



Spring temperature shapes West Nile virus transmission in Europe

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ABSTRACT

West Nile Virus (WNV) is now endemic in many European countries, causing hundreds of human cases every year, with a high spatial and temporal heterogeneity. Previous studies have suggested that spring temperature might play a key role at shaping WNV transmission. Specifically, warmer temperatures in April-May might amplify WNV circulation, thus increasing the risk for human transmission later in the year. To test this hypothesis, we collated publicly available data on the number of human infections recorded in Europe between 2011 and 2019. We then applied generalized linear models to quantify the relationship between human cases and spring temperature, considering both average conditions (over years 2003-2010) and deviations from the average for subsequent years (2011-2019). We found a significant positive association both spatial (average conditions) and temporal (deviations). The former indicates that WNV circulation is higher in usually warmer regions while the latter implies a predictive value of spring conditions over the coming season. We also found a positive association with WNV detection during the previous year, which can be interpreted as an indication of the reliability of the surveillance system but also of WNV overwintering capacity. Weather anomalies at the beginning of the mosquito breeding season might act as an early warning signal for public health authorities, enabling them to strengthen in advance ongoing surveillance and prevention strategies.

1. Introduction

West Nile Virus (WNV), a flavivirus that was first isolated in Uganda in 1937 (Smithburn et al., 1940), is one of the most recent emerging mosquito-borne pathogens in Europe. It is maintained in a bird-mosquito transmission cycle primarily involving *Culex* species mosquitoes of which the *Cx. pipiens* complex is thought to be the most important in Europe (Zeller and Schuffenecker, 2004). Mosquitoes acquire infection after biting an infectious bird and, after an incubation period, they become infectious and thus can transmit the virus through subsequent blood meals. Only lineage 1 and 2 of WNV have been associated with significant outbreaks in humans, which act as incidental hosts in the natural transmission cycle (Petersen et al., 2013). While most human infections are asymptomatic, about 25% of the infections develop symptoms such as fever and headache and less than 1% more severe neurological diseases (Petersen et al., 2013).

WNV (lineage 2) has most likely arrived in Europe first in Hungary thanks to migratory birds at the beginning of the century

(Hernández-Triana et al., 2014; Veo et al., 2019; Zehender et al., 2017), and since then it has spread to many European countries causing hundreds of human cases with a high heterogeneity in incidence both spatial (i.e. between and within different countries) and temporal (i.e. between different years) (European Centre for Disease Prevention and Control, 2020).

WNV transmission is largely affected by abiotic factors. First of all, mosquito population dynamics is strongly dependent on temperature (Chaves et al., 2011; Loetti et al., 2011; Ruybal et al., 2016). For instance, according to laboratory experiments, *Cx. pipiens* larvae take about three weeks to become adults at 15°C and only 8 days at 30°C (Loetti et al., 2011). Moreover, temperature is paramount at shaping viral circulation: warmer conditions increase mosquito biting rate (Ewing et al., 2016; Ruybal et al., 2016) and decrease the extrinsic incubation period (EIP) (Reisen et al., 2006), thus accelerating WNV transmission. For instance, EIP was estimated to decrease from about 20 days at 20°C to about 10 days at 25°C (Reisen et al., 2006). Also bird-mosquito transmission probability is temperature dependent and it

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was found that an increase of two degrees (from 15.5°C to 17.5°C) might even double it (from 0.005 to 0.01) (Vogels et al., 2016; Marini et al., 2020).

Several studies conducted in Europe, e.g. (Cotar et al., 2016; Ewing et al., 2016; Marcantonio et al., 2015; Marini et al., 2020; Paz et al., 2013; Rosà et al., 2014) and North America, e.g. (Chuang et al., 2011b; Chuang and Wimberly, 2012; Poh et al., 2019; Ruiz et al., 2010; Shand et al., 2016) highlighted the importance of temperature conditions both for *Culex* mosquito dynamics and WNV circulation. Recently, by calibrating an epidemiological model using detailed entomological data (mosquito abundance and WNV prevalence) gathered over several years in northern Italy, we found a strong positive association between average spring (April-May) temperature and WNV estimated prevalence both in mosquito and avian hosts during summer (Marini et al., 2020). We argued that such warm conditions at the beginning of the season might amplify earlier WNV transmission, resulting in a higher circulation during the following months. Here we investigate whether such relationship holds at a broader spatial and temporal scale. To this aim, we collated WNV epidemiological data publicly available from the ECDC database (now available in tabular format in the online public repository) and analyzed the relationship between spring temperature and WNV human infection at European level using regression analysis.

