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Impact of meteorological factors on *Legionella* colonisation of water systems and the incidence of Legionnaires' disease

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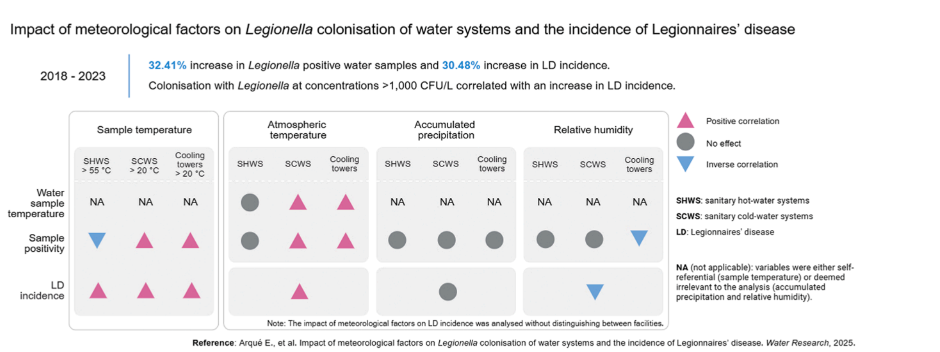
HIGHLIGHTS

- Atmospheric temperatures correlate with cold water system's *Legionella* positivity.
- Colonisations of *Legionella* at > 1000 CFU/L correlate with LD incidence.
- Atmospheric temperatures correlate with LD incidence.
- Relative humidity inversely correlates with LD incidence.
- No correlation was found between accumulated precipitation and LD incidence.

GRAPHICAL ABSTRACT



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ABSTRACT

Climate change will inevitably have consequences for the distribution and activity of infectious diseases. A clear example of this is Legionnaires' disease (LD), a pneumonia caused by the waterborne pathogen *Legionella* when inhaled from the environment in aerosol form. LD presents a seasonal pattern, with peaks in infection occurring during warmer periods of the year. Coincidentally, some man-made water systems easily reach the warm temperatures necessary for multiplication. In the present study, we analysed relationships between the temperature of the water in water samples, atmospheric temperature, accumulated precipitation and relative humidity on the one hand, and the presence of *Legionella* in water samples as well as LD incidence for the region of Catalonia

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during the years 2018–2023. To our knowledge, the present study is the first to assess the correlation between climatic factors and *Legionella* positivity in water systems using samples form routine testing. All LD cases reported to the Catalonia Public Health Agency during the study period were included. To analyse relationships between the variables, a maximum lag of 5 months was considered, and the estimated values of the cross-correlation function were plotted. The main findings from this study include evidence of an association between both atmospheric temperature and the temperature of the water in sanitary cold-water systems and cooling towers on the one hand, and sample positivity for *Legionella* on the other. We found that atmospheric temperature was also correlated with LD incidence. No correlation was found between accumulated precipitation and sample positivity or LD incidence, while the average relative humidity showed an inverse correlation with LD incidence. These results suggest that in Catalonia, meteorological factors are associated with the colonisation of water systems by *Legionella* and LD incidence. This study highlights that as climate change advances, an increase in LD incidence is likely to be observed.

1. Introduction

Climate change is having a major impact on natural ecosystems and therefore has the potential to impact human health. Progressive climate change can not only reduce water quantity and quality but also favour the activity and spread of microbial species in aquatic environments, including those that cause infectious diseases (Walker, 2018). An increase in pathogen levels in water bodies, colonisation by new pathogens and possible changes in pathogen properties will increase the incidence of waterborne infections (Dupke et al., 2023). Out of the 375 infectious diseases documented to have impacted humanity on a large scale, 58 % are believed to be aggravated by climatic hazards (Mora et al., 2022).

Climate change may also facilitate contact between people and waterborne pathogens as the leisure pastimes of the population move increasingly towards recreational water-related activities (Dupke et al., 2023; Mora et al., 2022). Furthermore, as populations struggle to maintain cool temperatures in buildings, the use of air cooling systems, fountains, water misters, and green infrastructure is likely to surge. If not appropriately managed, these cooling methods could become additional sources of exposure to pathogens.

As a waterborne microorganism, *Legionella* is bound to be impacted by climate change (Dupke et al., 2023; Walker, 2018). *Legionella* is an intracellular bacterium that is ubiquitous in natural aquatic environments as well as man-made water systems. Temperatures ranging from 25 °C to 42 °C are considered favourable for the bacterium's growth (Legionnaires' disease Annual Epidemiological Report for 2021, 2023), and man-made cold water systems easily reach such warm temperatures. Consequently, humans are at greater risk of *Legionella* infection in urban settings than in natural environments (Yao et al., 2024).

Infection takes place via inhaling aerosols from water sources harbouring *Legionella* (e.g. cooling towers, evaporative condensers, humidifiers, decorative fountains, hot tubs or showers) (Legionnaires' disease Annual Epidemiological Report for 2021, 2023). Given that no confirmed person-to-person infections have been reported, the environment is clearly the main source of infection. Infection can develop into either a mild Pontiac fever or Legionnaires' disease (LD), the latter being, in some countries, a reportable infection that presents in the form of pneumonia. According to the latest reports, in 2023 the European Union saw a total of 3.02 notifications of confirmed LD cases per 100, 000 population, the highest rate of any year under surveillance, and a 31.46 % increase compared to the previous year. Italy, France, Spain and Germany alone reported 72.49 % of the cases (Surveillance Atlas of Infectious Diseases, 2025).

LD peaks seasonally during the warmest seasons (Fisman et al., 2005; Legionnaires' disease Annual Epidemiological Report for 2021, 2023; Montagna et al., 2023; Walker, 2018). Many studies have shown that LD incidence correlates with various weather-related factors (Beauté et al., 2016; Halsby et al., 2014; Han, 2021). Among them, high temperature and precipitation, and more likely a combination of both (Beauté et al., 2016; Chen et al., 2014; Dupke et al., 2023; Han, 2021; Simmering et al., 2017).

As summers become longer, the greater duration of warmth is likely to lead to an increase in average water temperatures both in the natural environment and in man-made water systems, providing more optimal conditions for *Legionella* growth. As high temperatures extend into spring and autumn, it is expected that more cases of LD will be reported in these seasons (Dupke et al., 2023; Walker, 2018). Furthermore, recreational water-related activities and the use of evaporative cooling systems like cooling towers, which are the most well-known sources of LD outbreaks, will also be prolonged. This in turn is likely to raise the number of LD cases in some countries (Walker, 2018).

Although there exists research examining the correlation between LD incidence and the weather, little information is available about the impact of weather conditions on man-made water systems and their colonisation by *Legionella.* Nonetheless, it is of great importance to understand how *Legionella* colonisation in such systems react to seasonal changes in the weather, especially in those systems that are more exposed to atmospheric conditions. *Legionella* levels and phenotype patterns also play an important role in water system colonisation. In Spain, *Legionella* control is regulated by law and the different *Legionella* levels found in a water system determine the intensity of the measures to be taken (Real Decreto 487/2022 of June 21st, 2022). Furthermore, certain *Legionella* phenotypes are more reported than others for culture-confirmed cases of LD (Legionnaires' disease Annual Epidemiological Report for 2021, 2023). The aim of this study was therefore to analyse potential relation between atmospheric temperatures, accumulated precipitation and relative humidity and the presence of *Legionella* in man-made water systems in Catalonia, Spain, during the years 2018 to 2023, as well as to analyse potential relation of these weather conditions with the incidence rates of LD in this region during the same period. Additionally, we evaluate the possible impact of these atmospheric conditions on phenotyping patterns and levels of *Legionella* in man-made water systems.

