Science of the Total Environment 906 (2024) 167343

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Tracing the footprints of SARS-CoV-2 in oceanic waters

Giuseppina La Rosa [a,*,1], P. Mancini [a,1], M. Iaconelli [a], C. Veneri [a], G. Bonanno Ferraro [a], C. Del Giudice [a], E. Suffredini [b], the Sea Care team[2], A. Muratore [a], F. Ferrara [a], L. Lucentini [a], M. Martuzzi [c], A. Piccioli [d]

[a] National Center for Water Safety (CeNSia), Istituto Superiore di Sanità, Rome, Italy
[b] Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy
[c] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy
[d] Office of the Director General, Istituto Superiore di Sanità, Rome, Italy

* Corresponding author.
E-mail address: giuseppina.larosa@iss.it (G. La Rosa).
[1] These two authors contributed equally.
[2] Members are listed below.

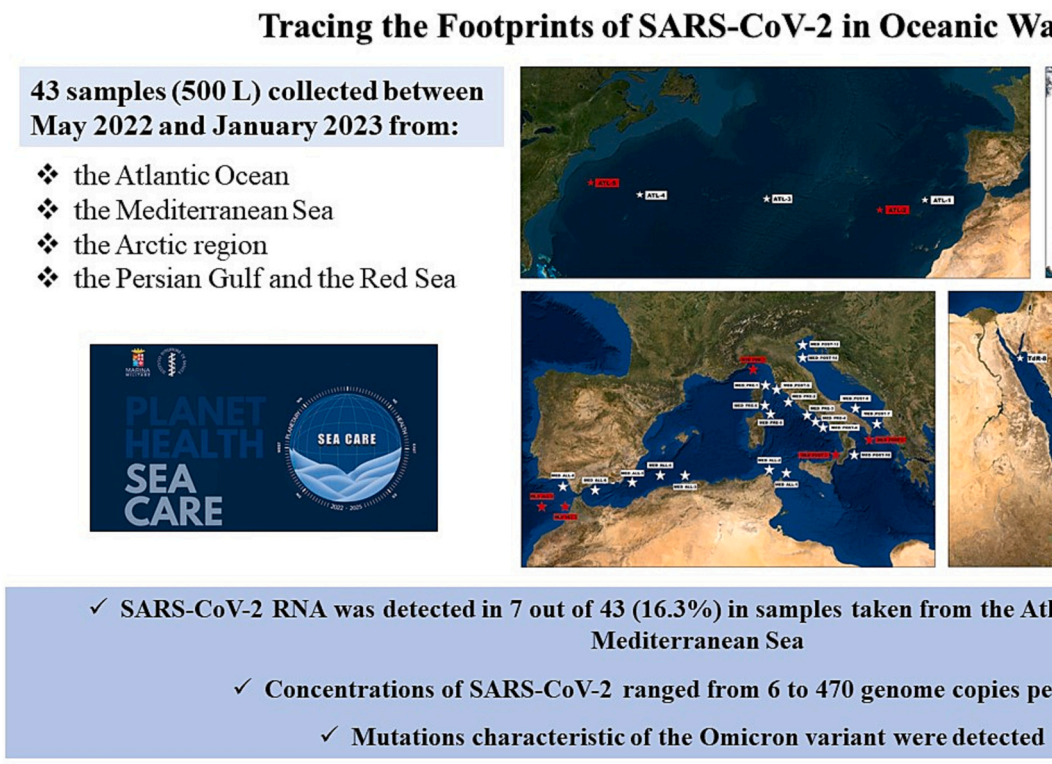
https://doi.org/10.1016/j.scitotenv.2023.167343

Available online 24 September 2023

HIGHLIGHTS

- An investigation into the presence of SARS-CoV-2 RNA in distant sea and oceanic waters is presented.
- The study reveals the existence of SARS-CoV-2 RNA in 16.3 % of samples, including remote oceanic waters.
- The Omicron variant was detected in samples from the Atlantic Ocean and the Mediterranean Sea.
- The findings emphasize the need for studies on virus circulation dynamics in marine environments.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Warish Ahmed

Keywords:
SARS-CoV-2 detection
Oceanic waters
Marine water samples
Molecular detection methods
Sea Care project

ABSTRACT

The detection of SARS-CoV-2 in water environments has predominantly focused on wastewater, neglecting its presence in oceanic waters. This study aimed to fill this knowledge gap by investigating the occurrence of SARS-CoV-2 in remote sea and oceanic waters, at large distances from the coastline. Forty-three 500-liter samples were collected between May 2022 and January 2023 from the Atlantic Ocean, the Mediterranean Sea, the Arctic region, the Persian Gulf and the Red Sea. Using molecular detection methods including real-time RT-qPCR and nested PCR followed by sequencing, we successfully detected SARS-CoV-2 RNA in 7 of the 43 marine water samples (16.3 %), and specifically in samples taken from the Atlantic Ocean and the Mediterranean Sea. The estimated concentrations of SARS-CoV-2 genome copies in the positive samples ranged from 6 to 470 per 100 l. The presence of mutations characteristic of the Omicron variant was identified in these samples by amplicon sequencing. These findings provide evidence of the unforeseen presence of SARS-CoV-2 in marine waters even at distances of miles from the coastline and in open ocean waters. It is important to consider that these findings only display the occurrence of SARS-CoV-2 RNA, and further investigations are required to assess if infectious virus can be present in the marine environment.

1. Introduction

The role of water bodies as potential reservoirs of viruses and pathogens has been increasingly recognized in recent years. Extensive research has focused on studying both surface waters and wastewater to detect different infectious agents. During the ongoing COVID-19 pandemic, significant efforts have been made to understand the transmission dynamics and environmental persistence of the causative agent of COVID-19, the SARS-CoV-2 virus (Atoui et al., 2023; Mahlknecht, 2022; Dutta et al., 2022; Amahmid et al., 2023; La Rosa et al., 2020).

While numerous studies have successfully detected SARS-CoV-2 in wastewater samples (Ciannella et al., 2023; Li et al., 2022; Tamáš et al., 2022; Mazumder et al., 2022; Keshaviah et al., 2023; Lundy et al., 2021; Bonanno Ferraro et al., 2022), little attention has been given to assessing its presence and persistence in offshore waters. This knowledge gap is particularly compelling considering the vastness and ecological significance of the world's oceans, which cover approximately 70 % of the Earth's surface.

Although some studies have examined viruses in nearshore marine waters, particularly in coastal and recreational areas (Bonadonna et al., 2019; Wyer et al., 2012; Wyn-Jones et al., 2011), investigations conducted in the open seas and oceans are relatively limited. This is due to logistical challenges associated with sampling in remote offshore waters, which often require specialized equipment, research vessels, and complex expedition planning. As a result, our understanding of the presence and behaviour of human viruses, including SARS-CoV-2 in the open oceans remains incomplete.

This paper aims to bridge this knowledge gap by investigating the occurrence of SARS-CoV-2 in marine waters, and specifically in regions far from the coastline and in oceanic waters.

This research is part of the Sea Care project, a collaborative initiative between the Italian Military Navy and the National Institute of Health - Istituto Superiore di Sanità (ISS). Spanning three years (2022–2025), the project involves monitoring activities that collect samples along the regular routes of naval units of the Italian Military Navy in territorial and international waters. The aim of the project is to study health risks associated with the environment and climate change from a "planetary health" perspective. Specifically, the primary objective is to gather data on the health status of the seas, contributing to a comprehensive understanding of the potential health risks posed by environmental factors.

