



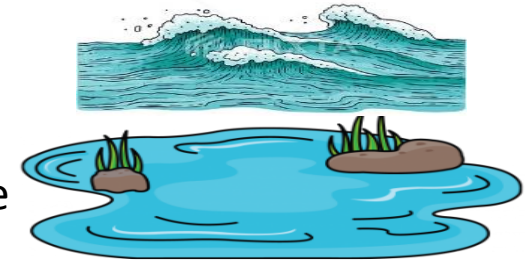
# National cartography of water points for the presence of *Vibrio* spp. in Belgium

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## Introduction

- Non-toxicogenic *Vibrio cholerae* and most *Vibrio* spp. are found in aquatic environment and are generally non-pathogenic.
- **A few species can cause sporadically illnesses** such as wound infections, otitis, bacteremia and gastroenteritis. Rarely they can cause collective food poisoning events.
- Invasive clinical cases of vibriosis have been described in Belgium after contact with recreational water (*De Keukeleire et al., 2018*).
- Recently, the number of reports of human infections, which can be life-threatening, involving non-O1, non-O139 *V. cholerae* and other *Vibrio* spp. **has increased** in Northern Europe and in France, i.e. Very few data are available for Europe. In the United States and in Japan, they are a major public health problem.
- Waters for recreational use such as lakes and sea water are **not yet monitored** for *Vibrio* spp.



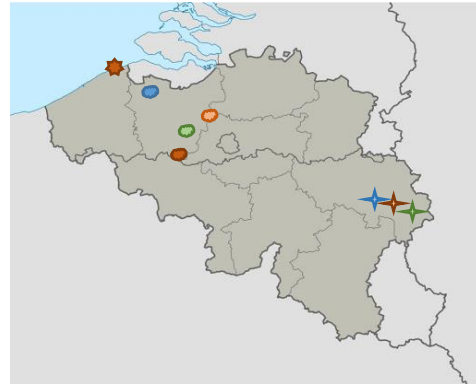
➔ The Belgian national reference center conducted a study, by doing a **cartography of Belgian water points** for screening the presence of ***Vibrio* spp.** in a few selected points to evaluate its possible impact on public health.

# Material and methods

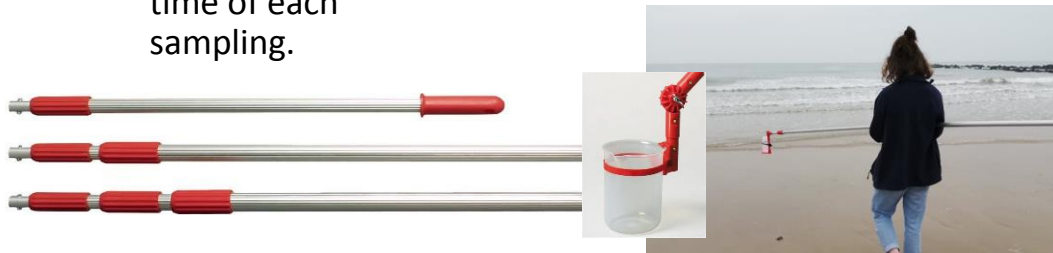
## Sampling

- According to recent clinical cases of vibriosis and to the distribution of recreational water locations, **8 areas were selected in Wallonia and Flanders including the North sea.**

- Boerekreek 9982
- Donkvijers 9700
- Blaarmeersen 9000
- Donk lake 9290
- Knokke-Heist 8300
- Butgenbach lake 4750
- Robertville lake 4950
- Warfaaz lake 4900