2. Methods

2.1. Water sampling and analysis

Since 2003, Spain has had measures in place to prevent and control outbreaks of LD, although the first guidelines were developed in the 1980s. Current Spanish legislation states that all water facilities that create aerosols and are thus susceptible of becoming sources of human exposure to *Legionella* must comply with a certain public safety regulations and procedures. The data for all the water samples included in this study were obtained as part of these mandatory procedures, which include the regular testing of water samples (Real Decreto 614/2024 of July 2nd, 2024; Real Decreto 487/2022 of June 21st, 2022; Real Decreto 865/2003 of July 4th, 2003). The samples were collected and analysed during the period from January 2018 to December 2023 by an accredited laboratory in Catalonia, Spain. Both sampling and analysis were performed as stipulated by the EN ISO norm 11,731 and methods of analysis were not modified in any way during the study period. Water sample data for the year 2020 were not included because during this time many of the water systems that are required to be tested were not in use due to the Covid-19 pandemic. If data from these samples had been included, since the water in the systems had remained stagnant for longer than usual, this would lead to atypically high values for the proportion of *Legionella* positive samples.

Each water sample was characterised by the sampling date, the average temperature of the sample, the type of facility from which it was taken and the results of testing for the presence of *Legionella* spp. As an exception, the average sample temperature in hot-water systems was measured once the temperature had stabilised after flushing. If *Legionella* was detected, parameters such as *Legionella* levels determined by plate count and the specific microorganisms present (*L. pneumophila* serogroup 1, *L. pneumophila* serogroups 2–14 and *Legionella* species) were determined as stipulated by the EN ISO norm 11,731.

The facilities comprise: sanitary hot-water systems (SHWS), sanitary cold-water systems (SCWS), cooling towers, recreational waters (e.g. swimming pools, jacuzzies and other types of baths), the water tanks of road sweeping vehicles, high aerosol producers (e.g., sprinkler systems, car washing hoses or decorative water fountains) and firefighting systems. The remaining uncategorised samples were defined as coming from "other facilities".

2.2. Reported LD cases

The Catalonia Public Health Agency (Agència de Salut Pública de Catalunya, ASPCAT) is responsible for gathering annual case data for all reportable infectious diseases, including LD, throughout Catalonia. All LD cases reported to the ASPCAT from 2018 to 2023 were included in this study.

2.3. Climatic data

The meteorological variables considered were the average atmospheric temperature, accumulated precipitation and relative humidity in Catalonia, aggregated on a monthly basis. These data were obtained from the "Open Data Catalogue of the Catalan Government" and the Meteorological Service of Catalonia (Servei Meteorològic de Catalunya).

2.4. Statistical analysis

Categorical variables were described by the frequency and percentage of each category. Continuous variables were described in terms of mean and standard deviation or median and interquartile range, depending on the distribution of the variables.

All time series analysed in the present study were represented graphically by month. Additionally, sample positivity rates (%) were classified by facility type and were plotted yearly.

To study the correlation between pairs of temporal series, a cross-correlation analysis was performed, considering a maximum lag of 5 months. The estimated values of the cross-correlation function (CCF) were plotted. A horizontal line was included to indicate values beyond which the cross-correlations are statistically significantly different from zero. For those lags with a significant CCF, the values of the pair of time series in their corresponding lags were plotted on a scatter plot to examine their association. LOESS regression was performed to visualize this association, and the estimated smooth curve was also plotted.

We applied this procedure to assess the cross-correlations between (i) sample positivity and LD incidence, considering lags in both directions, and (ii) each meteorological variable and both sample positivity and LD incidence, considering only lags of the meteorological variables.

The conditions of use of the models were validated and, whenever possible, confidence intervals at 95 % were calculated. All analyses were performed with the statistical program R version 4.4.0 (2024–04–24) for Windows.

3. Results

3.1. Descriptive data and temporal series

3.1.1. Sample classification by facility type

A total of 32,179 water samples gathered over the six-year study period were included in the study. Most of these samples were taken from SHWS (48.56 %), cooling towers (18.92 %) and SCWS (14.18 %). Recreational waters, road sweepers, high aerosol producers, firefighting systems and other facilities represented the remaining 18.34 % of the total samples.

3.1.2. Legionella positivity for each facility type

Over the course of the study period, the rate of *Legionella* positivity in routine analyses significantly increased by 32.41 %, from 6.51 % positivity in 2018 to 8.62 % in 2023 (2-sample test for equality of proportions with continuity correction with 95 % CI of 1.2 %, 3.0 % and p-value of <0.001). In SCWS, cooling towers and high aerosol producers the increasing trend was more pronounced (see Fig. 1).

3.1.3. Legionella levels in positive samples

The *Legionella* levels in positive samples also varied over the study period. Low levels (≤100 CFU/L) increased from 19.94 % in 2018 to 30.44 % in 2023. In turn, high levels (>1000 CFU/L) decreased from 49.86 % to 41.46 %. Intermediate levels (100 to 1000 CFU/L) remained constant, with 30.20 % and 28.10 % in 2018 and 2023, respectively (see Supplementary Table 1).

3.1.4. Legionella phenotypes in positive samples

The most prevalent *Legionella* phenotypes in our samples were *L. pneumophila* serogroups 2–14 (49.84 %) and serogroup 1 (38.12 %), well above other *Legionella spp.* (12.04 %). These proportions did not vary much between 2018 and 2023, with a 3 %−4 % difference in each phenotype.

3.1.5. Water temperature and sample positivity

SHWS did not show much variability in water temperature, with an average between 52 °C and 60 °C. Contrarily, SCWS and other facilities presented an average water temperature ranging from 15 °C to 29 °C. This variation seems to follow the same pattern as atmospheric temperatures, which ranged from a daily average of 5 °C to 25 °C (see Fig. 2).

Table 1 shows data for the relationship between water temperature and the presence of *Legionella* in water samples. *Legionella* positivity of samples taken from SHWS dropped dramatically when the water temperature exceeded 60 °C. For SCWS, positivity gradually increased as the water temperature approached values ideal for bacterial growth (25 °C to 42 °C (Surveillance Atlas of Infectious Diseases, 2025)).

3.1.6. LD cases in Catalonia between 2018 and 2023

LD cases in Catalonia rose over the study period from 5.74 to 7.49 cases per 100.000 population in 2018 and 2023, respectively, representing a 30.49 % increase. This percentage is almost identical to the increase in *Legionella* positivity in samples taken from water systems over the same period.