2. Materials and methods

From May 2022 to January 2023, during four sampling campaigns, a total of 43 marine water samples (Table 1) were collected while navigating with four different military naval vessels: Amerigo Vespucci, Caio Duilio, Alliance, and Thaon di Revel. The routes crossed the Mediterranean Sea, the Persian Gulf, the Gulf of Oman, the Gulf of Aden and the Red Sea, the Atlantic Ocean, and the Arctic region. The personnel embarked on the naval units for the sampling had been selected among researchers and technicians of the Istituto Superiore di Sanità (ISS), Rome, researchers from the Regional Environmental Protection Agency of Emilia-Romagna, PhD students in Analytical Chemistry of the Sapienza University of Rome, and Professors of the Chemical Faculty of the University of Padua, and had been specifically trained to carry out the activities to guarantee the same methodological approach. At each site, a sample of 500 l of marine water was collected using a submersible pump (Supplementary Fig. S1) from a depth of half a meter below the surface, and immediately filtered through electropositive cartridges (NanoCeram®, Argonide) at a filtration rate of 6 l per minute. Following filtration, the cartridges were promptly frozen on board. As soon as the campaign was over, the cartridges were delivered to the ISS under controlled temperature conditions. Simultaneously with the sample collection, data on salinity and temperature along a 50-meter water column were collected, as shown in Supplementary Figs. S3 and S4. Thawing was performed at +4 °C and the cartridges were then eluted with 400 ml of beef extract 3 % pH 9.5 by placing them in a 2-l beaker and keeping them under slow agitation (300 rpm) for 30 min. A known amount of mengovirus (strain MC_0) was added to the solution as process control before the secondary concentration step, carried out through organic flocculation according to the USEPA 1615 method (Fout et al., 2014). The pellet was resuspended in 4 ml of sodium phosphate buffer, which were split into 2 ml aliquots, one for testing and one as a backup sample, kept in storage at −30 °C. For the analysis, the samples were extracted using magnetic silica beads using the MagPurix® EVO 24 (Zinexts Life Science Corp.) instrument, a high throughput advanced extractor for automated nucleic acid purification. Nucleic acids were eluted in 100 μl of elution buffer, purified with ZYMO OneStep PCR

Table 1
Samples, sampling site and date, and naval vessel.

Sample name	Sampling date	Ocean/Sea	Site	Latitude	Longitude	Naval vessel
ATL-1	13-May-22	Atlantic Ocean	East Atlantic Ocean	35°7′00″N	16° 7′00″W	Caio Duilio
ATL-2	14-May-22	Atlantic Ocean	SW Azorre Islands	33°47′60″N	22° 6′00″W	
ATL-3	17-May-22	Atlantic Ocean	Central Atlantic Ocean	35°1′3.96″N	41°23′3.96″W	
ATL-4	20-May-22	Atlantic Ocean	mid-way between ATL3V&5V	35°54′16.80″N	62°9′47.40″W	
ATL-5	21-May-22	Atlantic Ocean	West Atlantic Ocean (Norfolk)	36°49′6.00″N	69°38′0.00″W	
HN 22_002_SW_001	5-Jul-22	Artic Ocean	Svalbard Island south	76°26.650′N	013°58.941′E	Alliance
HN 22_012_SW_003	7-Jul-22	Artic Ocean	Svalbard Island north west	79°00.810′N	008°11.322′E	
HN 22_046_SW_006	11-Jul-22	Artic Ocean	Svalbard Island north east	80°59.011′N	017°10.927′E	
HN 22_085_SW_007	16-Jul-22	Artic Ocean	Svalbard Island north	80°30.832′N	009°06.059′E	
HN 22_093_SW_008	17-Jul-22	Artic Ocean	Svalbard Island north west	78°50.145′N	005°03.232′E	
MED all 1	11-Jul-22	Mediterranean Sea	Strait of Sicily	37°27′7.86″N	11°31′50.10″E	Amerigo Vespucci
MED all 2	15-Jul-22	Mediterranean Sea	near Tunisi	37°30′48.00″N	10° 1′24.00″E	
MED all 3	21-Jul-22	Mediterranean Sea	Algeri	37°1′18.00″N	3°11′6.00″E	
MED all 4	22-Jul-22	Mediterranean Sea	Algeria, (meridian 0.8°)	37°3′36.00″N	0°49′48.00″E	
MED all 5	23-Jul-22	Mediterranean Sea	Alboran Sea	36°42′12.00″N	1°23′28.20″W	
MED all 6	24-Jul-22	Mediterranean Sea	Strait of Gibraltar	36°19′30.00″N	4°31′24.00″W	
MED all 7	2-Aug-22	Mediterranean Sea	Casablanca	35°28′60.00″N	8°20′60.00″W	
MED all 8	3-Aug-22	Mediterranean Sea	Morocco	35° 1′3.60″N	6°34′54.00″W	
MED all 9	10-Aug-22	Mediterranean Sea	Cádiz	36°18′37.80″N	6°34′60.00″W	
MED all 10	29-Aug-22	Mediterranean Sea	Catanzaro	38°43′0.00″N	16°49′0.00″E	
MED post 3	22-Oct-22	Mediterranean Sea	Strait of Messina	38°21′30.00″N	15°38′24.00″E	
MED post 4	7-Aug-22	Mediterranean Sea	Capri	40°22′60.00″N	14°31′60.00″E	
MED post 5	9-Sep-22	Mediterranean Sea	Elba	42°34′0.00″N	10°45′0.00″E	
MED post 6	29-Sep-22	Mediterranean Sea	Leuca	39°30′36.00″N	18° 8′60.00″E	
MED post 7	19-Oct-22	Mediterranean Sea	Otranto	40°16′60.00″N	18°40′60.00″E	
MED post 8	15-Oct-22	Mediterranean Sea	Monopoli	41°8′42.00″N	17°18′0.00″E	
MED post 12	10-Oct-22	Mediterranean Sea	Venice	45°13′0.60″N	12°44′49.20″E	
MED post 14	1-Oct-22	Mediterranean Sea	Ravenna	44°27′16.20″N	12°44′36.00″E	
MED pre 1	6-Jun-22	Mediterranean Sea	Corsica Channel	43°1′14.98″N	9°36′37.29″E	
MED pre 2	7-Jun-22	Mediterranean Sea	Civitavecchia	41°54′18.00″N	11°38′21.00″E	
MED pre 3	7-Jun-22	Mediterranean Sea	Gaeta	41°9′20.01″N	13°13′15.89″E	
MED pre 4	13-May-22	Mediterranean Sea	Ischia	40°41′53.00″N	13°43′25.00″E	
MED pre 5	17-Jun-22	Mediterranean Sea	Sardinia, La Maddalena	41°7′12.00″N	9°55′22.00″E	
MED pre 6	18-Jun-22	Mediterranean Sea	Corsica Island South East	41°23′43.00″N	9°28′27.00″E	
MED pre 7	19-Jun-22	Mediterranean Sea	Ligurian Sea	43°41′41.00″N	8°40′18.00″E	
TDR 22-1	10-Nov-22	Persian Gulf	Dubai	25°20′42.18″N	54°49′53.52″E	Thaon di Revel
TDR 22-2	11-Nov-22	Oman Gulf	Hormuz	24°57′45.90″N	57° 0′54.96″E	
TDR 22-3	13-Nov-22	Persian Gulf	Lavan	26° 4′23.88″N	53°13′1.14″E	
TDR 22-4	24-Nov-22	Persian Gulf	Doha (Qatar)	25°51′21.18″N	52°30′39.18″E	
TDR 22-5	10-Jan-23	Arabic Sea	Oman	22°56′26.40″N	59°56′24.60″E	
TDR 22-6	12-Jan-23	Aden Gulf	Aden	12°49′0.00″N	46°10′60.00″E	
TDR 22-7	15-Jan-23	Red Sea	central Red Sea	18°23′30.00″N	40° 2′48.00″E	
TDR 22-8	19-Jan-23	Red Sea	Sharm El Sheikh	27°37′42.00″N	34°26′18.00″E	

n.a. = not available.