2. Materials and Methods

2.1. WNV cases

We collated all publicly available data on laboratory confirmed WNV human cases from the ECDC website (www.ecdc.europa.eu, accessed on February 18, 2020), aggregated by year and administrative unit at the NUTS (Nomenclature of territorial units for statistics) 3 level (Eurostat, 2020). Data are available from 2011 to 2019 for a total of 186 NUTS3 areas in 15 EU Member States and neighboring countries. Cases are reported according to the European Commission Decision of 28/IV/2008, which provides that cases are confirmed through laboratory test (e.g. virus isolation in blood and/or WNV antibody detection).

We denote the number of human cases for area i and year y with $H_{y,i}$. Administrative units appear for the first time in the database at different years (when for the first time at least one human infection is recorded) and only if there are cases. Therefore, as only strictly positive numbers are presented, we introduced zeros in the following way: if a given NUTS3 area i appears in the database for the first time in year \hat{y} , then for every year $y > \hat{y}$ with no reported cases (i.e. area i does not appear in the ECDC dataset) we assumed $H_{y,i}=0$.

2.2. Temperature data

For each NUTS3 region and year between 2003 and 2019, daily average temperature data were obtained from the gap-free Moderate Resolution Imaging Spectroradiometer (MODIS) Land Surface Temperature (LST) maps (Metz et al., 2014). The MODIS data are downloaded automatically from NASA's Land Processes Distributed Active Archive Center (LP DAAC) located at the USGS Earth Resources Observation and Science (EROS) Center using pyModis software and processed using GDAL and GRASS GIS software. The daily tiles at 1000 m resolution for entire Europe are mosaicked and masked using the quality layer

provided by MODIS products. Subsequently, working at spatial resolution of 250 m, a temporal weighted gap filling was applied and eventually last empty pixels were reconstructed with a spatial bspline interpolation. Finally, daily temperature values for each region were computed as average of the daily tiles covering the area of interest.

2.3. Statistical models

The relationship between temperature and the number of human infections was investigated in a regression modelling framework applying a zero-inflated model. Specifically, we developed a mixture model including two generalized linear models (GLMs), one assuming a Bernoulli distribution with logit link and one assuming a Negative Binomial (NB) distribution with log link. Therefore, the model assumes a zero-inflated negative binomial (ZINB) distribution for the response variable $H_{y,i}$, which provides a convenient interpretation of the zeros observed in the dataset. Specifically, the zeros are modelled as coming from two different processes: the "true" zeros modelled by the count process (NB GLM), and the "false" zeros modelled by the binary process (Bernoulli GLM) which are zeros that cannot be explained by the count process and may be due to under reporting or lack of detection (Zuur et al., 2009). We considered three possible explanatory variables both for the count and the binomial part:

- 1 \hat{T} : the average, over years 2003-2010, of the mean April-May temperature, the same temporal window highlighted in (Marini et al., 2020). In that study, the average host and mosquito prevalence estimated by a temperature-dependent epidemiological model in June was found to be significantly associated with the average temperature of the two previous (April and May) months. Be $\omega_{y,i}$ the average April-May temperature for area i and year y , then $\hat{T}_i = \frac{\sum_{y=2003}^{2010} \omega_{y,i}}{8}$. Such variable can thus be interpreted as an indication of the average conditions of a given region and a proxy for its climatic suitability for viral circulation.
- 2 STD_ANOMALY: spring temperature anomaly, given as the difference of the average April-May temperature of a given year with the 2003-2010 average divided by the standard deviation. So $\text{STD_ANOMALY}_{y,i} = (\omega_{y,i} - \hat{T}_i) / \text{sd}_{2003-2010}(\omega_i)$ for $y \geq 2011$. This variable can be interpreted as an indication of how warmer or colder spring is during year y , in a region, with respect to its average condition.
- 3 WNV_BEFORE: a variable set to "NR" (Not Recorded) for each year that is the first one in which the administrative unit appears in the database, set to 0 for each year when in the previous year no WNV cases were reported within the administrative unit (but the current year is not the first one in which the administrative unit appears in the database), set to 1 otherwise (there were WNV cases during the previous year).