3.1.7. Atmospheric variables

The temporal series shown in Fig. 3 for LD incidence (3A) seems to display a seasonal fluctuation pattern. LD incidence usually peaks two months after summer atmospheric temperatures (3D) peak.

Comparing series 3A and 3E, there is no apparent association between LD cases and rainfall, but Catalonia suffered a drought during part of the study period, lasting from 2021 to 2024.

The mean relative humidity in Catalonia during the study period was 70.73 %, with a maximum >85 % and a minimum <55 %. Relative humidity presents an inverted pattern compared to atmospheric

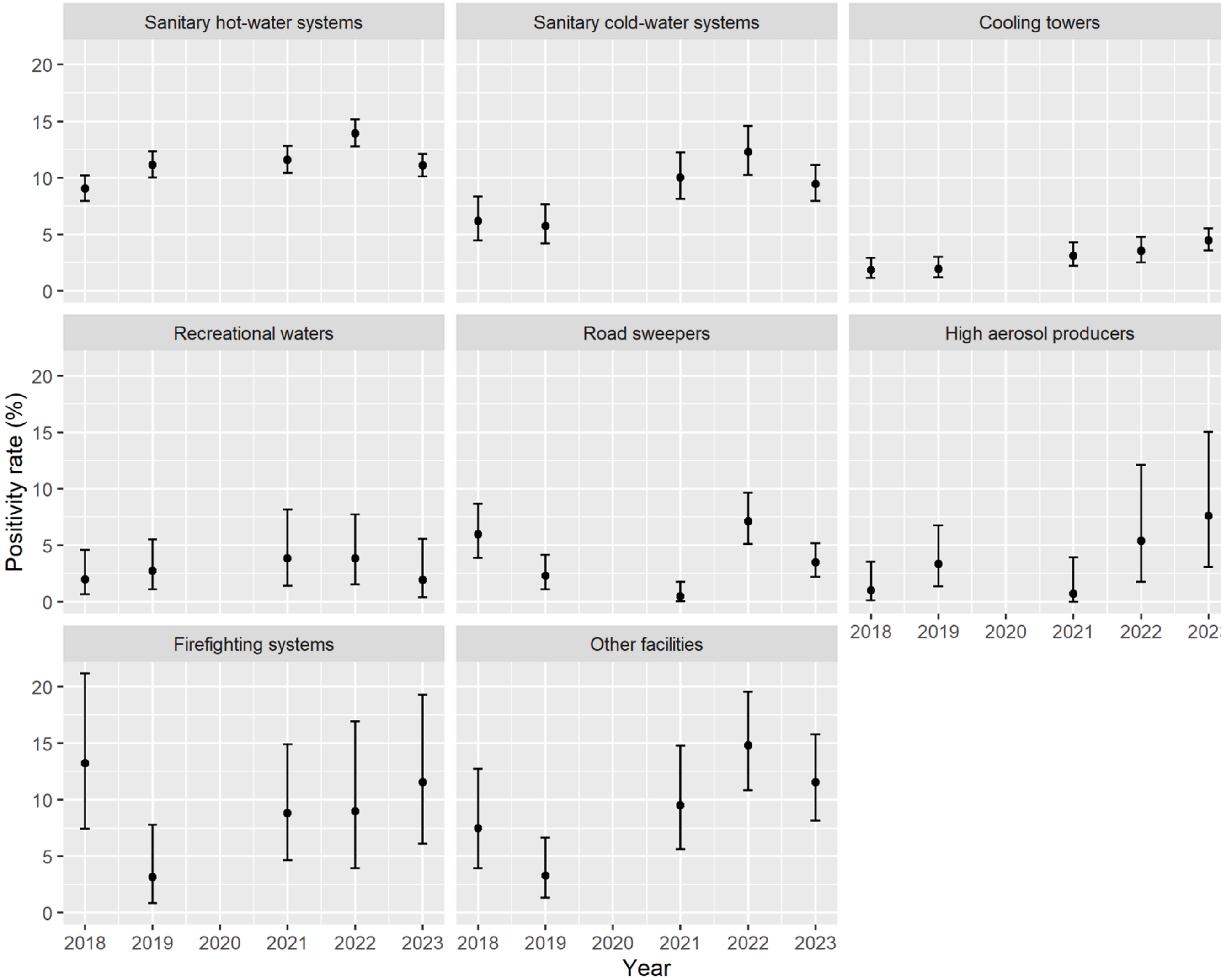

Fig. 1. Full temporal series of the proportion of *Legionella* positive samples (%) classified by facility type.

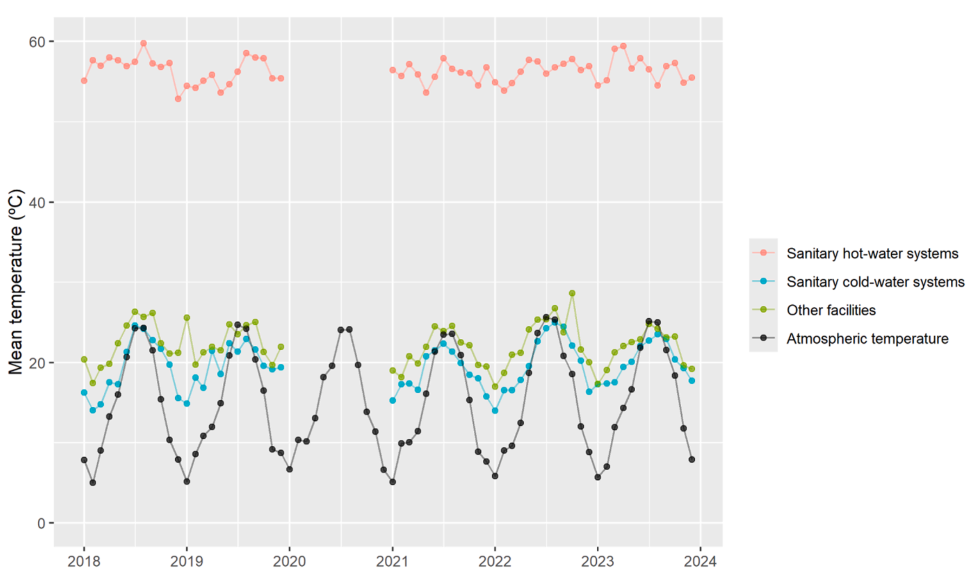

Fig. 2. Temporal series of the monthly average atmospheric temperature in Catalonia and monthly average temperature of water in water systems over the study period.

Table 1
Positivity for *Legionella* and type of *Legionella* present in water samples taken from SHWS and SCWS, split into several temperature ranges.