Inhibitor Removal Kits (Zymo Research) and immediately stored at −80 °C. Analysis was performed by real-time RT-PCR using an assay targeting the ORF1b region of the virus (nsp14, 3′-to-5′ exonuclease; positions 18,600–18,699 of GenBank accession number NC_045512), designed at the beginning of the pandemic and used in Italy for the environmental surveillance of SARS-CoV-2 in urban wastewater (La Rosa et al., 2021a; Cutrupi et al., 2022). Reactions for quantitative analysis were performed in duplicate on a Quant Studio 12 K Flex instrument (Thermo Scientific), using the AgPath-ID One-Step RT-PCR kit (Life Technologies). The amplification conditions were: reverse transcription for 30 min at 50 °C, inactivation for 5 min at 95 °C, and 45 cycles of 15 s at 95 °C and 30 s at 60 °C. An external inhibition control (in vitro synthetized RNA containing the region targeted by the PCR) was used to rule out false negative results. Standard curves for quantification were built using a double-strand fragment of the target region (Eurofins Genomics) quantified by Qubit (Thermo Scientific). Results were considered acceptable if inhibition was ≤75 % (ΔCt between sample and reference assay ≤2.00) and if standard curves displayed a slope between −3.1 and −3.6 and a $R^2 \geq 0.98$ (Hougs et al., 2017). Samples uncompliant due to excessive inhibition, were tested in a 1:10 dilution. Specificity and sensitivity (LOD_{50} and LOQ) of the RT-PCR assay were evaluated in a previous work (La Rosa et al., 2021a). Viral recovery was assessed using a previously described real-time assay for mengovirus (Costafreda et al., 2006).

Attempts were made to obtain sequences for further confirmation and variant identification. For this purpose, a nested PCR approach was adopted, amplifying a long fragment (PCR_980, approximately 1600 bps) of the spike protein-coding gene, spanning amino acids 58 to 573. This assay had previously been designed to amplify a fragment of the spike region containing multiple mutations (amino acid changes or deletions), enabling differentiation among different SARS-CoV-2 variants/subvariants (La Rosa et al., 2021b; La Rosa et al., 2021c; La Rosa et al., 2023). Since amplifying such long fragments in environmental samples is challenging, two additional PCR assays were used targeting shorter fragments (PCR_986, 545 bps and PCR_1030, 573 bps) in the same region, to increase the chances of obtaining sequences. The primers used for sequencing purposes are shown in Supplementary Table 1. After amplification, the products were run on a QIAxcel Connect System (Qiagen), a capillary electrophoresis instrument for rapid and accurate fragment analysis. The PCR products of the expected size were purified using a Montage PCR 96 Microwell Filter Plate (Millipore) and sequenced by Sanger sequencing through an external service (Bio-Fab Research, Rome, Italy). Consensus sequences were generated using MEGA X software and compared to the NCBI database using BLAST analysis.

In order to ensure accuracy of the results, the backup aliquots of three samples testing positive for SARS-CoV-2 were also extracted and analysed by real-time RT-PCR in another ISS laboratory, different from the one testing the first aliquots, and the obtained RNAs were further subjected to confirmation by nested PCR followed by sequencing. Furthermore, all positive samples were subjected to the detection of the human RNAseP gene to evaluate the possibility of viruses originating from anthropogenic source in the tested wasters. The analyses were undertaken using the internal control of the Logix Smart™ COVID-19 Test kit (Co-Diagnostics, UT, USA) according to manufacturer's instruction.

3. Results

In the majority of the samples (29/43), the observed inhibition significantly exceeded 75 %. Consequently, an additional RNA purification step was executed using the ZYMO OneStep PCR Inhibitor Removal Kits, leading to a satisfactory reduction of inhibition levels in fourteen samples. The remaining 15 samples still affected by high inhibition – prevalently collected in the Mediterranean Sea – were tested in a 1:10 dilution. Overall, average inhibition in the samples at their testing dilution was 36.6 %, (range: 0 % to 73.5 %). Viral recovery in the secondary concentration and in the extraction procedure, evaluated using the mengovirus process control, was on average 3.4 %, ranging from <0.1 % to 14.5 %.

SARS-CoV-2 RNA was detected in 7 out of 43 (16.3 %) marine water samples. Of these, 6 were found positive by both real-time PCR and nested PCR followed by sequencing of the spike gene. These positive samples were all collected in 2022, specifically on May 14th and May 21st (ATL-2 and ATL-5) in the Atlantic Ocean, and on June 19th (MED PRE 7), August 2nd and 10th (MED ALL 7 and MED ALL 9), and October 22nd (MED POST 3) in the Mediterranean Sea. Additionally, one sample from the Mediterranean Sea, collected on September 29th (MED POST 6), tested positive using the two short-nested PCR, despite being negative using the real-time RT-PCR. The results of the first three samples testing positive (ATL-2, ATL-5 and MED POST 6) were confirmed on the second sample aliquot in an independent test.

Fig. 1 shows a map depicting the collection sites of the 43 samples analysed in this study. The samples that tested positive for SARS-CoV-2 are highlighted in red. The estimated concentrations of SARS-CoV-2 genome copies (g.c.) per 100 l (g.c./100 l) in the positive samples ranged from 6 to 470 (Table 2) and all positive samples displayed the presence of human DNA (RNAseP gene). While the long PCR yielded negative results, amplification and successful sequencing were achieved for all positive samples using the short PCR assays (either one or both assays). Blast analysis confirmed that the obtained sequences belonged to SARS-CoV-2. From the 7 positive samples, four different SARS-CoV-2 sequences were obtained. The identified mutations are illustrated in Table 2. The mutation package H69-V70del, G142D, V213G, G339D, either with or without the R346T mutation, was found in ATL-2, MED PRE 7, MED ALL 7, MED ALL 9, and MED POST 3. The package G142D, V213G, G339D (without the 69/70 deletion and the R346T mutation) was detected in MED POST 6 and in ATL-5. All of these mutations are characteristic of the Omicron variant (specifically, the Omicron clade GRA-B.1.1.529 + BA.x), which has been prevalent worldwide since January 2022. A more accurate identification of the subvariants could not be achieved due to the limited length of the amplified fragments. SARS-CoV-2 sequences obtained in this study were submitted to GenBank under Accession numbers OR242184-OR242196.

