Inferring the seasonal dynamics and abundance of an invasive species using a spatio-temporal stacked machine learning model

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Abstract

Various modelling techniques are available to understand the temporal and spatial variations of the phenology of species. Scientists often rely on correlative models, which establish a statistical relationship between a response variable (such as species abundance or presence-absence) and a set of predominantly abiotic covariates. The modelling approach choice, i.e. the algorithm, is a crucial factor for addressing the multiple sources of variability that can lead to disparate outcomes when different models are applied to the same dataset. This inter-model variability has led to the adoption of ensemble modelling techniques, among which stacked generalisation, which has recently demonstrated its capacity to produce robust results. Stacked ensemble modelling incorporates predictions from multiple base learners or models as inputs for a meta-learner. The meta-learner, in turn, assimilates these predictions and generates a final prediction by combining the information from all the base learners. In our study, we utilized a recently published dataset documenting egg abundance observations of *Aedes albopictus* collected using ovitraps. This dataset spans various locations in southern Europe, covering four countries - Albania, France, Italy, and Switzerland - and encompasses multiple seasons from 2010 to 2022. Utilising these ovitrap observations and a set of environmental predictors, we employed a stacked machine learning model to forecast the weekly average number of mosquito eggs. This approach enabled us to i) unearth the seasonal dynamics of *Ae. albopictus* for 12 years; ii) generate spatio-temporal explicit forecasts of mosquito egg abundance in regions not covered by conventional monitoring initiatives. Beyond its immediate application for public health management, our work presents a versatile modelling framework adaptable to infer the spatio-temporal abundance of various species, extending its relevance beyond the specific case of *Ae. albopictus*.

Keywords

Arthropod, *Aedes albopictus*, forecast, invasive species, population dynamics, time-series.

Author Contributions

Daniele Da Re, Beniamino Caputo, Alessandra della Torre and Roberto Rosà conceived the study; Daniele Da Re and Roberto Rosà designed the methodology, with relevant contributions from Carmelo Bonannella, Giovanni Marini, Fabrizio Laurini and Mattia Manica; Nikoleta Anicic, Alessandro Albieri, Paola Angelini, Daniele Arnoldi, Federica Bertola, Beniamino Caputo, Claudio De Liberato, Enkelejda Velo, Eleonora Flacio, Alessandra Franceschini, Perparim Kadriaj, Valeria Lencioni, Irene Del Lesto, Francesco La Russa, Riccardo Paolo Lia, Fabrizio Montarsi, Francesco Gradoni, Gregory L'Ambert, Federico Romiti, Gionata Stancher, Fabiana Zandonai collected the data; Daniele Da Re, Carmelo Bonannella, Giovanni Marini, Fabrizio Laurini, Mattia Manica and Roberto Rosà analysed the data; Daniele Da Re led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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1. Introduction

Understanding the phenology of species, the study of periodic events in biological life cycles influenced by seasonal and annual climate fluctuations, is of paramount importance across various domains such as agriculture (e.g., for crop yield forecasts; Fand et al., 2014), nature conservation (e.g., for assessing species responses to global changes; Ettinger, Chamberlain, and Wolkovich, 2022), and addressing public health concerns related to allergens and emerging infectious diseases carried by arthropod vectors (Burkett-Cadena et al., 2011). Ecologists have therefore developed and tested several modelling approaches, i.e. the mechanistic and correlative approaches, to infer the phenology of species and how it varies over space and time. The mechanistic approach employs laboratory or field observations about the influence of biotic or abiotic factors on the targeted life history traits (e.g. the effect of temperature on a juvenile form development rate) to parametrise mathematical models inferring the life cycle of the species of interest (e.g. Tran et al., 2013; Marini et al., 2019; Pfab et al., 2018; Da Re et al., 2022). Although generally accurate, mechanistic models often require estimating multiple parameters, constrained by the availability of ecological observations in the scientific literature (Tjaden et al., 2013; Da Re et al., 2022). As an alternative, ecologists frequently turn to correlative models, which establish statistical relationships between a response variable (e.g., species abundance or presence-absence) and a set of mostly abiotic covariates (Guisan et al., 2017; Edwards and Crone, 2021).

