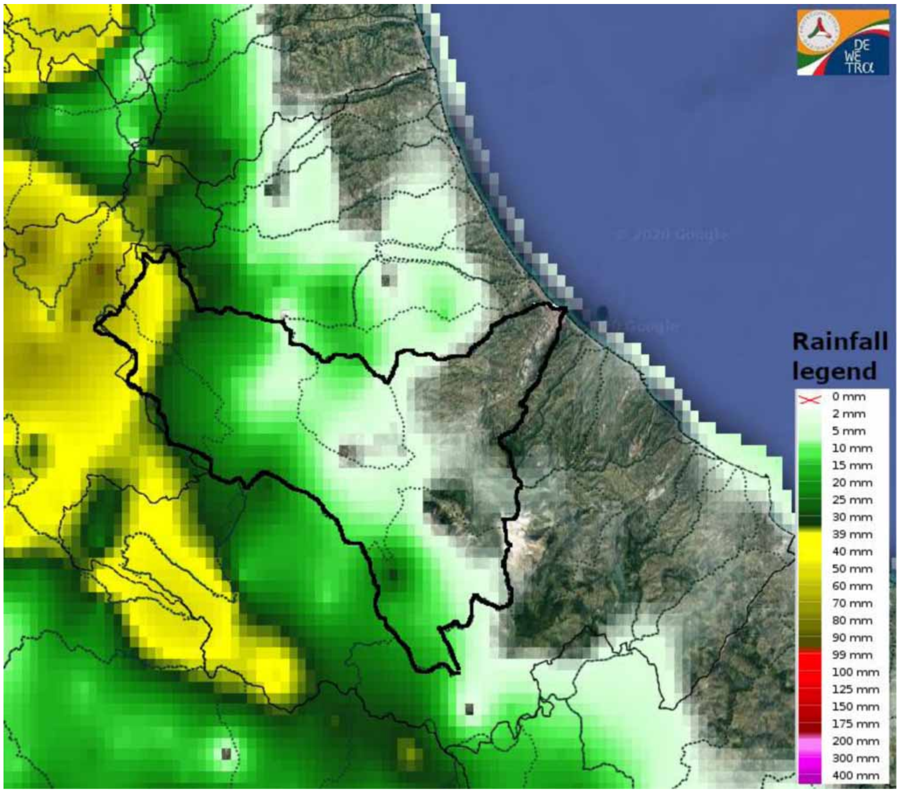

Figure 6 | Spatial interpolation of accumulated rainfall over Central Italy from 11 to 14 January 2016 based on local rain gauges (image from the Dewetra platform). Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wh.2021.203.