4. Discussion

While previous research has predominantly focused on detecting the virus in wastewater samples, our study extends the investigation to open seas, in regions far from the coastline, therefore targeting areas of paramount relevance, given the amplitude and ecological significance of the world's oceans. This study provides the first evidence of the presence of SARS-CoV-2 in oceanic waters. Detection of SARS-CoV-2 in seawater is not entirely surprising, as previous research has provided evidence of SARS-CoV-2 RNA presence in coastal waters. Polo and co-workers detected SARS-CoV-2 RNA in 25 % of estuarine sediment samples in Galicia, Spain, with concentrations ranging from <LOQ to 3.60 Log gc/g sediment (Polo et al., 2021). In another ongoing preprint study, Cárdenas-Calle and collaborators demonstrated the presence of SARS-CoV-2 RNA in seawater and outfalls of estuaries along the Ecuadorian Coast (Cárdenas-Calle et al., 2022). In addition, several studies have confirmed occurrence of detectable levels of SARS-CoV-2 RNA in bivalve molluscs (Mancusi et al., 2022; Polo et al., 2021; Desdouits et al., 2021; Le Guernic et al., 2022; Lombardi et al., 2023), which indirectly indicates its presence in the surrounding seawaters. What is particularly novel and surprising in this study, is the discovery of viral RNA in oceanic waters at considerable distances from the coastline. For instance, the most remarkable finding lies in the samples collected from the Atlantic Ocean sites ATL2 and ATL5, which are located at distances of 234 and 270 nautical miles (433 and 500 km) from the coast, respectively. Positive samples were found under variable conditions of both temperature and salinity, as shown in Supplementary Figs. S3 and

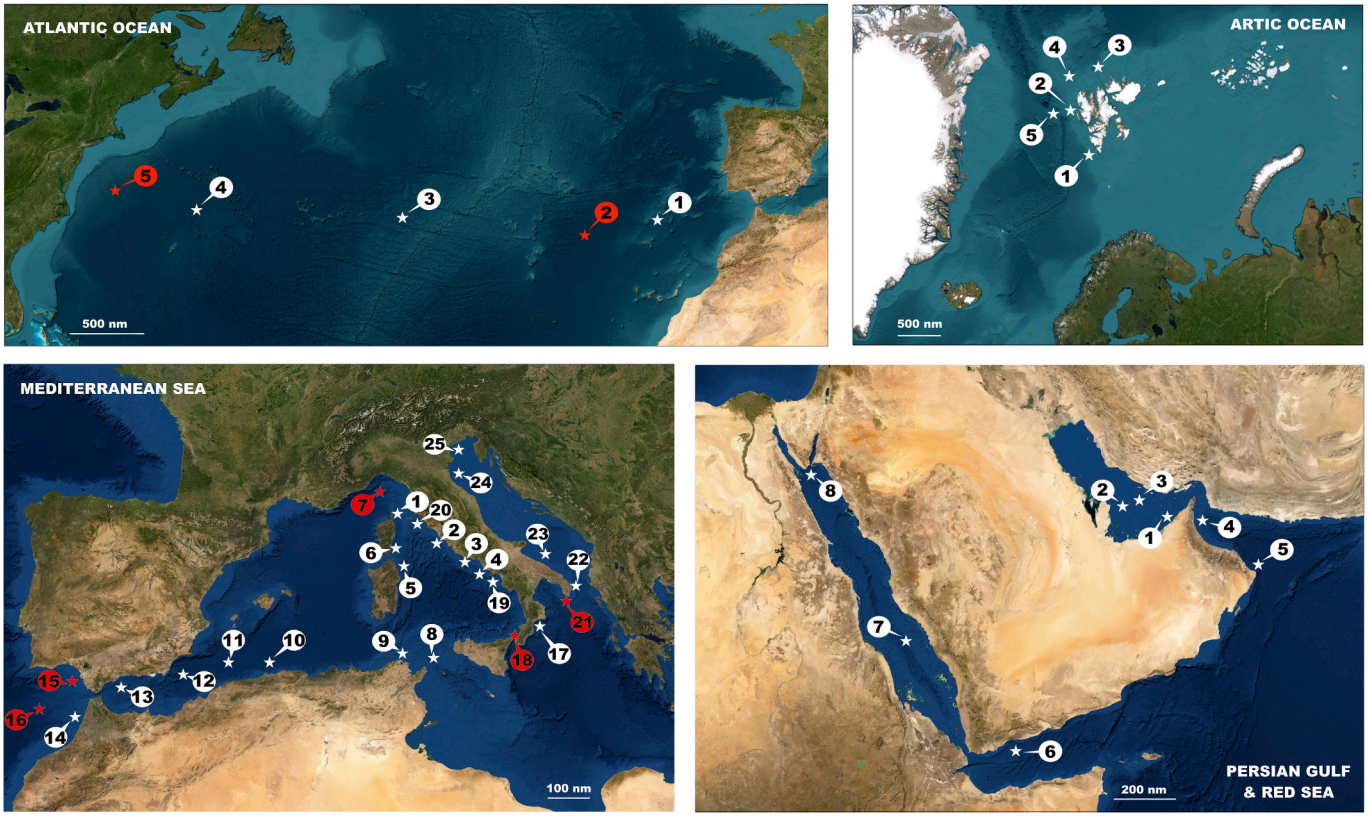


Fig. 1. Map depicting the collection sites of the 43 samples analysed in this study:
Atlantic Ocean: 1 = ATL-1; 2 = ATL-2; 3 = ATL3; 4 = ATL4 Artic Ocean: 1 = HN 22_002_SW_001; 2 = HN 22_012_SW_003; 3 = HN 22_046_SW_006, 4 = HN 22_085_SW_007; 5 = HN 22_093_SW_008 Persian Gulf and Arabic sea: 1 = TDR 22-1; 2 = TDR 22-2; 3 = TDR-22-3; 4 = TDR 22-4; 5 = TDR 22–5; 6 = TDR 22-6; 7 = TDR 22-7; 8 = TDR 22-8 Mediterranean Sea: 1 = MED pre 1; 2 = MED pre 2; 3 = MED pre 3; 4 = MED pre 4; 5 = MED pre 5; 6 = MED pre 6; 7 = MED pre 7; 8 = MED all 1; 9 = MED all 2; 10 = MED all 3; 11 = MED all 4; 12 = MED all 5; 13 = MED all 6; 14 = MED all 7; 15 = MED all 8; 16 = MED all 9; 17 = MED all 10; 18 = MED post 3; 19 = MED post 4; 20 = MED post 5; 21 = MED post 6; 22 = MED post 7; 23 = MED post 8; 24 = MED post 12; 25 = MED post 14. The samples that tested positive for SARS-CoV-2 are highlighted in red.

Table 2
Real-time PCR (ORF1b region; nsp14; 3′-to-5′ exonuclease) and sequencing results (spike region) for the six positive samples.

Sample name	Sea	Date	SARS-CoV-2 g.c./100 l	Viral recovery	Detection of human RNAseP gene (Ct)	Mutation detected by amplicon sequencing (spike region, PCR 986)	Mutation detected by amplicon sequencing (spike region, PCR 1030)
ATL-2	Atlantic Ocean East	14/05/2022	470	9.9 %	33.63	H69-V70del, G142D, V213G	V213G, G339D, R346T
ATL-5	Atlantic Ocean West	21/05/2022	160	3.8 %	33.43	G142D[a]	V213G, G339D
MED PRE 7	Mediterranean Sea	19/06/2022	6	7.8 %	33.60	H69-V70del, G142D, V213G	Negative
MED ALL 7	Mediterranean Sea	02/08/2022	7	6.7 %	30.89	H69-V70del, G142D, V213G	V213G, G339D
MED ALL 9	Mediterranean Sea	10/08/2022	15	2.9 %	32.70	H69-V70del, G142D, V213G	V213G, G339D, R346T
MED POST 3	Mediterranean Sea	22/10/2022	45	<0.1 %	36.18	H69-V70del, G142D, V213G	V213G, G339D
MED POST 6	Mediterranean Sea	29/09/2022	Negative	6.0 %	34.61	G142D, V213G	V213G, G339D

[a] This sequence is incomplete at its 3′ end due to low-quality sequence electropherograms.

S4.