The full model can thus be represented by the following equations:

$$H_{y,i} \sim \text{ZINB}(\mu_{y,i}, k, \pi_{y,i})$$

$$\text{Count model : } \log \mu_{y,i} = \beta_0 + \beta_1 \cdot \hat{T}_i + \beta_2 \cdot \text{STD_ANOMALY}_{y,i} + \beta_3 \cdot \text{WNV_BEFORE_1}_{y,i} + \beta_4 \cdot \text{WNV_BEFORE_NR}_{y,i}$$

$$\text{Binomial model : } \text{logit}(\pi_{y,i}) = \alpha_0 + \alpha_1 \cdot \hat{T}_i + \alpha_2 \cdot \text{STD_ANOMALY}_{y,i} + \alpha_3 \cdot \text{WNV_BEFORE_1}_{y,i} + \alpha_4 \cdot \text{WNV_BEFORE_NR}_{y,i}$$

$$E(H_{y,i}) = (1 - \pi_{y,i}) \cdot \mu_{y,i}$$

$$E(H_{y,i}) = (1 - \pi_{y,i}) \cdot \mu_{y,i} \quad \text{Var}(H_{y,i}) = (1 - \pi_{y,i}) \cdot \mu_{y,i} \cdot \left(1 + \mu_{y,i} \cdot \left(\pi_{y,i} + \frac{1}{k}\right)\right)$$

Where $\pi_{y,i}$ represents the probability of getting zero in the binomial model while $\mu_{y,i}$ (mean) and k (dispersion parameter) characterize the NB distribution in the count model. Note that with this formulation we are defining 0 as reference level for WNV_BEFORE.

We computed all models with every possible combination of the covariates in the binomial and the count parts. We ranked them by the Akaike Information Criterion (AIC) and then selected as best model the most parsimonious one, i.e. the one with lowest AIC and all significant coefficients. All analysis, including plots creation, was carried out using R v3.6.0 (R Core Team, 2020). The dataset and the R code to reproduce the analysis are available at https://github.com/giomarini/WNV_spring_Europe.

3. Results

The distribution of $H_{y,i}$ is presented in Figure 1, also aggregated by administrative area and year. We gathered a total of 977 observations, of

