# First report on the presence of potentially pathogenic *Vibrio parahaemolyticus* in farmed mussels (*Mytilus galloprovincialis*) from the Istrian aquatorium by microbiological and molecular methods

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## Abstract

Microbiological and molecular PCR techniques were used to analyse 328 samples of mussels (*Mytilus galloprovincialis*) cultured in Istrian waters. Molecular testing was more effective than microbiological testing, detecting the presence of the bacterium *Vibrio parahaemolyticus* in 21.34% of samples, compared to 2.44% detected through microbiological methods. The probability of detecting the presence of this bacterium was 7.19 times higher when the sea surface temperature was above 15°C, while thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) positive samples were detected at sea temperatures above 22.5°C and 24.7°C, respectively. TDH- and TRH-positive samples constituted 10% of the total *V. parahaemolyticus*-positive samples. As there is no legal obligation to monitor the presence of this bacterium, consuming raw or insufficiently cooked mussels can lead to illness.

**Key words:** *mussels; Vibrio parahaemolyticus; microbiology; PCR*

## Introduction

Bivalve molluscs are filter-feeding organisms capable of filtering up to 8 litres of water per day, thereby bioaccumulating naturally occurring or anthropogenic pathogens (Lees, 2000; Ribarić et al., 2012). The cultivation of mussels (*Mytilus galloprovincialis*) in Istrian waters requires two warm water seasons to reach commercial

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size, taking an average of one and a half years (Džafić, 2022). Mussels are nutritionally valuable due to their rich composition of proteins, essential amino acids, polyunsaturated fatty acids, astaxanthin and other carotenoids, vitamin B12, and other vitamins, many minerals, and other components (Krešić et al., 2020). Various bacteria and viruses are indigenous microflora of marine habitats where molluscs are farmed. Potentially pathogenic halophilic vibrios are of particular concern, as there are no statutory controls on their presence at the European level (Regulation (EC) 853/2004; Regulation (EC) 2073/2005). The bacterium *Vibrio parahaemolyticus* is a Gram-negative, halophilic, straight or curved rod bacterium with a polar flagellum that is motile in liquid media. Most *V. parahaemolyticus* strains isolated from environmental samples and seafood are non-pathogenic. In contrast to such strains, clinical strains can produce an enzyme that lyses erythrocytes. The hemolytic activity of pathogenic strains of this bacterium on Wagatsuma agar is known as the Kanagawa phenomenon, resulting from the presence of the thermostable direct hemolysin (TDH) (Nelapati et al., 2012). Some Kanagawa-negative strains produce a toxin similar to TDH, called TDH-related hemolysin (TRH). These TDH and TRH hemolysins are encoded by the *tdh* and *trh* genes and are considered important virulence factors. Among the various serotypes of *V. parahaemolyticus* that can cause infections in humans, serotype O3:K6 is the most common. The *trh* gene is also found in other members of the *Vibrio* genus such as *Vibrio alginolyticus* (Rosec et al., 2012), which is why it is not reliable for the identification of *V. parahaemolyticus*. The *ToxR* gene was initially discovered as a regulatory gene of the cholera toxin operon, and later was shown to have a regulatory function in *V. parahaemolyticus* (Kim et al., 1999). The bacterium *V. parahaemolyticus* has been isolated from various marine organisms (Ripabelli et al., 1999; Ottaviani et al., 2005; Normano et al., 2006; Vernocchi et al., 2007; Suffredini et al., 2014), as well as from seawater and sediments (Schets et al., 2011; Yu et al., 2013). While most environmental strains are non-pathogenic, some can cause foodborne infections. The pathogenesis of *V. parahaemolyticus* is based on the production of hemolysin that causes hemolysis of human erythrocytes (Lozano-Leon et al., 2003).

Due to the lack of previous research in the Republic of Croatia, studies conducted on the Italian side of the Adriatic served as the basis for this study, given the specificity of the Adriatic Sea as a shallow sea, particularly in the extreme north where it is filled with alluvium from the Po River.