It's important to note that the primary and secondary concentration methods used in this study have not been specifically validated for processing large volumes of oceanic water. This limitation has resulted in relatively low recoveries, as outlined in the results section, along with notable inhibition issues. Therefore, further studies are recommended to establish the most suitable approaches for these types of waters and the associated substantial testing volumes. However, the overall analytical strategy (use of electropositive cartridges for sample concentration in conjunction with real-time RT-PCR and nested RT-PCR assays), achieved SARS-CoV-2 detection in oceanic waters and allowed the determination of its genetic profile. Notably, in relation to the RT-qPCR herein adopted (La Rosa et al., 2021a), this study confirms the successful use of this nsp14 assay - originally designed for wastewater samples - also to marine waters. This assay, currently employed in official environmental surveillance in Italy (https://zenodo.org/record/5758725#.ZF

kcbRHP3D4), has been previously proven effective in detecting SARS-CoV-2 in other complex environmental matrices, such as bivalve molluscs (Lombardi et al., 2023), solid waste (Di Maria et al., 2021) and air particulate matter (Pivato et al., 2022).

It is important to note that the concentrations detected in this study in positive samples (6 to 470 g.c./100 l) were significantly lower compared to those found in wastewater samples, that may reach 4.6×10^8 g.c./l (Bonanno Ferraro et al., 2022). This observation aligns with our expectations, considering the phenomenon of dilution that naturally occurs in the marine environment. Additionally, this study only detected nucleic acids, making it challenging to determine whether the virus present in the marine environment is infectious or not. The marine environment presents challenges for SARS-CoV-2 due to factors such as elevated pH and salinity, which can impact the virus's infectivity and integrity. Exposure to ultraviolet (UV) radiation through the upper layers of the aquatic environments can also affect the survival of SARS-CoV-2. Indeed, solar heat and UV radiation destroy the virus infection ability (Efstratiou and Tzoraki, 2021). All these environmental conditions may contribute to the reduction in viral viability in marine waters. Indeed, based on our knowledge, there is no available data on the presence of infectious SARS-CoV-2 in seawater. However, previous investigations with artificial seawater have demonstrated that SARS-CoV-2 can persist and remain viable in this matrix for short periods. Sun et al. showed that SARS-CoV-2 retained its infectivity about 3 days in artificial seawater at 22 °C (Sun et al., 2022). Similarly, Sala-Comorera and colleagues examined the rate of decline of viable and infectious SARS-CoV-2 in seawater at 4 °C and 20 °C. Their findings revealed the rapid inactivation of infectious SARS-CoV-2, as evidenced by the calculated T90 values, 1.1 days at 20 °C and 2.2 days at 4 °C (Sala-Comorera et al., 2021). Given these studies, the likelihood of infectious risk from SARS-CoV-2 in these aquatic environments appears to be minimal.

Nucleic acids present in seawater also undergo degradation when exposed to inactivating factors. In our study, the absence of positive results with the long PCR, despite obtaining positive results with the real-time PCR and the short PCRs, suggests the presence of fragmented RNA in the samples. It is crucial to note that current scientific evidence does not support the detection of infectious SARS-CoV-2 in untreated or treated wastewater or in the aquatic environments. Therefore, we should be cautious about overestimating the risk represented by SARS-CoV-2 presence in water matrices based solely on the detection of SARS-CoV-2 RNA (Maal-Bared et al., 2021).

Interestingly, the sequencing confirmed the presence SARS-CoV-2 and non–identical sequences were obtained, sign of a certain genetic variability in the different samples, although all of them referable to the Omicron variant. Unfortunately, the sequenced fragments were too small to accurately characterize the specific subvariants.

The presence of the virus in remote oceanic waters may be explained through different hypotheses. One possible explanation is the introduction of the virus through untreated wastewater discharges or inadequate sanitation practices near the coast. Such sources can introduce SARS-CoV-2 into marine waters, allowing it to spread over larger distances, following sea streams. Guo et al. (2021) developed a model suggesting that continuous discharge of domestic sewage carrying SARS-CoV-2 could contaminate vast sea areas, spanning tens of thousands of square kilometres. The movement of winds, waves, and ocean currents indeed plays a significant role in the virus's dissemination. Wind can carry aerosolized droplets containing the virus across the ocean, potentially contaminating marine environments. Similarly, waves can transport viral particles by carrying them across the water surface. Additionally, ocean currents contribute to the dispersal of the virus, carrying it to distant locations beyond the immediate vicinity of the source.

In addition to the previously mentioned hypothesis, human activities may also play a significant role in the spread of the virus in marine environments. This notion is substantiated by the detection of human DNA in all samples testing positive for SARS-CoV-2, a strong evidence of an anthropic impact in the tested areas. Various maritime activities, such as shipping, recreational boating, and fishing, can contribute to this phenomenon. Shipping activities involve the movement of different types of vessels, including cargo ships, tankers, passenger and cruise ships, fishing vessels, and military ships, among others. These ships engage in diverse operations and may discharge wastewater or effluents into the ocean. Wastewater from ships can contain various contaminants, including human waste, which may carry the virus. Based on maritime traffic data from www.vessel.com/IT, it is evident that all surveyed areas exhibit clear signs of naval activity. Some zones display intense levels of traffic, while others show reduced activity, particularly noticeable in the Arctic region, as illustrated in Supplementary Fig. 2. This observation further supports the hypothesis that human activities, including maritime traffic, can contribute to the introduction and spread of SARS-CoV-2 in marine environments.

Finally, the presence of SARS-CoV-2 in remote oceanic waters may also be explained by the existence of unexplored sources or reservoirs of the virus. For example, certain marine organisms or ecological niches could potentially serve as hosts or carriers for the virus, allowing it to persist and circulate in marine ecosystems. The virus could potentially infect marine organisms, including marine mammals, fish, and invertebrates (Yang et al., 2022; Li et al., 2022). A few studies have hypothesized that marine mammal species, including whales, dolphins, and seals, may be susceptible to SARS-CoV-2 infection due to the presence of ACE2 receptors with a binding affinity comparable to humans, suggesting the possibility of virus exposure (Audino et al., 2021; Mathavarajah et al., 2021).

5. Conclusion

This study represents a pioneering effort to investigate the presence of SARS-CoV-2 in oceanic waters, addressing a significant knowledge gap that exists in understanding the virus occurrence in remote offshore environments. The detection of viral RNA in seawater, though not unexpected due to prior evidence of its presence in coastal waters, emphasizes the relevance of exploring its distribution in open seas and oceanic regions. Indeed, what is particularly novel in this study, is the discovery of viral RNA at considerable distances from the coastline. It is essential to note that the presence of RNA does not necessarily indicate the presence of an infectious virus, as the unique conditions of the marine environment can impact virus viability.

Future research targeting SARS-CoV-2 should aim to expand the sampling efforts in open oceanic regions, considering a larger number of samples, and advanced modelling approaches for viral particle dispersion in water bodies, to contribute to the broader understanding of virus occurrence and persistence in water bodies. Moreover, as research moves forward, continued efforts will be pivotal for refining methodologies for processing large volumes of oceanic water.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

Very special thanks go to Prof. Silvio BRUSAFERRO, President of the Italian National Health Institute, and to the Italian Navy and her Chief of Staff, Vice Admiral Enrico Credendino, as Heads of the Memorandum of Understanding between the Italian Navy and the ISS.

Special thanks are also due to Rear Admiral Cristiano Nervi for his

promotion of the project and supporting its approval, to Vice Admiral Roberto Dattola (EP&OHS General Office of Navy General Staff), who guarantees the maximum support to the activities and the success of the project, and to Rear Admiral (LH) Massimiliano Lauretti for the decisive stimulus impressed for the implementation of the project and in the Amerigo Vespucci's World Tour Campaign.