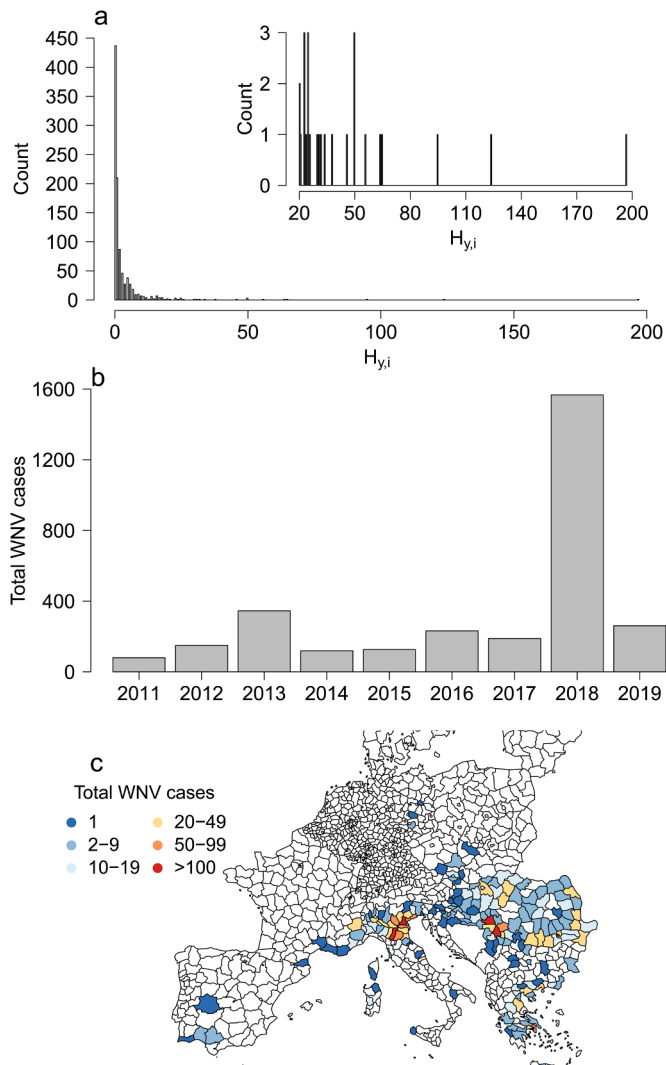


Fig. 1. WNV cases recorded in Europe between 2011 and 2019. a) Distribution of $H_{y,i}$, the number of recorded confirmed WNV human cases in area i during year y ; b) total number of cases by year; c) total number of cases by administrative area (NUTS3 level).

Table 1

Estimates, standard errors and p-values of the parameters of the selected best model. Reference level for WNV_BEFORE is 0 (no cases in the previous year).

Count model			
Parameters	Estimate	Standard Error	p-value
\hat{T}	0.42	0.04	<0.001
STD_ANOMALY	0.35	0.04	<0.001
WNV_BEFORE: 1	0.4	0.15	0.009
WNV_BEFORE: NR	0.08	0.17	0.64
Log(k)	-0.3	0.09	<0.001
Binomial model			
Parameters	Estimate	Standard Error	p-value
STD_ANOMALY	-0.52	0.12	<0.001
WNV_BEFORE: 1	-1.99	0.31	<0.001
WNV_BEFORE: NR	-19.7	976.16	0.98

which 437 are introduced zeros (44%), with an average of 3.1 (5.7 excluding the zeros), a median of 1 (2 excluding the zeros) and a maximum value of 197 cases. During 2018 large WNV outbreaks occurred in several areas, resulting in 1567 confirmed cases all over Europe, about the same as the total number of infections recorded during all other years (1502, see Figure 1b).

After ranking all computed models according to their AIC score, we selected as best model the second one as \hat{T} is not significant in the binomial part of the full model (see Appendix A). The coefficients of the selected best model are presented in Table 1. In the count model, both temperature covariates have a positive significant coefficient. So outbreaks are likely to be larger in warm regions; moreover warmer than usual springs are associated with a higher number of recorded cases (see Figure 2a, b). There are substantially fewer expected cases if WNV was not detected in the previous year.

Conversely, WNV detection during the previous year and anomalously warmer spring decrease the probability of a zero of the binomial model. This latter negative relationship taken together with the results from the count model suggests that with warmer spring conditions the probability of recording no cases substantially decreases (see Figure 2c, d).

4. Discussion

By analyzing WNV cases recorded in Europe during last decade, we found that warmer springs result in a likely higher risk for human spillover. Several studies (e.g. (Chuang and Wimberly, 2012; Cotar et al., 2016; Ewing et al., 2016; Marcantonio et al., 2015; Marini et al., 2020; Paz et al., 2013; Rosà et al., 2014)) suggest that early spring temperature conditions may be particularly important for activating the mosquito breeding season, reducing the extrinsic incubation period and thus accelerating virus amplification in the avian and mosquito populations. Nonetheless, such studies might be temporally or spatially limited. For instance, (Cotar et al., 2016; Marini et al., 2020; Rosà et al., 2014) use data gathered in a relatively small area (a single region of one country), while (Marcantonio et al., 2015; Paz et al., 2013) rely on data recorded over relatively few years (no more than three). Instead, we built our dataset using all data available so far at ECDC, namely 9 years of data gathered over 15 European countries, and our statistical analysis corroborates such previous suggestions. Note that the results are similar when analyzing the original ECDC dataset (see Appendix A) through a linear model with log-transformed data, without introducing the zeros using the procedure explained in section 2.1.

Warmer springs might help amplifying virus transmission through different mechanisms: by increasing the biting rate (Ewing et al., 2016; Ruybal et al., 2016), the host-to-vector transmission probability (Holicki et al., 2020; Vogels et al., 2016) and by accelerating the mosquito viral incubation period (Reisen et al., 2006). Moreover, favorable