SHWS	< 50 °C *N* = 2270	50 °C-55 °C *N* = 2605	55 °C-60 °C *N* = 4205	> 60 °C *N* = 6366
***Legionella* positivity**	374 (16.48 %)	536 (20.58 %)	552 (13.13 %)	307 (4.82 %)
***Legionella* phenotype**				
Serogroup 1	138 (38.66 %)	209 (40.50 %)	141 (25.78 %)	101 (33.44 %)
Serogroups 2–14	155 (43.42 %)	275 (53.29 %)	369 (67.46 %)	154 (50.99 %)
L. species	64 (17.93 %)	32 (6.20 %)	37 (6.76 %)	47 (15.56 %)
SCWS	**< 20 °C*N*** = 2430	**20 °C-25 °C*N*** = 1249	**25 °C-30 °C*N*** = 586	**> 30 °C*N*** = 91
***Legionella* positivity**	169 (6.95 %)	127 (10.17 %)	84 (14.33 %)	15 (16.48 %)
***Legionella* phenotype**				
Serogroup 1	70 (42.42 %)	58 (46.40 %)	28 (35.00 %)	8 (61.54 %)
Serogroups 2–14	72 (43.64 %)	52 (41.60 %)	43 (53.75 %)	4 (30.77 %)
L. species	23 (13.94 %)	15 (12.00 %)	9 (11.25 %)	1 (7.69 %)

temperatures, with increased humidity in colder months.

When data on these weather variables are analysed as a whole, there does not seem to be any apparent relationship between the atmospheric temperatures recorded and the positivity of samples. It is important to note that SHWS, which accounted for 48.56 % of all samples, cannot be impacted by atmospheric temperature since they are designed to generate their own heat and maintain water in the system within a particular temperature range. When SHWS are excluded, as shown in series 3C, sample positivity tended to rise in warmer months, while in colder months it diminished.

3.2. Statistical analysis

3.2.1. Correlation between water sample positivity and LD incidence in Catalonia

As seen in Fig. 4, no correlation was found between water sample positivity and the incidence of LD cases in Catalonia during the study period. However, colonisations in levels higher than 1000 CFU/L did positively correlate with an increase in LD cases after two months. This relationship of positivity and levels with the LD rate after two months is plotted in the Supplementary Figure A. When the water sample data was limited to those that were positive for *L. pneumophila* serogroup 1 in levels higher than 1000 CFU/L, no correlation was found with LD

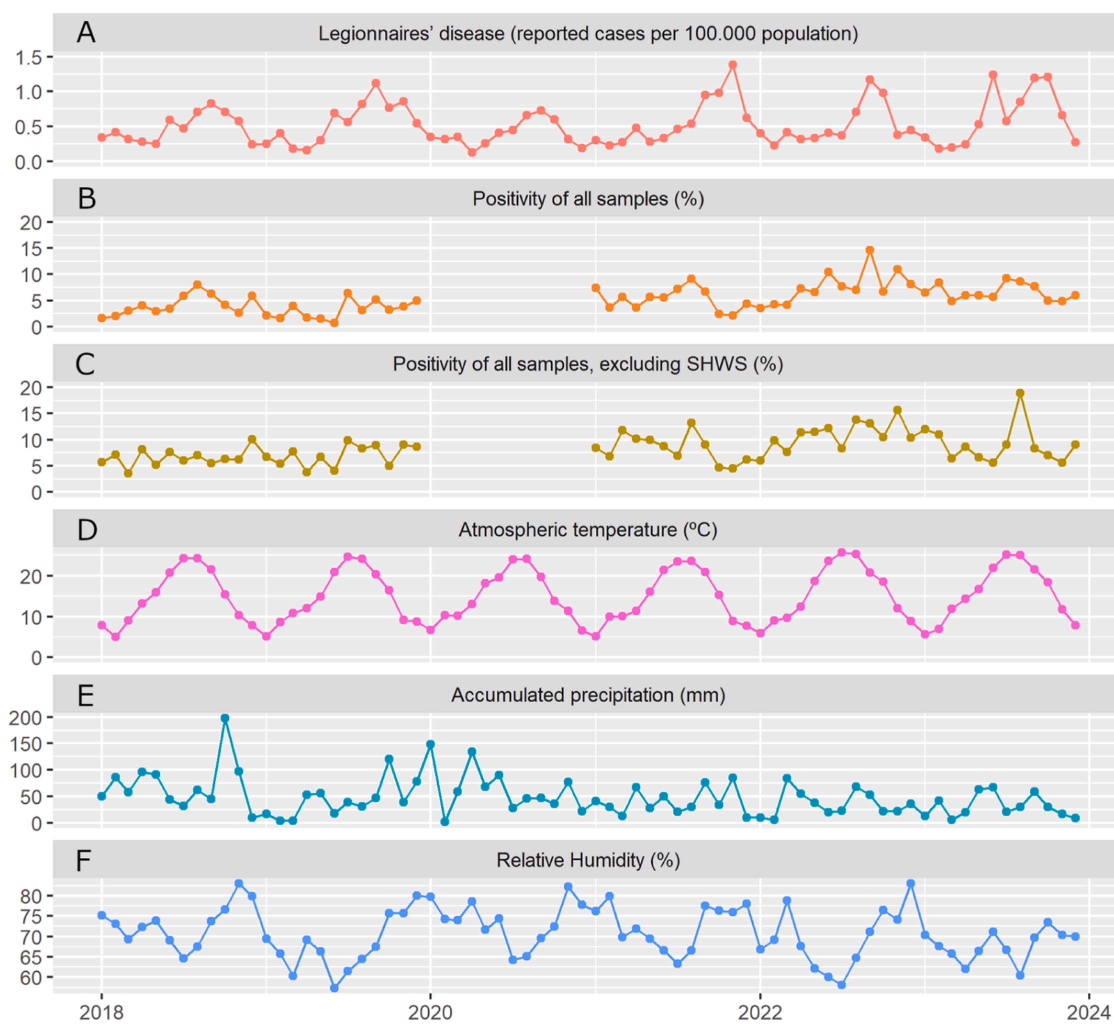

Fig. 3. Temporal series showing LD incidence, *Legionella* positivity of water samples, and meteorological variables over the study period in Catalonia.

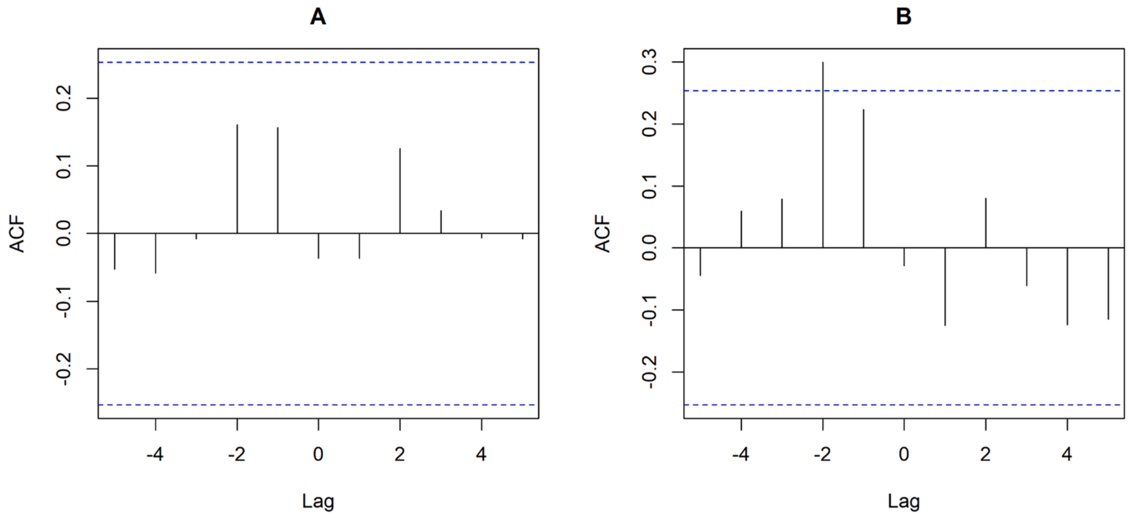

Fig. 4. CCF of sample positivity vs. LD incidence in Catalonia. Graph A shows results for all positive samples and B shows results for all positive samples at levels >1000 CFU/L.

incidence (Supplementary Figure B). However, there was a correlation when the phenotype was *L. pneumophila* serogroups 2–14 in levels above 1000 CFU/L (Supplementary Figures B and C)

3.2.2. Correlation of sample positivity with water temperature and meteorological variables

Statistical analysis of data from facilities other than SHWS, SCWS, and cooling towers was not possible due to an insufficient number of samples.