We also express our gratitude to the commanders of the naval vessels that hosted up-to-date the Sea Care project teams: Capt. Jacopo Rollo (Destroyer Caio Duilio), Capt. Massimiliano Siragusa, Capt. Luigi Romagnoli (Training Ship Amerigo Vespucci), Cdr. Maurizio Demarte (Navy Hydrographical Institute), and Cdr. Emanuele Morea (Offshore Patrol Vessel Thaon di Revel). Their experience and professionalism were of paramount importance in completing all planned activities, considering that the military vessels are not designed for research purposes.

We would also like to thank Cdr. Giuseppe Aceto for his invaluable support in coordinating and facilitating relations within the Naval General Staff and High Commands, as well as with all the naval vessels in which the scientific teams were embedded, and for his attitudes toward problem-solving. Finally, we express our gratitude to all the military shipborne and on shore personnel who passionately and professionally collaborated with the researchers by providing all the necessary support.

This project has been made possible through the financial support of the "Sea Care project: Health, Environment and Climate Research in the Vision of Planetary Health", a collaborative initiative between the Italian Navy and the National Institute of Health - Istituto Superiore di Sanità (ISS), with the main contribution from the Italian Navy. We also thank the European Union – NextGenerationEU under the National Recovery and Resilience Plan (NRRP) PE13 INF-ACT.

The Sea Care team:

Laura Barone – Department of Chemistry, University of Rome "La Sapienza".

Sara Bogialli – Department of Chemical Sciences, University of Padua, Padua, Italy.

Lucia Bonadonna – Formerly affiliated with the Department of Environment and Health, ISS, Rome, Italy.

Giuseppe Bortone –Environmental Protection Agency of Emilia-Romagna, Bologna, Italy.

Eleonora Brancaleone National Center for Water Safety, ISS, Rome, Italy.

Rossella Briancesco- National Center for Water Safety, ISS, Rome, Italy.

Roberto Cammarata – Central Directorate of Human and Economic Resources, ISS, Rome, Italy.

Mario Cerroni – National Center for Water Safety, ISS, Rome, Italy.

Anna Coccia – National Center for Water Safety, ISS, Rome, Italy.

Fortunato D'Ancona – Department of Infectious Disease, ISS, Rome, Italy.

Stefania de Angelis – National Center for Water Safety, ISS, Rome, Italy.

Roberta di Gioia – National Center for Water Safety, ISS, Rome, Italy.

Antonio Dondolini_Poli – Italian Navy's Health Inspectorate, Rome, Italy.

Antonella Filippi – National Center for Water Safety, ISS, Rome, Italy.

Giuseppina Gullifa – Department of Chemistry, University of Rome "La Sapienza".

Camilla Marchiafava – National Center for Water Safety, ISS, Rome, Italy.

Lorenzo Martellone – National Center for Water Safety, ISS, Rome, Italy.

Daniela Mattei – National Center for Water Safety, ISS, Rome, Italy.

Giorgia Mattei – National Center for Water Safety, ISS, Rome, Italy.

Cristina Mazziotti – Oceanographic Facility "Daphne", Environmental Protection Agency of Emilia-Romagna, Bologna, Italy.

Susanna Murtas – National Center for Water Safety, ISS, Rome, Italy.

Federica Nigro di Gregorio – National Center for Water Safety, ISS, Rome, Italy.

Elena Papa – Department of Chemistry, University of Rome "La Sapienza", Rome, Italy.

Flavia Riccardo – Department of Infectious Disease, ISS, Rome, Italy.

Roberta Risoluti – Department of Chemistry University of Rome "La Sapienza", Rome, Italy.

Clara Sette – National Center for Water Safety, ISS, Rome, Italy.

CRediT authorship contribution statement

La Rosa G., Mancini P., Iaconelli M., Veneri C., Bonanno Ferraro G., Del Giudice C., Suffredini E., The Sea Care team, Muratore A, Ferrara F., Lucentini L., Martuzzi M. and Piccioli A.

Conceptualization GLR, LL

Data curation GLR, PM, MI, CV, GBF, CDG, ES, AM, FF

Funding acquisition LL, MM, AP

Investigation GLR, PM, MI, CV, GBF, CDG, ES, AM, The Sea Care team, FF

Methodology GLR, PM, MI, CV, GBF, CDG, ES, AM, FF

Supervision LL, MM, AP

Writing - Original Draft GLR, PM

Writing - Review & Editing GLR, PM, MI, CV, GBF, CDG, ES, AM, The Sea Care team, FF, LL, MM, AP

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.167343.

References

Amahmid, O., El Guamri, Y., Rakibi, Y., Ouizat, S., Yazidi, M., Razoki, B., Kaid Rassou, K., Touloun, O., Asmama, S., Bouhoum, K., Belghyti, D., 2023. Assessment of SARS-CoV-2 stability in human and environmental matrices, and potential hazards. Int. J. Environ. Health Res. 33 (1), 1–14. https://doi.org/10.1080/09603123.2021.1996541 (Epub 2021 Oct 27; Jan).

Atoui, A., Cordevant, C., Chesnot, T., Gassilloud, B., 2023. SARS-CoV-2 in the environment: contamination routes, detection methods, persistence and removal in wastewater treatment plants. Sci. Total Environ. 881, 163453. https://doi.org/10.1016/j.scitotenv.2023.163453 (Epub 2023 Apr 12; Jul 10).

Audino, T., Grattarola, C., Centelleghe, C., Peletto, S., Giorda, F., Florio, C.L., Caramelli, M., Bozzetta, E., Mazzariol, S., Di Guardo, G., Lauriano, G., Casalone, C., 2021. SARS-CoV-2, a threat to marine mammals? A study from Italian seawaters. Animals (Basel) 11 (6), 1663. https://doi.org/10.3390/ani11061663. PMID: 34204885; PMCID: PMC8226612; Jun 3.

Bonadonna, L., Briancesco, R., Suffredini, E., Coccia, A., Della Libera, S., Carducci, A., Verani, M., Federigi, I., Iaconelli, M., Bonanno Ferraro, G., Mancini, P., Veneri, C., Ferretti, E., Lucentini, L., Gramaccioni, L., La Rosa, G., 2019. Enteric viruses, somatic coliphages and Vibrio species in marine bathing and non-bathing waters in Italy. Mar. Pollut. Bull. 149, 110570. https://doi.org/10.1016/j.marpolbul.2019.110570. Epub 2019 Sep 8. PMID: 31542593; Dec.

Bonanno Ferraro, G., Veneri, C., Mancini, P., Iaconelli, M., Suffredini, E., Bonadonna, L., Lucentini, L., Bowo-Ngandji, A., Kengne-Nde, C., Mbaga, D.S., Mahamat, G., Tazokong, H.R., Ebogo-Belobo, J.T., Njouom, R., Kenmoe, S., La Rosa, G., 2022. A state-of-the-art scoping review on SARS-CoV-2 in sewage focusing on the potential of wastewater surveillance for the monitoring of the COVID-19 pandemic. Food Environ. Virol. 14 (4), 315–354. https://doi.org/10.1007/s12560-021-09498-6. Epub 2021 Nov 2. PMID: 34727334; PMCID: PMC8561373; Dec.

Cutrupi, F., Cadonna, M., Manara, S., Postinghel, M., La Rosa, G., Suffredini, E., Foladori, P., 2022 Nov. The wave of the SARS-CoV-2 Omicron variant resulted in a rapid spike and decline as highlighted by municipal wastewater surveillance. Environ. Technol. Innov. 28, 102667 https://doi.org/10.1016/j.eti.2022.102667. Epub 2022 May 21. PMID: 35615435; PMCID: PMC9122782.