The aim of this study was to determine the presence of *V. parahaemolyticus* and the *tdh* and *trh* genes in the farming areas in Istrian waters, considering farming conditions (location and sea temperature) as factors that increase the public health risk of shellfish consumption.

## Material and methods

Mussel samples ($n$=328) were collected monthly from nine sampling points in six production areas over three periods. The first period (February 2017 to March 2018) comprised 108 samples, the second (April 2018 to March 2019) 106, and the third (April 2019 to March 2020) included 114 samples. The locations of the farming areas and the sampling points are described in the Monitoring plan for marine and shellfish quality in live bivalve mollusc production areas and nurseries (2020) and shown in Figure 1. Upon delivery to the laboratory, the mussels were washed with clean running water and opened under

aseptic conditions. Twenty-five grams of tissue and intershell fluid were collected for analysis. Figure 2 illustrates the procedure for the detection of the presence of potentially pathogenic *Vibrio* spp. and the isolation of DNA from enriched samples and suspect colonies and control bacterial strains (HRN EN ISO 21872-1: 2017 Microbiology in the food chain - A horizontal method for the determination of *Vibrio* spp. - Part 1: Detection of the presence of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae,* and *Vibrio vulnificus* (Anonymous, 2017).

Briefly, polymerase chain reaction (PCR) was used to identify suspect colonies and to test pre-enriched samples, using the primers and amplification cycles specified in HRN EN ISO 21872-1:2017. The control reference strain *V. parahaemolyticus* ATCC 17802 and the environmental isolate *V. parahaemolyticus tdh*/*trh*+env, Vp Tox Primers (Metabion International AG, Germany) and *tdh*/*trh* Primers (Macrogen Humanizing Genomics, South Korea) were used. They were prepared according to the manufacturer's instructions to obtain 100 μM solution. Working solutions with a concentration of 10 μM were prepared from the starting stock solution. *ToxR* gene was used for the specific detection of *V. parahaemolyticus*.