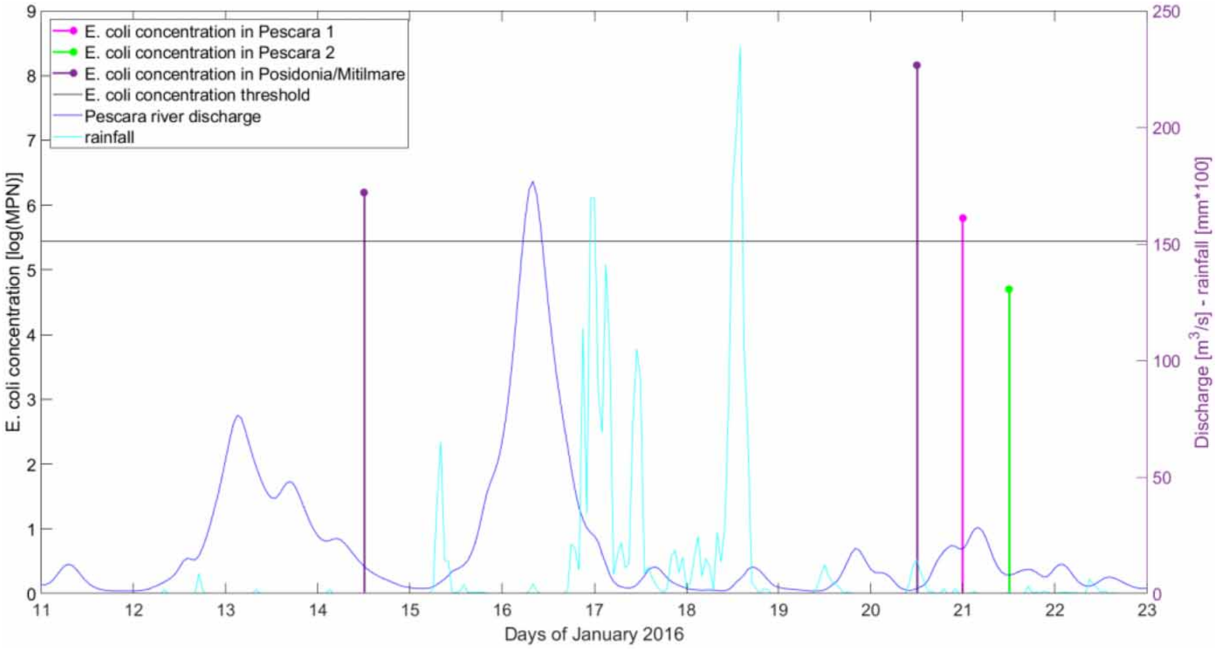

Figure 7 | Hourly river discharge from the Pescara River (blue line), hourly precipitation at Pescara River mouth (cyan line), and *E. coli* concentrations measured during January 2016 at the 'Pescara1' (pink line), 'Pescara2' (green line), and 'Posidonia/Mitilmare' (purple line) sampling stations. The *E. coli* concentration threshold is also shown (black flat line). All *E. coli* concentrations are natural logarithm-transformed. *X*-axis, days of January 2016; *y*-axis, left: natural logarithm of *E. coli* concentration in MPN; *y*-axis, right: river discharge at the Pescara River outlet and hourly precipitation, multiplied by 100. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wh.2021.203.

CS2: 15–20 January 2016

The second meteorological event in the Abruzzo Region occurred between 15 and 20 January 2016. During this period, rainfall was mainly concentrated over the coastal area, with precipitation maxima of 40 mm over 5 days observed close to the Pescara River mouth (Figure 8). The rest of the catchment received little to no precipitation. The high concentration of *E. coli* (3500 MPN per 100 g) detected on 21 January was preceded by a discharge maximum of 170 m^3/s 4 days before the sampling date. This higher discharge peak resulted in increased bacterial transportation offshore, where *E. coli* observations greater than the threshold were detected up to 4 days after the discharge peak (Figure 7).

The bacterial concentration in Posidonia/Mitilmare on 21 January was higher than the previously measured concentration on 14 January, potentially due to the combined contribution of both the first discharge peak on 13 January and the second discharge peak on 16 January. Moreover, the precipitation pattern during this second case study was different from the first case study, as precipitation mainly fell in coastal areas around the Pescara River mouth (Figure 8), which are characterized by significant suspended solid transport, high levels of urbanization, and other anthropogenic pressures.

CS3: 1–20 April 2016

The first 20 days of April 2016 were a period of drought relative to the seasonal climatological average. During this period, *E. coli* concentrations never exceeded the threshold value. For most of April, the Abruzzo region was under a high-pressure system due to the presence of a stable ridge over Italy that originated from Africa. A deep cut-off low-pressure system originated from an Arctic trough at the end of the first week of April (between 6 and 10 April) and quickly crossed over Italy the following 2 days, moving rapidly eastward and bringing a pattern of sparse precipitation to the entire Abruzzo region. The second