As can be observed in Fig. 5A, an increase in water temperature correlates with a decrease of positivity in the case of SHWS in the same month (see supplementary Figure D).

We observe the opposite with SCWS and cooling towers. There is a positive correlation between water temperature and positivity, in the same month or a month after for SCWS, and in the same month and up to two months after for cooling towers (see supplementary Figures E and F).

When sample positivity in SHWS and atmospheric temperature were tested for correlations (Fig. 5B), none was found. Atmospheric temperatures measurements never reached the lowest average temperature detected at this facility type, which was 51 °C.

Since the water in SCWS and cooling towers is unheated, it is presumably heavily associated with the atmospheric temperatures. This is consistent with our data, which showed that for SCWS, an increase in atmospheric temperature is associated to an increase of positivity in the same month and a month after, while for cooling towers this correlation happened in the same month and up to two months after (Fig. 5B and Supplementary Figs. G and H).

The average positivity rate during the study period was 11.45 % for SHWS and 9.07 % for SCWS. For SHWS, the average positivity rate in the coldest and warmest months was 12 % and 13 %, respectively. For SCWS, these average positivity rates were 6 % and 12 %, respectively. These differences observed were not statistically significant.

As shown in Fig. 5C, average rainfall did not correlate with sample positivity. Although statistical analysis shows an apparently negative correlation between positivity in cooling towers and accumulated precipitation after a month, when plotting these values on a scatter plot, it can be observed how this association is forced by a single month when higher accumulated precipitation and lower sample positivity than usual happened simultaneously (see Supplementary Figure I).

Relative humidity showed correlation with sample positivity only in cooling towers, where an increase in relative humidity correlated with a decrease in positivity in the same month and up to two months after (Fig. 5D and Supplementary Figure J).

3.2.3. Correlation of LD with sample temperature and meteorological variables

There was a positive correlation between water temperature in SHWS, SCWS and cooling towers and LD incidence (Fig. 6A and Supplementary Figures K, L and M). Atmospheric temperature also correlated positively with LD incidence (Fig. 6B and Supplementary Figure N), but accumulated precipitation did not (Fig. 6C). Finally, there was an inverse correlation between relative humidity and LD incidence (Fig. 6D and Supplementary Figure O).

4. Discussion

Our study suggests a correlation between LD incidence and the weather, specifically atmospheric temperatures. This is probably because higher atmospheric temperatures cause higher water temperatures which can favour *Legionella* colonisation and thus higher LD incidence. A few other studies have investigated the relationship between the climate and *Legionella* colonisation in man-made water systems. However, they evaluated facilities that were being investigated for LD incidents, so their results could not be generalised to such facilities as whole, including water systems with no reported connection to LD incidents (Barna et al., 2016; Buchholz et al., 2020; Montagna et al., 2023). Therefore, to our knowledge, the present study is the first to analyse the correlation between meteorological variables and *Legionella* colonisation of water systems in general using data from routine water sample testing. Moreover, the correlation between water sample data, and not only meteorological variables, and LD incidence was examined here as well.

4.1. Sample positivity and phenotypes

An increasing trend in the *Legionella* positivity of water samples was observed at all facilities during the study period. This correlated with an increase in LD incidence in Catalonia over the same period. Although LD tends to be an underdiagnosed and underreported infection, a similar increase in incidence has been reported for Europe and the United States in recent years (Han, 2021; Legionnaires' disease Annual Epidemiological Report for 2021, 2023).

Although our data show that the proportion of *Legionella*-positive water samples has increased in recent years, samples with low levels of the bacteria have increased while positive samples with higher levels