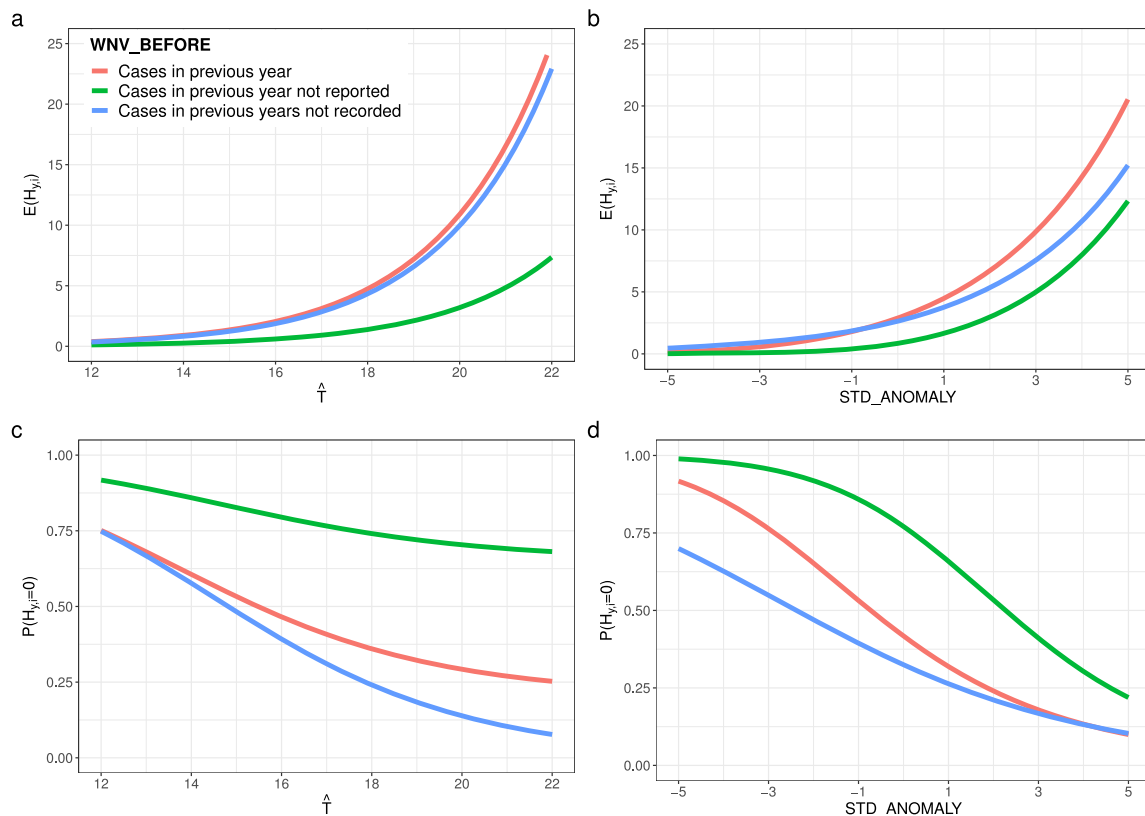


Fig. 2. Model predictions conditional to the temperature covariates and WNV_BEFORE. Expected values ($(1 - \pi_{y,i}) \cdot \mu_{y,i}$, panels a and b) and probability of recording zero cases ($\pi_{y,i} + (1 - \pi_{y,i}) \cdot P_{NB}(H_{y,i} = 0)$, panels c and d) as functions of \hat{T} (°C) (panels a and c) and STD_ANOMALY (panels b and d) according to the three possible values of WNV_BEFORE (0: green, 1: red, NR: blue).

conditions might increase mosquito population size during the season (Marini et al., 2016), possibly also by enhancing oviposition activity (Hayes and Hsi, 1975; Nguyen et al., 2012), thus resulting in a higher vector-to-host ratio and ultimately in a larger probability of enzootic transmission. The relevance of WNV as a public health threat at European level has peaked during the 2018 season when 15 countries reported cases and an unfortunate high number (180) of deaths (European Centre for Disease Prevention and Control, 2018). Therefore, it is essential to study the ecological mechanism that may signal an increased WNV risk of both spread to other countries and local transmission. Our results suggest that an increase in spring temperature is positively associated with an increase of WNV transmission and could be considered as an early warning to enhance surveillance and vector control. At present, the One Health approach to WNV surveillance, a system targeting humans, wild birds, horses, and mosquitoes, has been successful in mitigating the cost associated with WNV transmission (e.g. blood transfusion disruption and blood donor screenings) (Paternoster et al., 2017). Integrating also eco-climatic information collected before the start of the potential transmission season could further guide the disease mitigation policy by better tailoring the monitoring efforts.