Cárdenas-Calle, M., Patiño, L., Pernia, B., Erazo, R., Muñoz, C., Valencia-Avellan, M., Lozada, M., Regato-Arrata, M., Barrera, M., Aquino, S., Moyano, S., Fuentes, S., Duque, J., Velazquez-Araque, L., Carpio, B., Méndez-Roman, C., Calle, C., Cárdenas, G., Guizado-Herrera, D., Lucía Tello, C., Bravo-Basantes, V., Zambranod, J., Francis, J., Uyaguari, M., 2022. Detection of Fecal Coliforms and SARS-CoV-2 RNA in Sewage and Recreational Waters in the Ecuadorian Coast: A Call for Improving Water Quality Regulation medRxiv.

Ciannella, S., González-Fernández, C., Gomez-Pastora, J., 2023. Recent progress on wastewater-based epidemiology for COVID-19 surveillance: a systematic review of analytical procedures and epidemiological modeling. Sci. Total Environ. 878,

162953. https://doi.org/10.1016/j.scitotenv.2023.162953 (Epub 2023 Mar 21; Jun 20).
Costafreda, M.I., Bosch, A., Pintó, R.M., 2006. Development, evaluation, and standardization of a real-time TaqMan reverse ranscription-PCR assay for quantification of hepatitis A virus in clinical and shellfish samples. Appl. Environ. Microbiol. 72, 3846–3855.
Desdouits, M., Piquet, J.-C., Wacrenier, C., Le Mennec, C., Parnaudeau, S., Jousse, S., Rocq, S., Bigault, L., Contrant, M., Garry, P., Chavanon, F., Gabellec, R., Lamort, L., Lebrun, L., Le Gall, P., Meteigner, C., Schmitt, A., Seugnet, J.L., Serais, O., Peltier, C., Bressolette-Bodin, C., Blanchard, Y., Le Guyader, F.S., 2021. Can shellfish be used to monitor SARS-CoV-2 in the coastal environment? Sci. Total Environ. 778, 146270.
Di Maria, F., La Rosa, G., Bonato, T., Pivato, A., Piazza, R., Mancini, P., Bonanno Ferraro, G., Veneri, C., Iaconelli, M., Beccaloni, E., Scaini, F., Bonadonna, L., Vicenza, T., Suffredini, E., 2021. An innovative approach for the non-invasive surveillance of communities and early detection of SARS-CoV-2 via solid waste analysis. Sci. Total Environ. 801, 149743 https://doi.org/10.1016/j.scitotenv.2021.149743. Epub 2021 Aug 18. PMID: 34467913; PMCID: PMC8372477; Dec 20.
Dutta, H., Kaushik, G., Dutta, V., 2022. Wastewater-based epidemiology: a new frontier for tracking environmental persistence and community transmission of COVID-19. Environ. Sci. Pollut. Res. Int. 29 (57), 85688–85699. https://doi.org/10.1007/s11356-021-17419-0 (Epub 2021 Nov 11; Dec).
Efstratiou, M.A., Tzoraki, O., 2021. Coronavirus survival on beach sand: sun vs COVID-19. Mar. Pollut. Bull. 167, 112270.
Fout, S., Brinkman, N., Cashdollar, J., Griffin, S., McMinn, B., Rhodes, E., Varughese, E., Karim, M., Grimm, A., Spencer, S., Borchardt, M., 2014. Method 1615 Measurement of Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR. U.S. Environmental Protection Agency, Washington, DC.
Guo, W., Cao, Y., Kong, X., Kong, S., Xu, T., 2021. Potential threat of SARS-CoV-2 in coastal waters. Ecotoxicol. Environ. Saf. 220, 112409.
Hougs, L., Gatto, F., Goerlich, O., Grohmann, L., Lieske, K., Mazzara, M., Narendja, F., Ovesna, J., Papazova, N., Scholtens, I., Žel, J., 2017. Verification of analytical methods for GMO testing when implementing interlaboratory validated methods, 2017. EUR 29015 EN, Publication Office of the European Union, Luxembourg, ISBN 978-92-79-77310-5. https://doi.org/10.2760/645114. JRC 109940.
Keshaviah, A., Diamond, M.B., Wade, M.J., Scarpino, S.V., Global Wastewater Action Group, 2023. Wastewater monitoring can anchor global disease surveillance systems. Lancet Glob. Health 11 (6), e976–e981. https://doi.org/10.1016/S2214-109X(23)00170-5 (Jun).
La Rosa, G., Bonadonna, L., Lucentini, L., Kenmoe, S., Suffredini, E., 2020. Coronavirus in water environments: occurrence, persistence and concentration methods - a scoping review. Water Res. 179, 115899. https://doi.org/10.1016/j.watres.2020.115899 (Epub 2020 Apr 28; Jul 15).
La Rosa, G., Mancini, P., Bonanno Ferraro, G., Veneri, C., Iaconelli, M., Bonadonna, L., Lucentini, L., Suffredini, E., 2021a. SARS-CoV-2 has been circulating in northern Italy since December 2019: evidence from environmental monitoring. Sci. Total Environ. 750, 141711 https://doi.org/10.1016/j.scitotenv.2020.141711. Epub 2020 Aug 15. PMID: 32835962; PMCID: PMC7428442; Jan 1.
La Rosa, G., Mancini, P., Bonanno Ferraro, G., Veneri, C., Iaconelli, M., Lucentini, L., Bonadonna, L., Brusaferro, S., Brandtner, D., Fasanella, A., Pace, L., Parisi, A., Galante, D., Suffredini, E., 2021b. Rapid screening for SARS-CoV-2 variants of concern in clinical and environmental samples using nested RT-PCR assays targeting key mutations of the spike protein. Water Res. 197, 117104 https://doi.org/10.1016/j.watres.2021.117104. Epub 2021 Apr 2. PMID: 33857895; PMCID: PMC8018700; Jun 1.
La Rosa, G., Brandtner, D., Mancini, P., Veneri, C., Bonanno Ferraro, G., Bonadonna, L., Lucentini, L., Suffredini, E., 2021c. Key SARS-CoV-2 mutations of alpha, gamma, and eta variants detected in urban wastewaters in Italy by long-read amplicon sequencing based on nanopore technology. Water 13, 2503. https://doi.org/10.3390/w13182503.
La Rosa, G., Brandtner, D., Bonanno Ferraro, G., Veneri, C., Mancini, P., Iaconelli, M., Lucentini, L., Del Giudice, C., Orlandi, L., SARI network, Suffredini, E., 2023. Wastewater surveillance of SARS-CoV-2 variants in October-November 2022 in Italy: detection of XBB.1, BA.2.75 and rapid spread of the BQ.1 lineage. Sci. Total Environ. 873, 162339. https://doi.org/10.1016/j.scitotenv.2023.162339. Epub 2023 Feb 21. PMID: 36813191; May 15.
Le Guernic, A., Palos Ladeiro, M., Boudaud, N., Do Nascimento, J., Gantzer, C., Inglard, J.-C., Mouchel, J.-M., Pochet, C., Moulin, L., Rocher, V., Waldman, P., Wurtzer, S., Geffard, A., 2022. First evidence of SARS-CoV-2 genome detection in zebra mussel (Dreissena polymorpha). J. Environ. Manag. 301, 113866.