The PCR was carried out in a final volume of 20 μL in the Techne Thermal Cycler

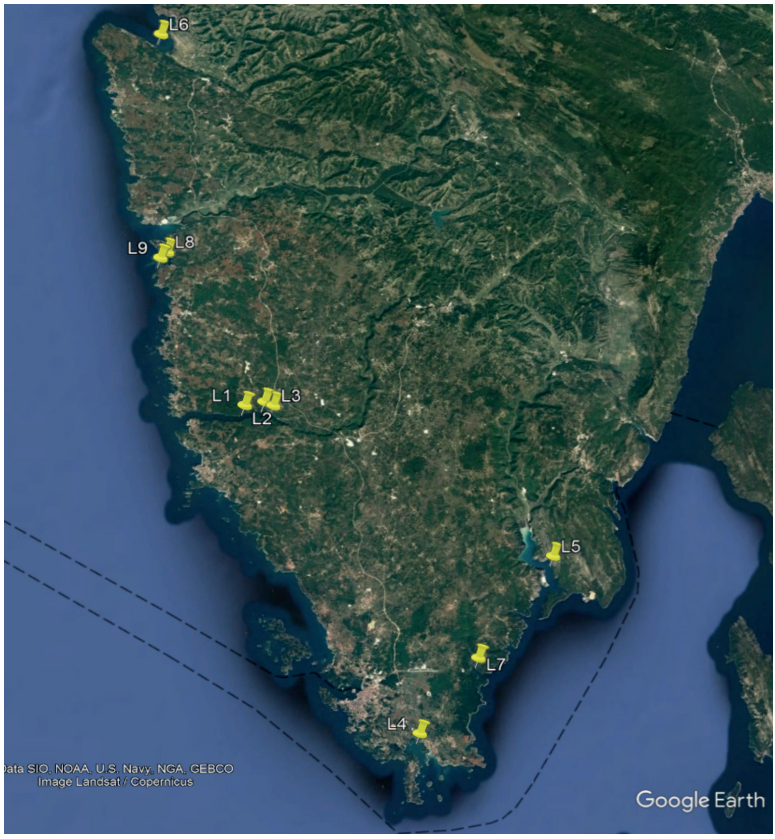
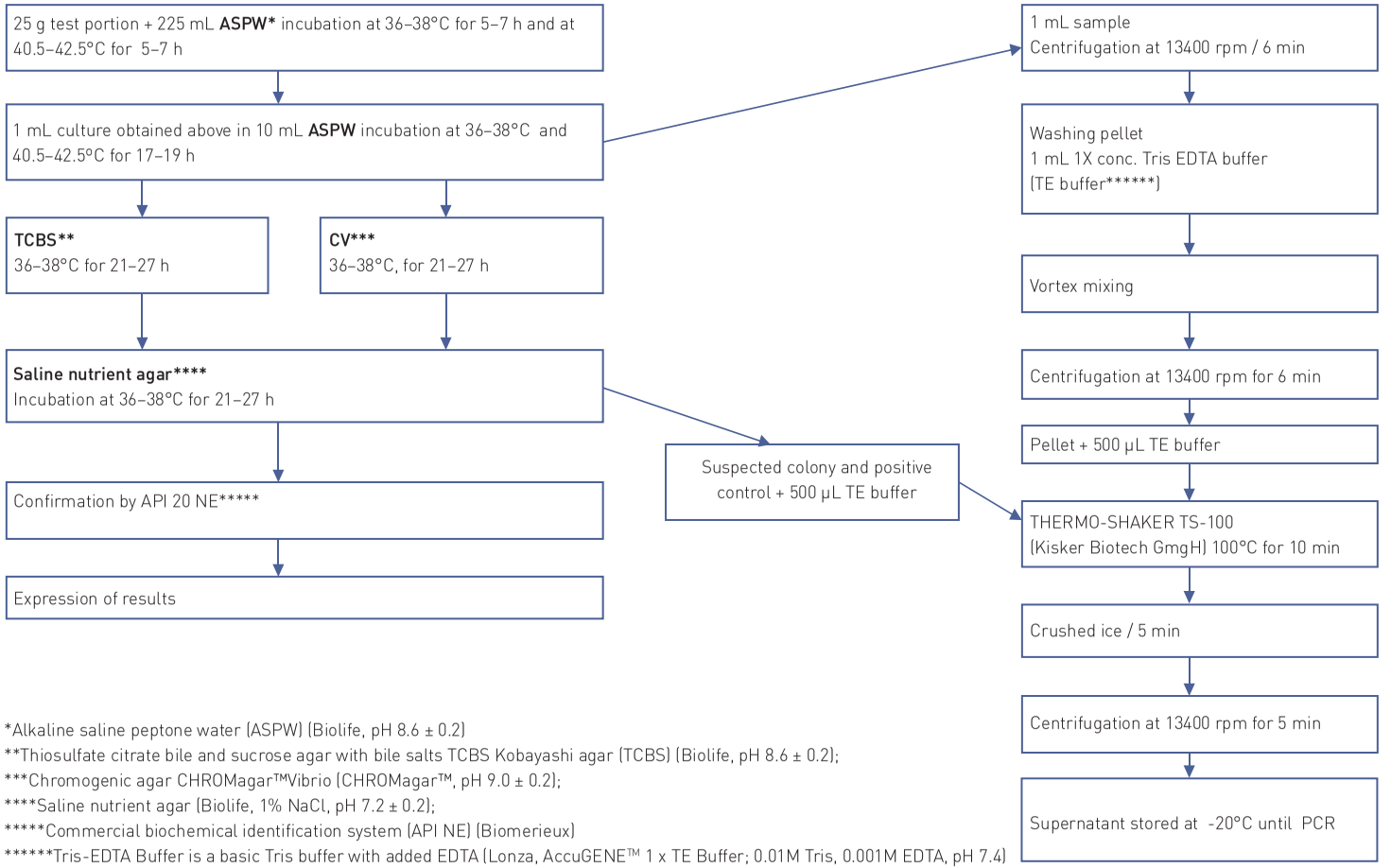