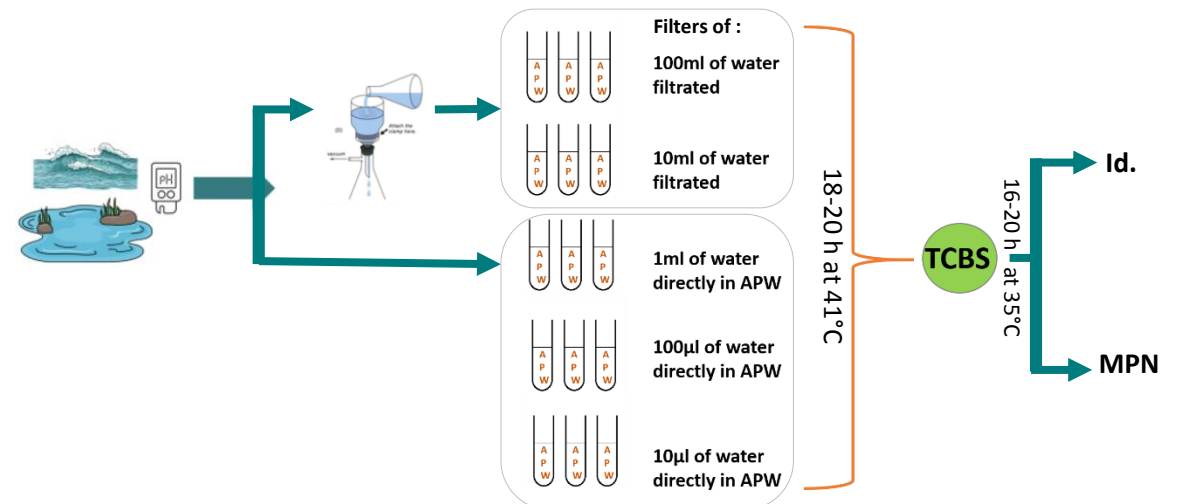


- Sampling** of water was done at each site once per month **between May and September 2021.**
  - Use of a telescopic device for collection of 1 litre sample
  - Water poured in sterile bottle, transported on ice and kept at 4°C until analysis within 24 hours.
  - Temperature and pH of water measured and recorded** at time of each sampling.

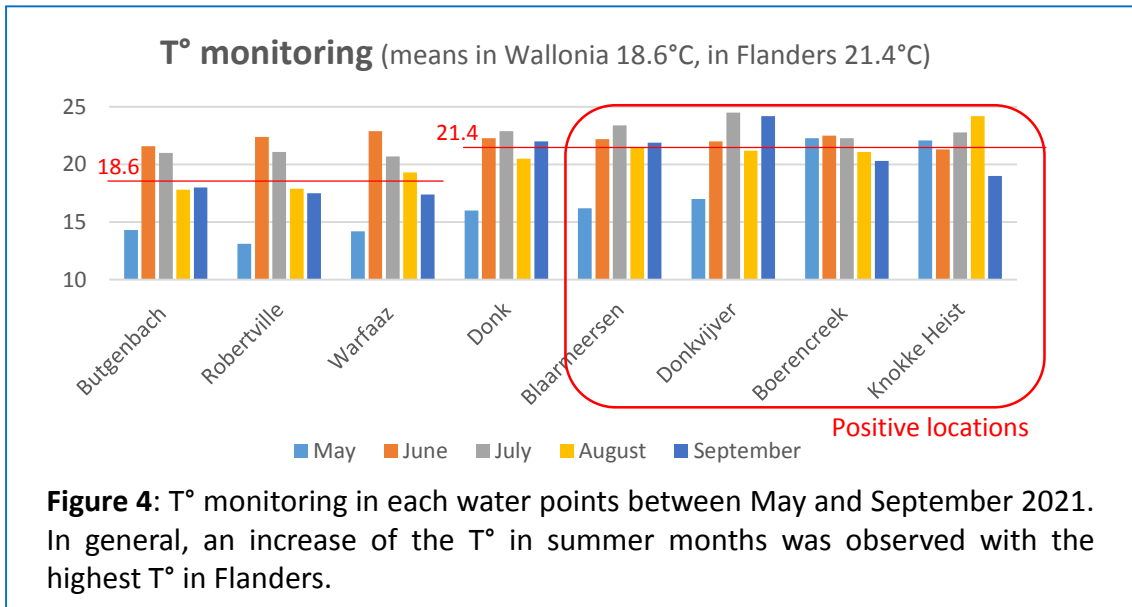
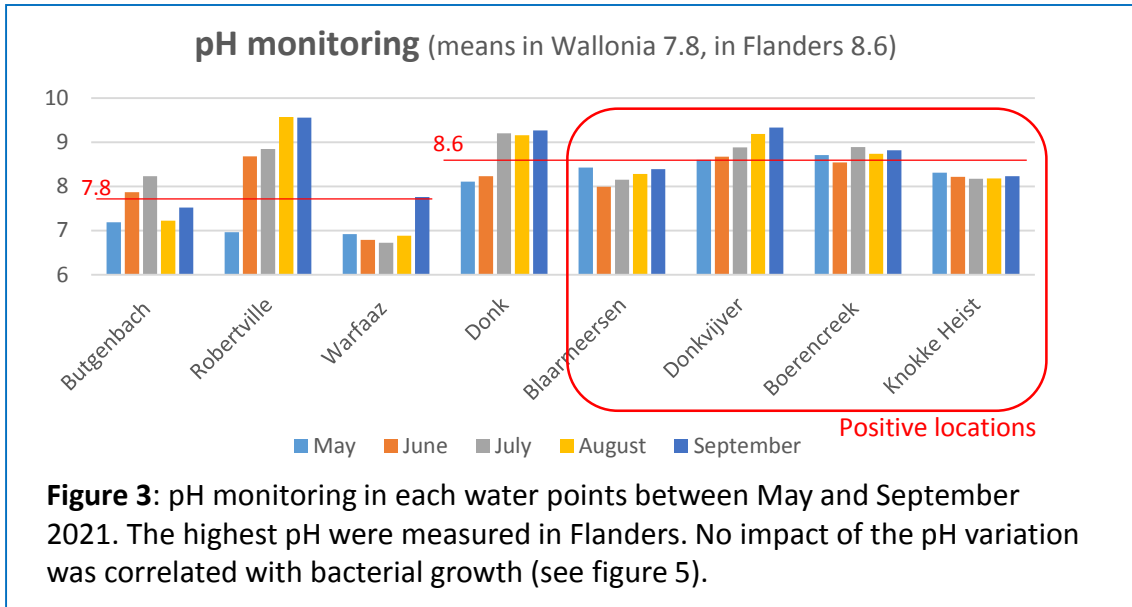