Li, S., Yang, R., Zhang, D., Han, P., Xu, Z., Chen, Q., Zhao, R., Zhao, X., Qu, X., Zheng, A., Wang, L., Li, L., Hu, Y., Zhang, R., Su, C., Niu, S., Zhang, Y., Qi, J., Liu, K., Wang, Q., Gao, G.F., 2022. Cross-species recognition and molecular basis of SARS-CoV-2 and SARS-CoV binding to ACE2s of marine animals. Natl. Sci. Rev. 9 (9), nwac122 https://doi.org/10.1093/nsr/nwac122. PMID: 36187898; PMCID: PMC9517163; Jun 23.
Lombardi, A., Voli, A., Mancusi, A., Girardi, S., Proroga, Y.T.R., Pierri, B., Olivares, R., Cossentino, L., Suffredini, E., La Rosa, G., et al., 2023. SARS-CoV-2 RNA in wastewater and bivalve mollusk samples of Campania, Southern Italy. Viruses 15, 1777. https://doi.org/10.3390/v15081777.
Lundy, L., Fatta-Kassinos, D., Slobodnik, J., Karaolia, P., Cirka, L., Kreuzinger, N., Castiglioni, S., Bijlsma, L., Dulio, V., Deviller, G., Lai, F.Y., Alygizakis, N., Barneo, M., Baz-Lomba, J.A., Béen, F., Cíchová, M., Conde-Pérez, K., Covaci, A., Donner, E., Ficek, A., Hassard, F., Hedström, A., Hernandez, F., Janská, V., Jellison, K., Hofman, J., Hill, K., Hong, P.Y., Kasprzyk-Hordern, B., Kolarević, S., Krahulec, J., Lambropoulou, D., de Llanos, R., Mackuľak, T., Martinez-García, L., Martínez, F., Medema, G., Micsinai, A., Myrmel, M., Nasser, M., Niederstätter, H., Nozal, L., Oberacher, H., Očenášková, V., Ogorzaly, L., Papadopoulos, D., Peinado, B., Pitkänen, T., Poza, M., Rumbo-Feal, S., Sánchez, M.B., Székely, A.J., Soltysova, A., Thomaidis, N.S., Vallejo, J., van Nuijs, A., Ware, V., Viklander, M., 2021. Making waves: collaboration in the time of SARS-CoV-2 - rapid development of an international co-operation and wastewater surveillance database to support public health decision-making. Water Res. 199, 117167 https://doi.org/10.1016/j.watres.2021.117167. Epub 2021 Apr 22. PMID: 34015748; PMCID: PMC8060897; Jul 1.
Maal-Bared, R., Sobsey, M., Bibby, K., Sherchan, S.P., Fitzmorris, K.B., Munakata, N., Gerba, C., Schaefer, S., Swift, J., Gary, L., Babatola, A., Bastian, R., Olabode, L., Reimers, R., Rubin, A., Kester, G., Casson, L., 2021. Letter to the Editor regarding Mathavarajah et al. (2020) Pandemic danger to the deep: The risk of marine mammals contracting SARS-CoV-2 from wastewater. Sci. Total Environ. 773, 144855 https://doi.org/10.1016/j.scitotenv.2020.144855. Epub 2020 Dec 31. PMID: 33423797; PMCID: PMC7833100; Jun 15.
Mahlknecht, J., 2022. Presence and persistence of SARS-CoV-2 in aquatic environments: a mini-review. Curr. Opin. Environ. Sci. Health 29, 100385. https://doi.org/10.1016/j.coesh.2022.100385 (Epub 2022 Jul 31; Oct).
Mancusi, A., Capuano, F., Girardi, S., Di Maro, O., Suffredini, E., Di Concilio, D., Vassallo, L., Cuomo, M.C., Tafuro, M., Signorelli, D., Pierri, A., Pizzolante, A., Cerino, P., La Rosa, G., Proroga, Y.T.R., Pierri, B., 2022. Detection of SARS-CoV-2 RNA in bivalve mollusks by droplet digital RT-PCR (dd RT-PCR). Int. J. Environ. Res. Public Health 19, 943.
Mathavarajah, S., Stoddart, A.K., Gagnon, G.A., Dellaire, G., 2021. Pandemic danger to the deep: the risk of marine mammals contracting SARS-CoV-2 from wastewater. Sci. Total Environ. 760, 143346.
Mazumder, P., Dash, S., Honda, R., Sonne, C., Kumar, M., 2022. Sewage surveillance for SARS-CoV-2: molecular detection, quantification, and normalization factors. Curr. Opin. Environ. Sci. Health 28, 100363. https://doi.org/10.1016/j.coesh.2022.100363 (Epub 2022 Apr 10; Aug).
Pivato, A., Formenton, G., Di Maria, F., Baldovin, T., Amoruso, I., Bonato, T., Mancini, P., Bonanno Ferraro, G., Veneri, C., Iaconelli, M., et al., 2022. SARS-CoV-2 in atmospheric particulate matter: an experimental survey in the province of Venice in Northern Italy. Int. J. Environ. Res. Public Health 19, 9462. https://doi.org/10.3390/ijerph19159462.
Polo, D., Lois, M., Fernández-Núñez, M.T., Romalde, J.L., 2021. Detection of SARS-CoV-2 RNA in bivalve mollusks and marine sediments. Sci. Total Environ. 786, 147534.
Sala-Comorera, L., Reynolds, L.J., Martin, N.A., O'Sullivan, J.J., Meijer, W.G., Fletcher, N.F., 2021. Decay of infectious SARS-CoV-2 and surrogates in aquatic environments. Water Res. 201, 117090 https://doi.org/10.1016/j.watres.2021.117090. Epub 2021 Mar 29. PMID: 34111729; PMCID: PMC8005746; Aug 1.
Sun, Z.P., Yang, S.Y., Cai, X., Han, W.D., Hu, G.W., Qian, Y., Wang, Y.Y., Zhang, R., Xie, Y.H., Qu, D., 2022. Survival of SARS-CoV-2 in artificial seawater and on the surface of inanimate materials. J. Med. Virol. 94 (8), 3982–3987. https://doi.org/10.1002/jmv.27807 (Epub 2022 May 5; Aug).
Tamáš, M., Potocarova, A., Konecna, B., Klucar, Ľ., Mackulak, T., 2022. Wastewater sequencing-an innovative method for variant monitoring of SARS-CoV-2 in populations. Int. J. Environ. Res. Public Health 19 (15), 9749. https://doi.org/10.3390/ijerph19159749. PMID: 35955106; PMCID: PMC9367975; Aug 8.
Wyer, M.D., Wyn-Jones, A.P., Kay, D., Au-Yeung, H.K., Gironés, R., López-Pila, J., de Roda Husman, A.M., Rutjes, S., Schneider, O., 2012. Relationships between human adenoviruses and faecal indicator organisms in European recreational waters. Water Res. 46 (13), 4130–4141. https://doi.org/10.1016/j.watres.2012.04.008. Epub 2012 Apr 19. PMID: 22633054; Sep 1.
Wyn-Jones, A.P., Carducci, A., Cook, N., D'Agostino, M., Divizia, M., Fleischer, J., Gantzer, C., Gawler, A., Girones, R., Höller, C., de Roda Husman, A.M., Kay, D., Kozyra, I., López-Pila, J., Muscillo, M., Nascimento, M.S., Papageorgiou, G., Rutjes, S., Sellwood, J., Szewzyk, R., Wyer, M., 2011. Surveillance of adenoviruses and noroviruses in European recreational waters. Water Res. 45 (3), 1025–1038. https://doi.org/10.1016/j.watres.2010.10.015. Epub 2010 Oct 29. PMID: 21093010; PMCID: PMC7112131; Jan.
Yang, Y., Li, J., Han, F., 2022. Focus on marine animal safety and marine bioresources in response to the SARS-CoV-2 crisis. Int. J. Mol. Sci. 23 (23), 15136. https://doi.org/10.3390/ijms232315136. PMID: 36499463; PMCID: PMC9737530; Dec 1.