**Figure 1.** Sampling locations (downloaded and modified from Google Earth Pro version 7.3.6)




*Alkaline saline peptone water (ASPW) (Biolife, pH 8.6 ± 0.2)
**Thiosulfate citrate bile and sucrose agar with bile salts TCBS Kobayashi agar (TCBS) (Biolife, pH 8.6 ± 0.2);
***Chromogenic agar CHROMagar™Vibrio (CHROMagar™, pH 9.0 ± 0.2);
****Saline nutrient agar (Biolife, 1% NaCl, pH 7.2 ± 0.2);
*****Commercial biochemical identification system (API NE) (Biomerieux)
******Tris-EDTA Buffer is a basic Tris buffer with added EDTA (Lonza, AccuGENE™ 1 x TE Buffer; 0.01M Tris, 0.001M EDTA, pH 7.4)

**Figure 2.** Flowchart of the procedure for the detection of potentially pathogenic *Vibrio spp.* and isolation of DNA from pre-enriched samples, suspected colonies and control bacterial strains.

**Table 1.** Primer sequences used for detection of genome expected product size

| Bacteria | Primers | Primer sequences | Product size (bp) |
|---|---|---|---|
| *V. parahaemolyticus* | Vp*ToxR* (FW) (Kim et al., 1999) | GTC TTC TGA CGC AAT CGT TG | 368 |
| | Vp*ToxR* (REV) (Kim et al., 1999) | ATA CGA GTG GTT GCT GTC ATG | |
| *V. parahaemolyticus tdh* | L-*tdh* (Bej et al., 1999) | GTA AAG GTC TCT GAC TTT TGG AC | 269 |
| | R-*tdh* (Bej et al., 1999) | TGG AAT AGA ACC TTC ATC TTC ACC | |
| *V. parahaemolyticus trh* | L-*trh* (Bej et al., 1999) | TTG GCT TCG ATA TTT TCA GTA TCT | 500 |
| | R*trh* (Bej et al., 1999) | CAT AAC AAA CAT ATG CCC ATT TCC G | |

Prime (Cole-Parmer Ltd, UK). The premix EmeraldAmp®MAX HS PCR Master Mix (Takara, Japan) was used to prepare the reaction mixture. The following volumes were used to prepare the PCR mixture: Master mix 10.0 µL; primer (FW) 0.5 µL (10 µM); primer (REV) 0.5 µL (10 µM); water 7.0 µL; DNA sample 2.0 µL.

For gel electrophoresis, 2% agarose (SeaKem® LE Agarose, Lonza, USA) in Tris-acetate-EDTA buffer (TAE) (AccuGENE®, Lonza, USA), pH 8.3 was used. The gel was stained with DNA dye (GelStar™, Lonza, USA) (5 µL dye per 50 mL gel) and ladder marker (DNA ladder Dye Plus, Takara, Japan) in a volume of 2 µL was used as a molecular size marker. The running conditions were 130 V for 65 to 80 minutes (Gator A2 Large System 24-Slot Rapid Load UVT Gel Trays, Thermo Scientific, USA). The gel documentation system UVIDOC HD 5 (UVITEC, Cambridge, UK) and the associated data processing program UVI TEC UVI 1-D (UVITEC, Cambridge, UK) were used for product visualisation.

Data on weather conditions and sea temperatures at the time of sampling were obtained from the Croatian Meteorological and Hydrometeorological Institute (DHMZ). The data were collected by meteorological ground observation at the main meteorological station in Pula. Sea surface temperature was measured at a permanent measuring point at a depth of 30 cm and then checked before data entry. Data on the average daily sea temperature was used, which was linked to the results of microbiological tests on the day of sampling. Other data on weather conditions relate to average daily air temperatures and precipitation.

To perform statistical analysis of the data obtained during the study, software Stata 13.1 (Stata Corp., USA) was used.