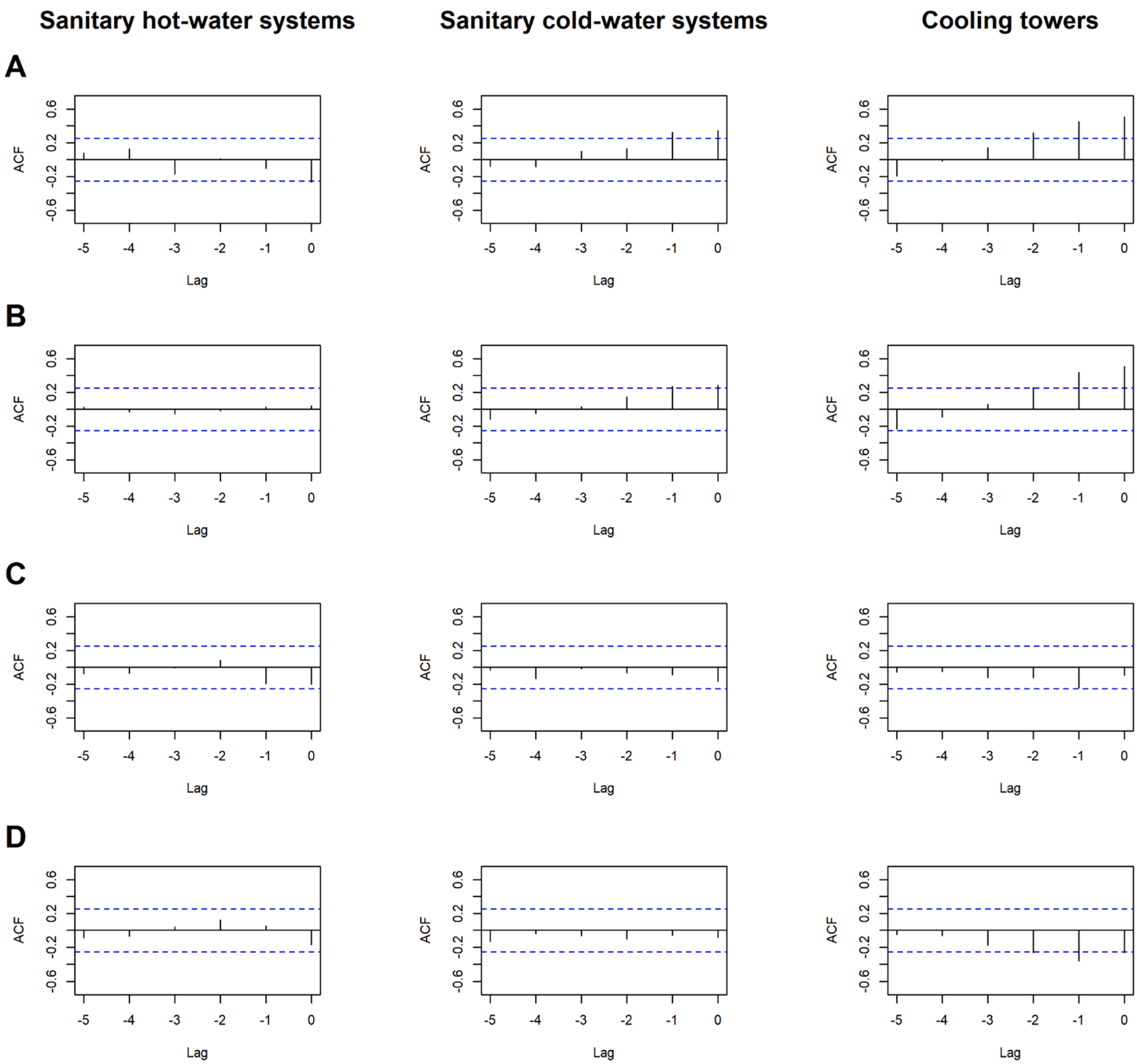

Fig. 5. CCF of sample positivity in different types of water systems with water temperature (row A), atmospheric temperature (row B), accumulated precipitation (row C), and relative humidity (row D).

have diminished. This apparent contradiction can perhaps be explained by the fact that recent legislation in Spain has brought about stricter monitoring of water systems where *Legionella* colonisation is likely to occur and facility managers are more likely to perform preventive treatments in water systems. However, some shock treatments, mainly hyperchlorination and high-temperature treatments, do not eradicate *Legionella* completely. This means that the positivity is not reduced, although the levels are (Li et al., 2014; Párraga-Niño et al., 2024).

L. pneumophila serogroup 1 was strongly represented in the water systems colonisations tested here, accounting for 38.22 % of all positive samples. Research shows that *L. pneumophila* alone accounts for 90 % of LD cases worldwide, with *L. pneumophila* serogroup 1 being the main source of infection in Europe, representing 82 % of all confirmed cases in 2021 (Legionnaires' disease Annual Epidemiological Report for 2021, 2023; Viasus et al., 2022). However, this fact should be treated with caution since *Legionella* urinary antigen (LUA) tests, which are the most widely used diagnostic tools for LD, can only detect *L. pneumophila* serogroup 1 infections, meaning that overall *Legionella* infections may well be severely underdiagnosed. Greater efforts should be directed to the implementation of more specific diagnostic tools for LD, especially since *L. pneumophila* serogroups 2–14 are the most prevalent in our studied environmental samples (49.84 %) and *L. pneumophila* serogroup 1 may be overestimated in current assessments of LD (Muyldermans et al., 2020). Indeed, a retrospective study by Wikén et al. mentions that in a ten-year period, 30 % of all LD infections were caused by serogroups 2–14. Furthermore, Wikén's study mentions that species specific PCR techniques are necessary to establish diagnosis, emphasising the importance of not relying solely on LUA tests (Wikén et al., 2025).

The correlation seen between colonisation of *L. pneumophila* serogroups 2–14 at levels > 1000 CFU/L with LD incidence but not seen with colonisation of *L. pneumophila* serogroup 1 at the same levels, poses a few questions. If LD diagnosis mainly detects infections caused by *L. pneumophila* serogroup 1, correlation should be seen with this phenotype. However, a possible theory is that detection of

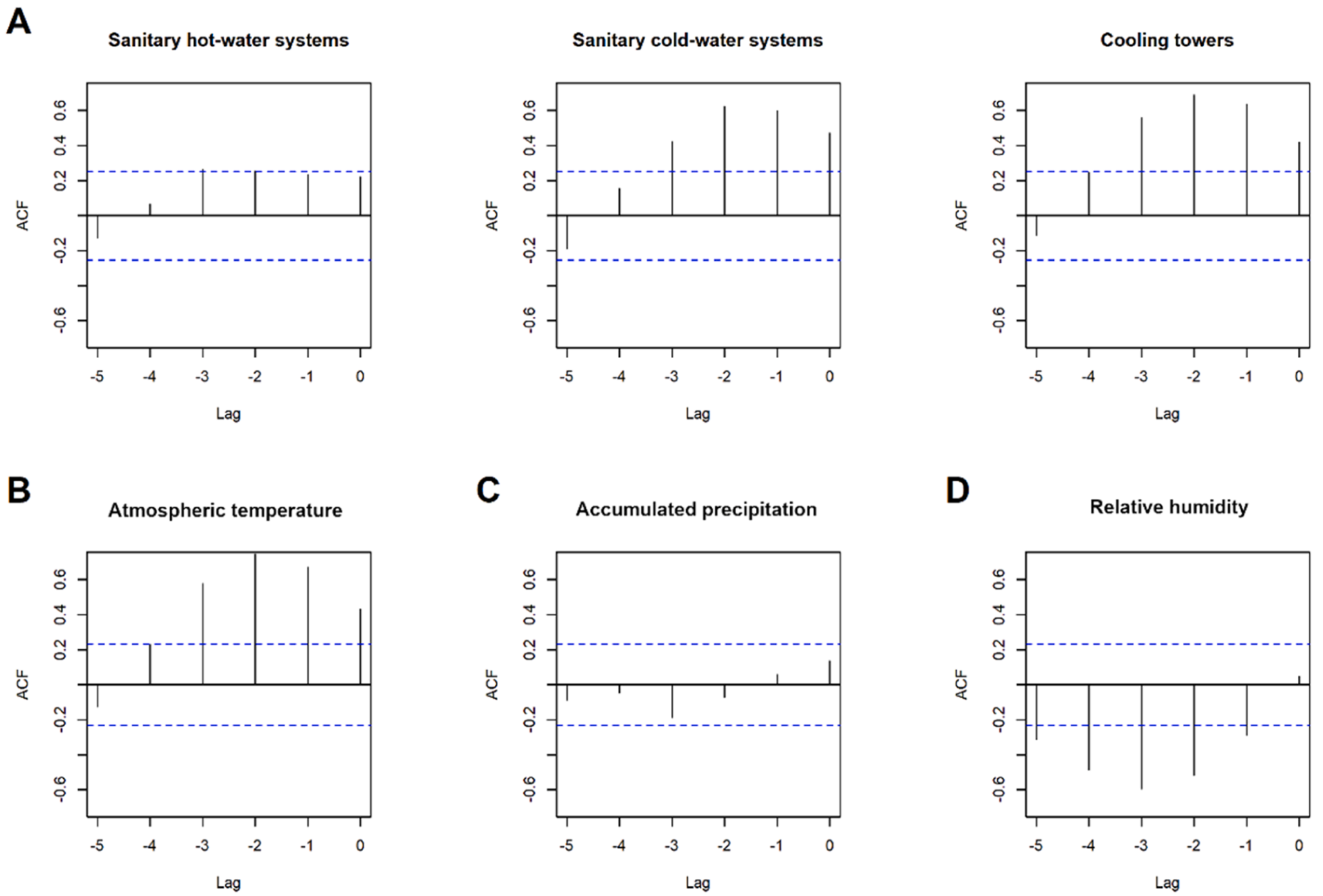

Fig. 6. CCF of LD incidence with sample temperature in different facilities and meteorological variables. The top row (A) shows LD incidence vs. water temperature in three types of water systems. The other graphs correspond to the correlation between LD incidence and atmospheric temperature (B), accumulated precipitation (C) and relative humidity (D) in Catalonia during the study period.