## Culture method

- Most Probable Number (MPN) culture method:** serial dilutions in alkaline peptone water (APW)
  - Upon reception in the laboratory, for each sample of water, in triplicate:
    - 10 mL and 100 mL were filtered (0.45 μ) and filters were inoculated in 50 mL of APW.
    - 10 μL, 100 μL and 1 mL were inoculated in 9 mL of APW
    - All inoculated APW were incubated 18-20h at 41°C and further sub-cultured (10 μL) on thiosulfate citrate bile saccharose agar medium (TCBS), then incubated 16-20h at 35°C.
- Identification and estimated quantification of *Vibrio spp***
  - Identification** of growing colonies on TCBS by MALDI-TOF mass spectrometry + agglutination and PCR for *V.cholerae*.
  - The positive TCBS plates originating from the serial dilutions in APW, allowed the estimation of the concentration of *Vibrio spp* in the different samples according to **MPN** interpretation.



# Results



	Blaarmeersen	Domaine de Donk	Boerecreek	Sea (Knokke)
May	/	110CFU/ml	7,5 CFU/ml	/
June	2.3CFU/ml	/	> 110 CFU/ml	110 CFU/ml
July	/	/	210 CFU/ml	110 CFU/ml
August	/	46CFU/ml	1,100 CFU/ml	> 11,000 CFU/ml
Septembre	/	/	460 CFU/ml	> 110 CFU/ml

Figure 5: Estimation of the concentration of *Vibrio cholerae* (non-01, non-0139) and *Vibrio spp* (MPN) in four water points in Flanders. No *Vibrio spp.* were detected in the Wallonian lakes. In the North sea, the monthly concentration of *Vibrio spp.* seemed to be correlated with an increase of the water temperature (see figure 4) as for example, in June (21,3°C) the *Vibrio cholerae* concentration was evaluated at 110 CFU/ml while in August (24.2°C), the concentration reached >11.000 CFU /ml.

	Butgenbach lake	Robertville lake	Warfaaz lake	Donk lake	Blaarmeersen	Donkviijers lake	Boerecreek	Knokke-Heist
May	/	/	/	/	/	<i>V.cholerae</i>	<i>V. cholerae</i>	/
June	/	/	/	/	/	/	<i>V. cholerae</i>	<i>V. cholerae</i> <i>V. alginolyticus</i>
July	/	/	/	/	<i>V. cholerae</i>	/	<i>V. cholerae</i>	<i>V. cholerae</i> <i>V. alginolyticus</i> <i>V. parahaemolyticus</i>
August	/	/	/	/	/	<i>V.cholerae</i>	<i>V. cholerae</i>	<i>V. cholerae</i> <i>V. alginolyticus</i> <i>V. parahaemolyticus</i>
September				/	/	/	<i>V. cholerae</i>	<i>V. cholerae</i> <i>V. alginolyticus</i>

Figure 6: Results of culture on TCBS and Mald-Tof MS identification for positive cultures. *Vibrio cholerae* (non-01, non-0139) was found in three lakes in Flanders and in the North sea. Other *Vibrio spp.* as *V. alginolyticus* and *V. parahaemolyticus* were also found in the North sea.



# Conclusion

- Our study demonstrate the presence of *Vibrio cholerae* (non-O1, non 0-139) and *Vibrio spp.* at concentrations able to cause human infections in different water points mostly in the North of Belgium.
- Mean temperatures and pH were higher in Flemish selected locations than in Walloon selected lakes. They can be favourable factors for the growth of *Vibrio spp.* Other factors such as salinity should be also included in future surveillance.
- This study supports the recommendation to include *Vibrio spp.* in water quality controls in order to define if water recreational activities are harmless for humans in Belgium.