Chi-square and Fisher's exact tests were used to check the association of the detection of vibrios with location and year of sampling. Environmental conditions (sea and air temperature) of locations where vibrios were and were not found were compared. Statistical results are presented as probabilities (P values). Probabilities less than 0.05 were considered statistically significant.

Variables significantly associated with the outcome (detection of vibrio) in univariate analysis were included in the

**Table 2.** Amplification cycles for detection of *V. parahaemolyticus (Tox-R)* and *tdh/trh*

| Step description | | Temperature and time | | Number of cycles |
|---|---|---|---|---|
| | | *V. parahaemolyticus Tox-R* | *V. parahaemolyticus tdh/trh* | |
| Pre-heating | | 96°C for 5 min | 94°C for 5 min | 1 |
| Amplification | Denaturation | 94°C for 1 min | 94°C for 1 min | 30 |
| | Annealing | 63°C for 1.5 min | 58°C for 1 min | |
| | Extension | 72°C for 1.5 min | 72°C for 1 min | |
| Post amplification | | 72°C for 7 min | 72°C for 5 min | 1 |
| Ending | | 4°C | 4°C | |

logistic regression model. Sea temperature and location were transformed into binary variables. Hence the odds of isolation of *V. parahaemolyticus* were statistically analysed between temperatures higher and lower than 15°C. Results of logistic regression are expressed as odds ratio (OR) with associated P values.

## Results and discussion

The microbiological examination of the mussel samples resulted in eight (2.44%) positive samples (Table 3), while the results of the PCR applied on the pre-enriched samples showed 70 positive samples (21.34%). The observed difference in the results between the two methods is probably due to the low selectivity of the TCBS agar used, as previously noted in several studies (Lotz et al., 1983; Kitaura et al., 1983; Massad and Oliver, 1987). Ivezić-Jakšić (1986) found that *V. alginolyticus* has an antagonistic effect on the growth of *V. parahaemolyticus* in mixed culture on TCBS agar, for which the author found no confirmation in the available literature. In the present study, we frequently observed abundant growth of large yellow colonies (on TCBS agar) and large milky white colonies (on CV agar) of the bacterium *V. alginolyticus*, which is the predominant species in mussels from the study area in period of year with higher temperature. This is consistent with the findings of Cavallo and Stabili (2002), who found that the most frequently isolated *Vibrio* species in mussel samples in Italy was *V. alginolyticus*. The prevalence of *V. parahaemolyticus* isolated in our study was 2.4%, while researchers on the Italian side of the Adriatic Sea demonstrated a prevalence of the same bacterium of 6.25% (Di Pinto et al., 2008), 7.83% (Normanno et al., 2006), 24% (Ottaviani et al., 2005) and up to 56.7% (Suffredini et al., 2014).

In the present study, *V. parahaemolyticus* was most frequently isolated from samples of the third period (four samples or 3.51%), while two samples were isolated in each of the other two periods observed. The differences in the frequency of isolation of this species between the different observation periods were not statistically significant (P=0.739).

Larger differences were observed in the molecular analyses. *V. parahaemolyticus* was most frequently detected by molecular methods in pre-enriched samples collected during the first period (24.07%) and

least frequently in samples from the third period (18.42%). However, the differences in the frequency of molecular detection of *V. parahaemolyticus* species between the study periods were not statistically significant (P=0.586). Many authors have compared the effectiveness of molecular techniques with microbiological detection methods (Blanco-Abad et al., 2009; Rosec et al., 2009, 2012). The absence of the same pathogen does not necessarily mean that it is not present, as *V. parahaemolyticus* can enter the "viable but non-culturable state" (VBNC) state in which it retains its metabolic activity but is undetectable by conventional microbiological methods (Oliver, 2005, 2009).