L. pneumophila serogroup 1 in water systems colonised by different serogroups might be more difficult due a lesser representation of the serogroup 1 phenotype in the sample (Messi et al., 2011). However, despite this discrepancy, large levels of *Legionella* are positively correlated with LD incidence after two months, which suggests that colonisation at high *Legionella* levels poses a greater risk of propagation than colonisation at lower levels. This finding aligns with Spanish legislation, which mandates that water systems with levels above 1000 CFU/L must implement stronger corrective measures (Real Decreto 487/2022 of June 21st, 2022).

4.2. *The effect of water temperature and atmospheric temperature*

Man-made water systems such as cooling towers and SHWS have been historically associated with a high risk of propagating *Legionella,* so legislation governing their use tends to be stricter. On one hand, Spanish legislation states that the facilities with sanitary water systems, must be sampled and tested on a quarterly basis for *Legionella*, whereas cooling towers must be tested every month (Real Decreto 614/2024 of July 2nd, 2024). Although the water samples from cooling towers in our data did not present high *Legionella* positivity, cooling towers are capable of producing contaminated aerosols that can travel several kilometres (Rebellato et al., 2023; Shivaji et al., 2014). These aerosols can reach large numbers of population and ultimately generate outbreaks, which explains why such facilities are subject to heightened monitoring. On the other hand, Spanish legislation considers SHWS and SCWS to be of unequal importance. For example, in a facility with 51 to 100 distal points, at least four hot water and two cold water distal points must be tested (Real Decreto 487/2022 of June 21st, 2022). Despite having similar annual positivity rates, SHWS' (11.45 %) are slightly higher than SCWS' (9.07 %), however, when taking into account the positivity during the coldest and warmest months, SCWS double their positivity (from 6 % to 12 %) in hot weather, reaching the same positivity rates found in SHWS, which are more stable during the year. Observing these results, a revision of the legislation that would assign the same importance to both systems should be considered.

The similar positivity rates in SHWS and SCWS also suggest that SHWS are not well protected by their high water temperatures. It is important to note that water temperature in SHWS is the maximum temperature reached by the system at the time it is sampled, and it is not indicative of the water's usual temperature, which tends to be lower, especially in sections of the system that are distant from the heating element (Barna et al., 2016). In fact, not all water samples complied with the regulations, which state that water at SHWS terminal points must reach 50 °C. A previous study by our group found that temperatures around 60 °C are needed to ensure a reduction in the viability of *Legionella* (Párraga-Niño et al., 2024). This coincides with the results obtained in the present study, which indicated a drop in positivity from a maximum of 20.25 % at temperatures lower than 60 °C to 4.82 % when the water showed temperatures above 60 °C. Furthermore, *Legionella* can survive in a viable but non-culturable (VBNC) state at temperatures ranging from 50 °C-70 °C (Cervero-Aragó et al., 2019; Párraga-Niño et al., 2024). In this state, bacteria will not grow on agar plates (Li et al., 2014), which becomes a problem given that environmental analyses are necessarily performed by plate count (Real Decreto 487/2022 of June 21st, 2022). This poses a huge public health risk since bacteria in a

VBNC state can regain virulence when conditions become more favourable (Li et al., 2014). The average maximum temperatures of SHWS range from 52 °C to 60 °C, so it is highly likely that the water sample analyses are underestimating the real presence of *Legionella* in these water systems.

In general, *Legionella*'s ideal growth temperatures range from around 25 °C to 42 °C (Legionnaires' disease Annual Epidemiological Report for 2021, 2023). This is evidenced by the increasing positivity in SCWS as the water temperatures rise from less than 20 °C to more than 30 °C. This warming in cold-water systems coincides with an increase in atmospheric temperatures in warm months, indicating that the increase in water temperature up to ideal growth temperatures is directly associated with atmospheric temperature. SCWS, cooling towers and high aerosol producers are the facilities with a more evident increase in *Legionella* positivity. Coincidentally, these water systems are the most exposed to the weather. Statistical analysis of the association of high aerosol producers with atmospheric temperature was not possible in this study due to the insufficient number of samples; however, for SCWS and cooling towers, an increase in either atmospheric temperature or in water temperature is statistically correlated with a higher positivity of samples. This correlation is not always immediate, and it may come after one to two months which, in good conditions, is the time necessary for substantial bacterial growth to take place. Atmospheric temperatures do not correlate with the positivity of water samples from SHWS because these systems are subject to legal restrictions and the temperature of water in them is artificially controlled to reach above 50 °C, so in principle they cannot be influenced by the climate.

In our data, we observe peaks in sample positivity during the summer, which are followed, two to three months later, by peaks in LD incidence. Our results also show that atmospheric temperatures correlate directly with LD incidence, after a lag of 0 to 4 months, which is sufficient time for *Legionella* colonisations to increase in bacterial levels, become dispersed in aerosols, and cause new LD cases. The same correlation occurs between water temperature and LD incidence for SCWS and cooling towers, with a 0 to 3- or 4-month lag. For SHWS, despite a high water temperature correlating with a decrease in positivity, there is a positive correlation between water temperature and LD incidence, with a 2- to 3-month lag. A possible explanation is that in SHWS a negative result might be due not to the lack of *Legionella* colonisation but rather to the presence of the bacteria in VBNC state (Li et al., 2014). If the results of testing indicate that the facility is apparently not colonised, no corrective measures will be performed. However, since there could be viable *Legionella* in the water system but, in accordance with regulations, another analysis will not take place until three months later, this would allow *Legionella* to regain its pathogenic potential and infect a suitable host.

4.3. The effect of accumulated precipitation

According to other studies, precipitation in warmer weather is usually linked to increased LD incidence (Dupke et al., 2023; Fisman et al., 2005; Walker, 2018). Our study did not find any correlation between accumulated precipitation and LD incidence; however, the drought period experienced between 2021 and 2024 might have influenced the results.

In our results, average accumulated precipitation showed no correlation with either water sample positivity or LD incidence. Some studies point to rainfall as the most critical factor for LD occurrence, rather than increased temperature (Chen et al., 2014; Fisman et al., 2005). Han et al. even state that drought suppresses LD (Han, 2021). However, according to our study, despite the fact that Catalonia was experiencing a drought for the last three years of the study, LD cases, as well as the number of facilities colonised by *Legionella,* increased over that period.