The negative coefficients in the binomial model decrease the probability that WNV cases are not reported due to shortcomings in the detection (“false” zeros). The negative coefficient estimated for STD_ANOMALY in the binomial model taken together with the results from the count model suggests that with warmer spring conditions the probability of recording no cases, possibly due to imperfect surveillance (binomial part), substantially decreases; yet when a zero is recorded under such conditions the model suggests that it is more likely due to absence of symptomatic human cases (so indeed a “true” zero) rather than under-detection or lack of reporting. In fact, the model seems to suggest that there is an unexpected excessive absence of symptomatic cases when

conditions for WNV circulation (at least the ones included in the models) are not optimal. This may be due to sporadic circulation that fails to be detected. On the other hand, when conditions are optimal, either there is a substantial number of cases or, for yet unexplained reasons (e.g. local extinction or local bird immunity) WNV circulation is limited or absent.

We also found a positive association with previous WNV detection. This may be due to several factors: once WNV is introduced in a new area, it will likely overwinter and reactivate every year, as already suggested by phylogenetical analyses (Veo et al., 2019; Ziegler et al., 2020); furthermore, a first detection of a WNV case in an area will increase the physicians’ awareness of the disease, thus improving the detection capacity of the surveillance system. These findings seem to indicate that once present WNV is there to stay, therefore urging public health authorities of neighboring WNV-positive areas to increase surveillance and vector control efforts. This task is however challenged by a lack of precise knowledge of the underlying mechanisms that drive WNV spread and re-emergence between countries and years.

Certainly, as already hinted above, many other abiotic factors could influence the occurrence of large WNV outbreaks in a given area. For instance, other climatic variables not explicitly considered in our analysis, such as precipitation or humidity, influence WNV transmission as well (Cotar et al., 2016; Kioutsoukakis and Stilianakis, 2019; Paz et al., 2013). Also, human activities can influence mosquito dynamics and pathogen circulation (Chaves et al., 2011; Chuang et al., 2012, 2011a). Moreover, yearly variations in WNV incidence may be due to the intrinsic dynamics of the epidemics; for instance, it is plausible that after a year with high incidence, both adult birds and humans will be immune to WNV, thus decreasing the incidence in the following year, independently of abiotic factors (Kwan et al., 2012; Paull et al., 2017; Ribeiro et al., 2020). Certainly model predictions could greatly benefit from including all these kinds of data, if available.

Nonetheless, temperature seems to be one of the most crucial factors at shaping both mosquito dynamics and viral circulation (Paz, 2015). During the cold season, temperature might critically affect survival of overwintering mosquitoes (Poh et al., 2019; Reisen et al., 2010), while during summer it can strongly influence mosquito abundance and dynamics (Chuang et al., 2011a; Marini et al., 2016; Rosà et al., 2014). Using a similar approach, we performed some additional statistical analyses to investigate whether summer average conditions and deviations significantly affect WNV transmission and found that, although unsurprisingly warm summers are associated with larger outbreaks, spring predictors better explain the observed European dynamics (see Appendix A). Such results thus confirm the importance of weather conditions during the beginning of the mosquito breeding season. In our study we used LST data, as previously done in several studies (Bisanzio et al., 2011; Candeloro et al., 2020; Chuang and Wimberly, 2012; Conte et al., 2015; Marcantonio et al., 2015; Rosà et al., 2014), although air temperature, as usually collected through ground stations above 2m from the ground, might represent a better predictor from a biological perspective. However, it seems reasonable to assume that such temperature is correlated with LST, so the latter might be a proxy for the former.

Conclusions

Despite its limitations, our study highlights that weather anomalies at the beginning of the mosquito breeding season might be considered as an early warning signal for public health authorities, in particular those already implementing surveillance programs. In fact, spring anomalies might prompt a strengthening of such ongoing surveillance efforts or an earlier application of control treatments.

CRediT authorship contribution statement

Giovanni Marini: Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Mattia Manica:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **Luca Delucchi:** Resources, Writing - review & editing. **Andrea Pugliese:** Conceptualization, Writing - review & editing. **Roberto Rosà:** Conceptualization, Writing - review & editing.

Declaration of Competing Interests

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.

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Supplementary materials

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