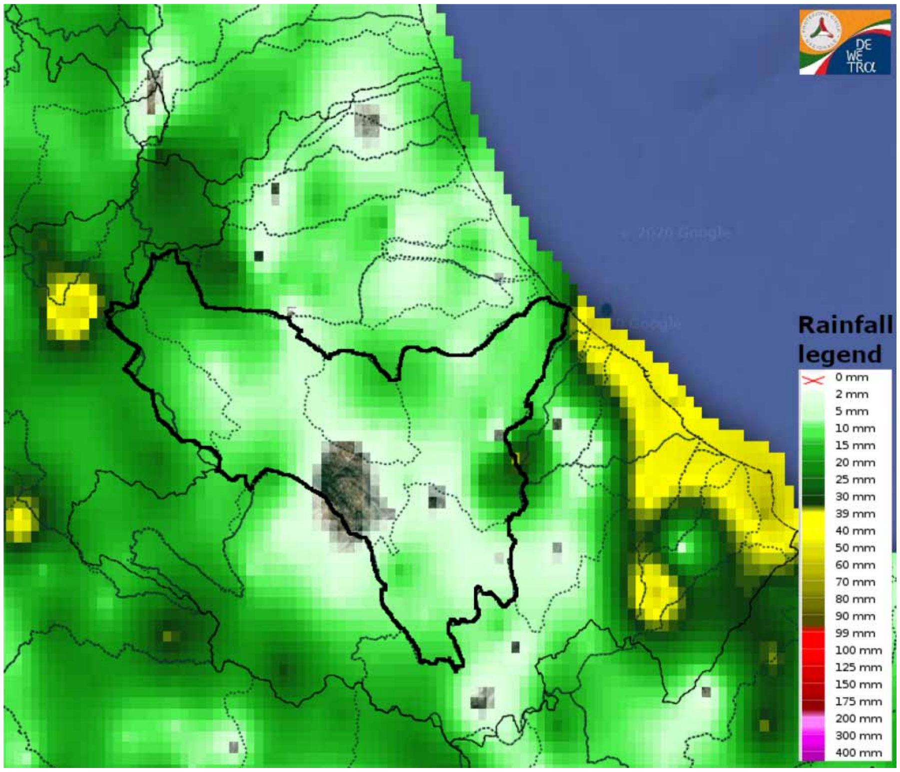

Figure 8 | Spatial interpolation of accumulated rainfall over Central Italy from 16 to 20 January 2016 based on local rain gauges (image from the Dewetra platform).

10 days of April were characterized by a strong high-pressure African ridge, which acted as a barrier for the Mediterranean basin and confined all Atlantic cyclogenesis to western Europe.

After the drought, a single precipitation event affecting the coastal areas of the Abruzzo region occurred between 24 and 25 April. The total 20-day accumulated rainfall over the course of this case study was approximately 20 mm, mainly concentrated in the northern part of the Abruzzo region, outside the Aterno-Pescara catchment (Figure 9).

Figure 10 shows the comparison between precipitation, discharge, and *E. coli* concentration for this case study. During this dry period in April 2016, detected *E. coli* concentrations were significantly below the maximum acceptable threshold. This case study represents the longest period of sub-threshold bacterial concentrations in the entire 6-month analysis window. The results of this case study also support the hypothesis that bacteria are fluvially transported from both the upper and lower regions of the Aterno-Pescara catchment.

CONCLUSIONS

We analysed 6 months of *E. coli* concentration data for bivalve molluscs collected from three pilot areas around the mouth of the Pescara River, with the aim of identifying correlations between hydrologic variables and bacterial concentrations. Because no river discharge data were available for our study period, we estimated streamflow through the Aterno-Pescara catchment by running a hydrological simulation using the CHyM and forcing the simulation with precipitation measurements from local rain gauges. Precipitation data were provided by the Civil Protection Department official station network and the Dewetra platform. Our results show that peak faecal coliform concentrations occurred within 6 days of a river discharge