4.4. The effect of relative humidity

It has been suggested by other studies that higher relative humidity would increase the risk of acquiring LD (Dupke et al., 2023; Fisman et al., 2005; Simmering et al., 2017). It has been described that *Legionella* strains survive better at relative humidity levels around 65 % (Walker, 2018). In Catalonia, the mean relative humidity is already high, at 70.73 %, so in order to reach ideal levels for bacterial survival, it would actually need to decrease, as our results suggest. Not many studies specify the relative humidity values in the regions evaluated, but Simmering et al. state that warm and humid weather with relative humidity above 80 % is a major risk factor for LD (Simmering et al., 2017). In Catalonia, relative humidity levels above 80 % are only reached in colder months, suggesting that atmospheric temperatures have a stronger association with LD incidence than relative humidity.

In our study, relative humidity was inversely correlated with sample positivity only in the case of cooling towers. The fact that this was true for only one type of facility suggests that perhaps this meteorological factor has a stronger association with the dispersion of aerosols than on *Legionella* colonisation in water systems. Given that the graphs in Fig. 3 for atmospheric temperatures (3D) and relative humidity (3F) show an inverse relationship, it may be theorised that the association of atmospheric temperature with sample positivity is stronger than the association with relative humidity. In support of this theory, Halsby et al. describe an association between relative humidity with LD, although the inclusion of other weather variables diminished it, implying that relative humidity exerts a weaker influence than other variables (Halsby et al., 2014).

4.5. Limitations and climatic context of Legionella dynamics

In this study we evaluated a five-year database of water sample test results. The first limitation derives from the fact that samples were not paired. However, since water testing took place at regular intervals over each year and our database comprises more than 30,000 samples, our data end up following a normal distribution.

Another limitation is that our data come from a single laboratory, so not all water system facilities in Catalonia were included. Additionally, the study period was not long enough to justify firm conclusions about the effect of the region's climate on *Legionella* colonisation or LD incidence. However, we feel that five years was sufficient to evaluate specific meteorological factors and seasonality patterns.

The combined effect of all meteorological variables was not studied, although such an approach helped assess inverse relationships between weather factors, such as atmospheric temperature and relative humidity. Furthermore, meteorological factors were analysed using average values for Catalonia, although the region encompasses diverse climatic conditions across its territories. Future studies should account for these climatic variations within the study area. Additionally, including the geographical location of the samples would allow for spatial epidemiological analyses.

A previously mentioned limitation is the fact that the amount of VBNC bacteria present in samples was not taken into account due to the analyses being performed by plate counts, as mandated by current Spanish legislation.

Finally, factors such as heightened awareness of LD in public health institutions in Catalonia, an increase in the number of aerosol-producing water systems, and possible changes in the characteristics of vulnerable populations were not assessed, although they could be variables that, together with meteorological factors, influenced LD incidence.

Studies have assessed the correlation of a wide variety of meteorological factors with LD incidence. An increase in LD cases has been linked to increased relative humidity (Dupke et al., 2023; Fisman et al., 2005; Simmering et al., 2017), increased precipitation (Beauté et al., 2016; Chen et al., 2014; Dupke et al., 2023; Fisman et al., 2005; Halsby et al., 2014; Han, 2021; Walker, 2018), increased atmospheric temperature (Beauté et al., 2016; Dupke et al., 2023; Halsby et al., 2014; Han, 2021; Montagna et al., 2023; Mora et al., 2022; Simmering et al., 2017; Walker, 2018), low atmospheric pressure (Beauté et al., 2016; Dupke et al., 2023) and decreasing UVB radiation (Halsby et al., 2014; Han, 2021). However, one study found no correlation between the weather and LD incidence (Dunn et al., 2013), while another study found that there was an association between rainfall and humidity and LD incidence in some regions of the US, but not in others (Wade and Herbert, 2024). LD incidence may vary with different weather conditions depending on the climatic features of each region. Furthermore, non-meteorological factors also play an important role in the occurrence of LD outbreaks (Timms et al., 2024). Water systems are designed differently and must comply with very different regulations depending on the legislation of each country. Water parameters such as residual disinfectant or pH should also be considered. This can affect the ability of *Legionella* to colonise them as they are critical determinants of *Legionella* colonisation. Additionally, the physical structure, age, and condition of water systems can also interact with the impact of meteorological and climatic factors. This would explain the fact that different conclusions have been reached by different studies.

What can be ascertained is that, in a region like Catalonia, atmospheric temperatures are associated with *Legionella* colonisation in cold-water systems. In turn, an increase in bacterial levels in these systems favours the dissemination of the bacteria through aerosols among susceptible populations, increasing the LD incidence. In addition, a decrease in relative humidity to levels around 65 % also favours aerosol transmission, and therefore LD incidence. Our study suggests that other regions with a climate, water system structures, and *Legionella* control legislation similar to Catalonia's would experience increasing LD incidence.

5. Conclusions

In the present study, we analysed potential relations between the temperature of water in water systems, atmospheric temperature, accumulated precipitation and relative humidity on the one hand, and, on the other, the presence and type of *Legionella* in those water systems and LD incidence. Our findings suggest a positive correlation between atmospheric temperature and *Legionella* colonisation in cold-water systems and cooling towers. A positive correlation was also detected between atmospheric temperatures and LD incidence and an inverse correlation was also detected with relative humidity. Further studies are needed to corroborate these findings, especially ones that examine the association of the weather on not only LD incidence but also *Legionella* colonisation in water systems in a variety of climate zones. Furthermore, water in water systems should be tested by techniques that will detect *Legionella* even if it is in a VBNC state. By the same token, diagnostic tools need to be able to detect *L. pneumophila* infections caused by *L. pneumophila* serogroups other than serogroup 1, as well as other *Legionella* species.

When atmospheric temperatures increase, the result may be the creation of optimal conditions for bacterial growth in water systems. Climate change is increasing the probability that such optimal conditions will occur in water systems and also that they will persist for longer periods of time, thereby increasing the number of facilities at risk of dispersing *Legionella* through aerosols and causing infections among the population.

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CRediT authorship contribution statement

Elisenda Arqué: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Sonia Ragull:** Writing – review & editing, Validation, Resources. **Daniel Villodres-Moreno:** Writing – review & editing, Resources, Investigation. **Adrià Alsina-Samaniego:** Writing – review & editing, Resources, Investigation. **Pau Satorra:** Writing – review & editing, Formal analysis. **Cristian Tebé:** Writing – review & editing, Formal analysis. **Gemma Rosell-Duran:** Writing – review & editing, Resources. **Dídac Pérez-Lallave:** Writing – review & editing, Resources. **Luisa Pedro-Botet:** Writing – review & editing, Conceptualization. **Miquel Sabrià:** Writing – review & editing, Supervision. **Noemí Párraga-Niño:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Sonia Ragull reports a relationship with Aqualab Asesoría y Análisis de Aguas S.L. that includes: board membership. Miquel Sabrià reports a relationship with Aqualab Asesoría y Análisis de Aguas S.L. that includes: board membership. Daniel Villodres Moreno reports a relationship with Aqualab Asesoría y Análisis de Aguas S.L. that includes: employment. Adrià Alsina Samaniego reports a relationship with Aqualab Asesoría y Análisis de Aguas S.L. that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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Data availability

Due to sample confidentiality, codified data will be made available on request.

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