The advantage of the PCR method in this study, as also reported elsewhere (Rosec et al., 2009, 2012), lies in its application after pre-enrichment of the sample. This two-step pre-enrichment procedure promotes the proliferation of the target bacterial species while also eliminating the inhibitory effect of mussel tissue on the PCR. This ensures optimal conditions for molecular methods, while the microbiological method faces challenges from the proliferation of other bacterial species present in the sample. These other species can mask or even antagonistically influence the growth of the targeted *Vibrio* spp. (Ivezić-Jakšić, 1986), leading to possible misidentification. Di Pinto et al. (2008) stated that out of 47 suspect isolates from culture media, 12 were confirmed as *V. parahaemolyticus* by biochemical identification, while only nine of those 12 were confirmed by the molecular PCR method.

All pre-enriched samples that tested VP *ToxR*-positive for *V. parahaemolyticus* in our study (21.3%) were further analysed for the presence of the *tdh* and *trh* genes that encode the enzymes responsible for the pathogenic effect. Of the 70 VP *ToxR*-positive samples, the *tdh* gene was detected in one pre-enriched sample (1.43%), while the *trh* gene was found in seven pre-enriched samples (10%). Previous studies on mussels in Croatia (Ivezić-Jakšić, 1986; Jakšić et al., 2002; Mikuš et al., 2010; Čanak et al., 2018) demonstrated the presence of *V. parahaemolyticus*, but to our knowledge, this is the first report of the presence of genes encoding the TDH and TRH toxins in the Croatian part of the Adriatic Sea. In the Adriatic Sea, the presence of *tdh*- and *trh*-positive *V. parahaemolyticus* was previously described in Italy by Ottaviani et al. (2005) who found that only one of their isolates was *tdh*-positive and three were *trh*-positive. Suffredini et al. (2014) detected *V. parahaemolyticus* in 56.7% of samples from Sardinia and in 27.5% of samples from Veneto, while the presence of potentially pathogenic *V. para-*

**Table 3.** Results in accordance to periods of sampling

| Period | Total | Microbiological results | | PCR results | | | |
|---|---|---|---|---|---|---|---|
| | | Negative N (%) | Positive N (%) | Negative N (%) | Positive N (%) | *tdh* | *trh* |
| 1st | 108 | 106 (98.15) | 2 (1.85) | 82 (75.93) | 26 (24.07) | 0 | 3 (11%) |
| 2nd | 106 | 104 (98.11) | 2 (1.89) | 83 (78.3) | 23 (21.7) | 0 | 0 |
| 3rd | 114 | 110 (96.49) | 4 (3.51) | 93(81.58) | 21 (18.42) | 1 | 4 (19%) |
| Total | 328 | 320 (100) | 8 (2.44) | 258 (100) | 70 (21.34) | 1(1.43%) | 7 (10%) |

**Table 4.** Correlation of sea and air temperature (°C) with the microbiological results

| Temperature | Microbiological test | N | Mean value °C | Standard deviation | *P* |
|---|---|---|---|---|---|
| Sea | Negative | 320 | 18.19 | 5.75 | 0.0067 |
| | Positive | 8 | 23.77 | 3.25 | |
| Air | Negative | 320 | 15.48 | 7.24 | 0.0116 |
| | Positive | 8 | 22.03 | 5.40 | |

*haemolyticus* was detected in six samples, including three *tdh*-positive and three *trh*-positive isolates. In the Spanish part of the Mediterranean, the prevalence of this bacterium was 14.2%, with pathogenic variants detected in 27.1% of samples (Lopez-Joven et al., 2015). In our study, PCR for the detection of *tdh* and *trh* genes in isolated *V. parahaemolyticus* colonies yielded negative results for these genes.

Early ecological studies showed that the occurrence of *V. parahaemolyticus* depends on water temperature. The pathogen is typically undetectable at water temperatures <14°C during the winter months, although it is present in the sediment (Kaneko and Colwell, 1973; Su and Liu, 2007). These studies confirm that as water temperatures rise from late spring to early summer, vibrios overwintering in the sediment are released from the bottom communities, attach to zooplankton via the enzyme chitinase (Kaneko and Colwell, 1975) and spread through the water column. The probability of detecting *V. parahaemolyticus* in our study was 7.19 times higher when the sea surface temperature was above 15°C (Table 7), which is in agreement with the literature.