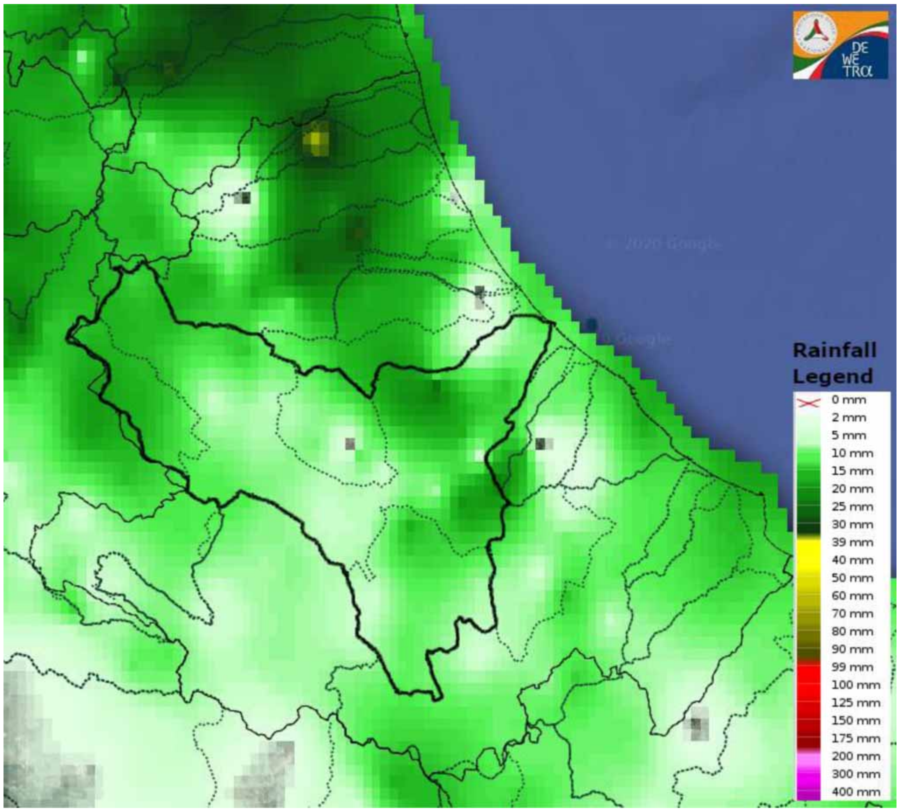

Figure 9 | Spatial interpolation of accumulated rainfall over Central Italy from 1 to 20 April 2016 based on local rain gauge data (image from the Dewetra platform).