In the present study, a statistically significant difference in sea temperature was found between positive and negative results (P=0.0067). The mean sea temperature at which *V. parahaemolyticus* was detected was 23.77°C (Table 4), whereas detection by PCR occurred at a mean sea temperature of 21.84°C (Table 5). In contrast to previous studies on mussels from the Adriatic Sea, Vernocchi et al. (2007) and Di Pinto et al. (2008) detected the presence of the bacterium *V. parahaemolyticus* in samples analysed in April 2002 as well as in January and February 2003 and in winter 2008, which is

**Table 5.** Correlation of sea and air temperature (°C) with the PCR results

| Temperature | PCR test | N | Mean value °C | Standard deviation | *P* |
|---|---|---|---|---|---|
| Sea | Negative | 258 | 17.37 | 5.66 | <0.0001 |
| | Positive | 70 | 21.84 | 4.70 | |
| Air | Negative | 258 | 14.75 | 7.07 | <0.0001 |
| | Positive | 70 | 18.91 | 7.06 | |

**Table 6.** Results in accordance to locations

| Sampling site | Number of samples | Microbiological results | | PCR results | |
|---|---|---|---|---|---|
| | | Negative | Positive (%) | Negative | Positive (%) |
| L1 | 38 | 36 | 2 (5.26) | 25 | 13 (34.21) |
| L2 | 38 | 37 | 1 (2.63) | 28 | 10 (26.32) |
| L3 | 38 | 38 | 0 | 26 | 12 (31.58) |
| L4 | 34 | 34 | 0 | 31 | 3 (8.82) |
| L5 | 34 | 32 | 2 (5.88) | 25 | 9 (26.47) |
| L6 | 38 | 37 | 1 (2.63) | 35 | 3 (7.89) |
| L7 | 34 | 34 | 0 | 29 | 5 (14.71) |
| L8 | 36 | 36 | 0 | 30 | 6 (16.67) |
| L9 | 38 | 36 | 2 (5.63) | 29 | 9 (23.68) |
| Total | 328 | 320 | 8 (2.5) | 258 | 70 (21.34) |

in compliance with our results, where the lowest sea temperature at which we could detected the bacterium *V. parahaemolyticus* by PCR was 10.3°C (February 2019, individual results not shown). The odds ratio for the detection of *V. parahaemolyticus* in our study was 7.19 times higher when the sea temperature was above 15°C ($P < 0.001$). However, the odds of detecting *V. parahaemolyticus* were not statistically related to the study periods (Table 7).

As mentioned above, all samples that tested positive for *V. parahaemolyticus* by PCR were also tested for the presence of the *tdh* and *trh* genes. It was found that these genes were only detected in samples where the sea temperature at the time of sampling was above 24.7°C (*trh* gene) or 22.5°C (*tdh* gene). Flynn et al. (2019) stated that the strongest positive correlation between sea surface temperature and three genetic markers (*tlh*, *tdh*, and *trh*) was found up to a threshold of 22°C, with the observed correlation for *tdh* and *trh* influenced by the total density of *V. parahaemolyticus*. This threshold of 22°C, above which no increase in bacterial numbers was observed, was determined for the Pacific Northwest region

**Table 7.** Logistic regression analysis results

| Factor | OR | $SE_{OR}$ | z | *P* | 95% confidence interval |
|---|---|---|---|---|---|
| Time period | 0.86 | 0.14 | -0.87 | 0.383 | 0.61–1.20 |
| Temperature (>15 ≤15°C) | 7.19 | 3.00 | 4.72 | 0.000 | 3.17–16.32 |

OD-odds ratio; SE-standard error; z z-value; P -probability

of the USA. Air temperature, for which a statistically significant difference between positive and negative samples (P = 0.0116) was also found, could have a significant effect on the potential increase in bacterial counts after shellfish harvesting. At the same time, storage conditions at temperatures of up to 26°C favour the rapid multiplication of *V. parahaemolyticus* by 50 to 790 times the initial value within 24 hours (Su and Liu, 2007). Cross-contamination between contaminated and uncontaminated mussels is also possible, emphasising the necessity for continuous improvement of hygiene procedures during distribution until consumption (Hara-Kudo et al., 2012). In regions with large intertidal fluctuations in sea level where farming areas are located, mussels remain exposed to the ambient temperature allowing for potential proliferation of *V. parahaemolyticus* (Flynn et al., 2019).

In the present study, *V. parahaemolyticus* was isolated from samples from five locations (Table 6 and Figure 1), with 1 to 2 positive samples at those locations. The observed differences in the frequency of detection of this bacterium by classical microbiological tests were not statistically significant (P=0.454). The highest relative frequency of detection of *V. parahaemolyticus* by PCR was recorded at location 1. The observed differences in the frequency of detection of *V. parahaemolyticus* by PCR between the different sampling locations were statistically significant ($P = 0.043$).

Locations L1, L2 and L3 had the highest proportion of PCR-positive results. They are located in a deep, relatively narrow bay with low-intensity maritime traffic, designated as a Special Marine Protected Area. Conversely, location L5 is located in a bay with heavy maritime traffic, adjacent to a terminal for general cargo, livestock, and timber. Location L9, a large shoal, stands out with over 20 positive results. The remaining four locations included in this study had fewer than 20% positive results, with none of the location confirmed to be negative by PCR.

During the first study period, three *trh*-positive samples were detected at sites L2, L5, and L7, while in the third period, two samples each were detected at sites L4 and L7 and *tdh*-positive at site L3. Interestingly, despite having a lower overall frequency of positive samples (8.8% and 14.7%), sites L4 and L7 exhibited the highest relative frequency of *trh*- and *tdh*- positive samples.

## Conclusion

The absence of active monitoring of the presence of Vibrios in food, particularly seafood, in the European Union suggests that the current threat to human health may be underestimated. In the era of global warming, where significant increases in air and sea surface temperatures occur, favourable conditions are created for the proliferation of pathogens in the environment. As a result, the geographical areas where human gastrointestinal diseases caused by potentially pathogenic *Vibrio* bacteria occur have shifted to include the Mediterranean and the Adriatic Sea. Through this study, we have identified the presence of *Vibrio parahaemolyticus*, along with its pathogenic variants, in mussel samples intended for human consumption. So far, no cases of illness caused by these bacteria have been reported in the Republic of Croatia, possibly due to the fact that there is no mandatory reporting.

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# Prvo izvješće o prisutnosti potencijalno patogene bakterije *Vibrio parahaemolyticus* u uzgojenih dagnji (*Mytilus galloprovincialis*) istarskoga akvatorija primjenom mikrobioloških i molekularnih metoda

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Primjenom mikrobioloških i molekularnih tehnika, pretraženo je 328 uzoraka školjkaša dagnji (*Mytilus galloprovincialis*), uzgojenih na području istarskoga akvatorija. Rezultati ukazuju na smanjenu osjetljivost mikrobiološke tehnike (2.44 %) u odnosu na molekularnu dijagnostiku kojom je dokazana prisutnost bakterije *V. parahaemolyticus* u 21,34 % uzoraka. Izgledi za dokazivanje prisutnosti ove bakterije su 7,19 puta veći kada je površinska temperatura mora veća od 15 °C, dok su geni za kodiranje termostabilnog direktnog hemolizina (TDH) i hemolizina ovisnog o TDH (TRH) u uzorcima dokazani pri temperaturama mora iznad 22,5 °C, odnosno 24,7 °C. Udio TDH i TRH pozitivnih uzoraka iznosio je 10 % od ukupnih 70 *V. parahaemolyticus*-pozitivnih uzoraka. S obzirom na nedostatak zakonske obveze praćenja prisutnosti ove bakterije moguće je oboljevanje ljudi nakon konzumacije sirovih ili termički nedostatno obrađenih školjkaša.

**Ključne riječi:** dagnje, *Vibrio parahaemolyticus*, *mikrobiološka metoda*, *PCR*