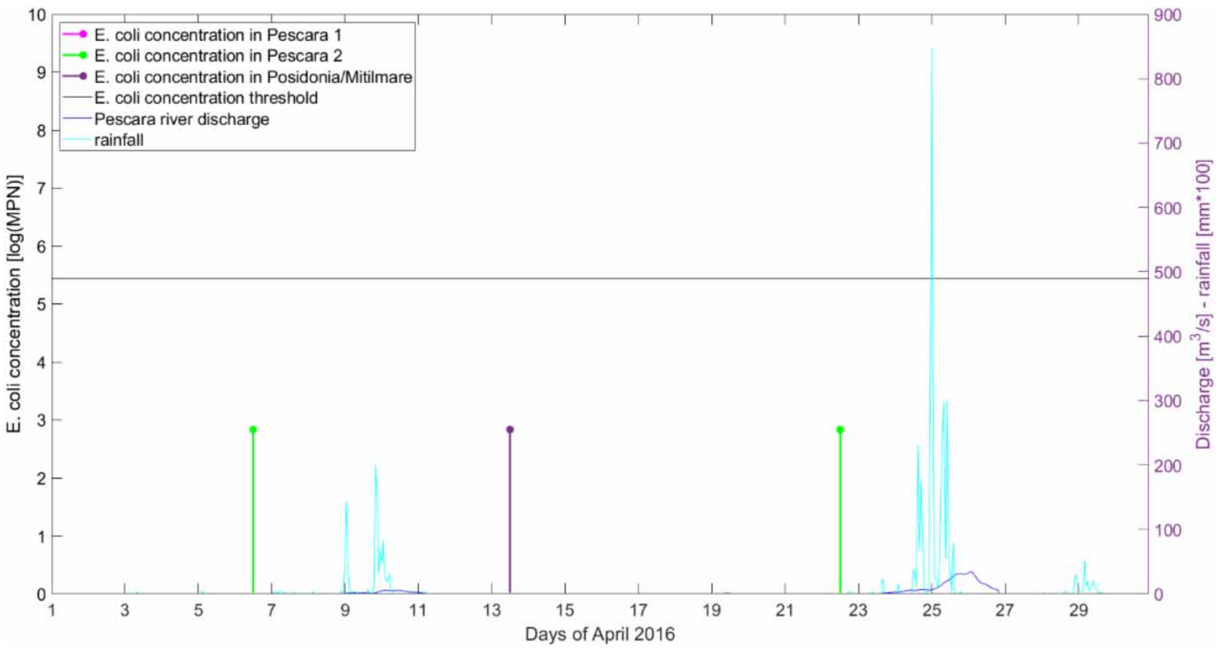

Figure 10 | Hourly river discharge from the Pescara River (blue line), hourly precipitation at the Pescara River Mouth (cyan line), and *E. coli* concentrations measured during December 2015 at the 'Pescara1' (pink line), 'Pescara2' (green line), and 'Posidonia/Mitilmare' (purple line) sampling stations. The *E. coli* concentration threshold is also shown (black flatline). All *E. coli* concentrations are natural logarithm-transformed. *X*-axis, days of April 2016; *y*-axis, left: natural logarithm of *E. coli* concentration in MPN; *y*-axis, right: river discharge at the Pescara River outlet and hourly precipitation, multiplied by 100. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wh.2021.203.

maximum for most of our simulated case studies. Moreover, increased flow through the Pescara River outlet was linked to 87% of cases of high bacterial concentrations, while increased precipitation was linked to almost 60% of cases.

These results suggest that systematically comparing discharge rates and *E. coli* concentrations would be a straightforward way to validate our hypothesis that the Pescara River discharge is the main hydrometeorological driver of *E. coli* concentrations in harvested bivalves. We therefore recommend more bivalve sampling and bacterial measurements, especially after discharge peaks, to enable a more robust statistical analysis. The case-study analysis may be useful to evaluate these effects, as the source of bacterial contaminants may often be human activities occurring in the inland areas of the simulated region. Anthropogenic microbiological contamination of coastal marine waters from freshwater streams and runoff presents an important public health risk, as humans may be exposed to these bacterial contaminants while bathing or if they consume bivalve molluscs.

This work demonstrates that, especially at the mouths of rivers, a holistic approach based on correlations between rainfall data (intensity and location) and variation in river flow rates can be used to predict the times and places of exposure to microbiological contamination. The combined evaluation of abiotic factors (physical and chemical), hydrometeorological components, and biotic factors (bivalve organisms considered in this study) also provides holistic information on the overall state of the ecosystem. In this study, the relationship between biotic and abiotic factors highlights the role of bivalve molluscs as biological indicators, able to provide responses with a high synthesis capacity, but with poor analytical ability.

Supplementing our experimental data with additional observations of *E. coli* concentrations will enable the development of a model that can predict areas of potential microbiological contamination at river mouths based on forecasts of rainfall and river flow rates. This information will allow competent authorities to intervene promptly before and during potential contamination events. This integrated information may additionally contribute to a deeper understanding of the estuarine system and could even be used to develop a decision support system for aquaculture activities if the hydrometeorological model was combined with ocean and dispersion models to form an integrated deterministic prediction system.

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AUTHORS CONTRIBUTION

V.C., A.L., B.T., and F.D.G. was involved in the draft preparation; V.C., A.L., B.T., and F.D.G conceptualized the study; V.C., A.L., B.T., F.D.G, C.I., C.G., and A.M.C. performed the formal analysis; V.C., F.D.G., A.L., B.T., C.I., C.G., M.L., G.M., L.D.R., M.B., A.C., N.F., and M.V. was involved in the draft revision; and N.F. and B.T supervised the study.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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