Despite the utility of correlative models, their outputs are subject to various sources of variability, such as sampling location bias and model tuning (Hortal et al., 2008; Fourcade 2021; Bazzichetto et al., 2023; Da Re et al., 2023a). The choice of modelling method, in particular, has proven influential, as different models applied to the same dataset can yield distinct results (Araújo & New, 2007; Pearson et al., 2006, Marmion et al., 2009). This inter-model variability has prompted the use of ensemble modelling techniques, also called consensus modelling, which involves fitting multiple independent algorithms on the same input data and then aggregating the individual models' outputs to produce a final prediction, reducing the risk of overfitting and extrapolation issues (Marmion et al., 2009). While simple aggregation methods like averages and weighted averages have been traditionally used (Marmion et al., 2009; Hao et al., 2019), more advanced ensemble techniques, such as stacking or stacked generalisation (Wolpert, 1992), have recently demonstrated superior performance (Bonannella et al., 2022; 2023). In stacked ensemble modelling, multiple base models' predictions serve as inputs for a meta-learner, which learns from these predictions and generates the ultimate prediction by combining information from all the base models.

Motivated by these considerations, our study introduces a spatio-temporal stacked model to infer the abundance of Aedes albopictus' eggs in Southern Europe from 2010 to 2022. The "Asian tiger mosquito" (*Aedes* (*Stegomyia*) *albopictus* (Skuse, 1895)) serves as a compelling case study because it is an invasive alien species with a rapidly expanding range (Roche et al., 2015; Ibáñez-Justicia, 2020) and it has medical interest due to its role of vector in several outbreaks of vector-borne diseases occurred in Mediterranean Europe during the last two decades (Rezza et al., 2007; Venturi et al., 2017; Brady et al., 2019; Barzon et al., 2021).

Local public health authorities have established surveillance and monitoring initiatives to gain deeper insights into the distribution, abundance and seasonality of this

vector, facilitating the development of proactive strategies for population and disease control. Consequently, the collection of these monitoring data is necessary for the implementation of passive surveillance systems, as outlined by Caputo and Manica (2020). These systems encompass modelling techniques that can reliably calculate and predict vector abundance and seasonal patterns, offering invaluable assistance to local public health agencies. While correlative models have been widely used to infer the geographic distribution of *Ae. albopictus* in support of surveillance and monitoring activities (Lippi et al., 2023), to the best of our knowledge this is the first application of a stacked spatio-temporal model on this species. This study not only contributes to the first application of a stacked spatio-temporal model to *Ae. albopictus* but also offers specific results to support public health authorities in planning vector control activities and assessing disease risk. The model enables the inference of seasonal abundance even in areas lacking active surveillance, providing crucial support for resource allocation in monitoring and surveillance efforts. Beyond its immediate application, our work presents a versatile modelling framework adaptable to infer the spatio-temporal abundance of various species, extending its relevance beyond the specific case of *Ae. albopictus*.

2. Methods

2.1 Biological observations and area of interest

We used *Ae. albopictus*' egg counts obtained from monitoring activities conducted with ovitraps as the response variable in our models. Ovitraps are cheap and efficient monitoring tools consisting of a dark container filled with water and a substrate where container-breeding mosquitoes can lay their eggs. The stick is collected on a weekly or biweekly basis, depending on the local protocol adopted by the stakeholders, and the number of eggs laid on the stick counted.

We collected ovitraps observations from four European countries (Albania, France, Italy, and Switzerland) that had active monitoring and surveillance programs of *Ae. albopictus* utilising ovitraps between 2010 and 2022 (see Da Re et al. 2023b for a detailed description of the sampling protocols and the observations pre-processing). We chose the week as the fundamental temporal unit of our study; therefore, if the monitoring period extended beyond one week, the observed egg counts were distributed randomly over the period of trap activity using a binomial draw with a probability equal to 1/n weeks of activation. This means that if a trap was active for 2 weeks and collected 500 eggs, the observed 500 eggs would be randomly assigned to each week with a probability p=1/2, resulting in, e.g. 256 eggs collected during the 1st week and 244 collected during the second. In addition, we aggregated the ovitraps by the median using a grid of 9x9 km spatial resolution (i.e., the native spatial resolution of the ERA5Land climatic datasets; Muñoz-Sabater et al., 2021) to reduce the inherent variability related to the microclimatic conditions to whom the single ovitraps are exposed, and mild the artefacts produced by different sampling strategies and size of the ovitraps.

The collected ovitraps observations are located in a geographical extent spanning from 6° to 21° E and from 36° to 47° N (Fig. 1). According to Cervellini et al. (2020), this area is characterised by three main biogeographical regions, namely Alpine, Continental, and Mediterranean (Fig. 1). Since the location of the ovitraps well represents these three biogeographical regions, we decided to limit the geographical area of extrapolation of the model to the abovementioned geographical extent and these biogeographical regions only.

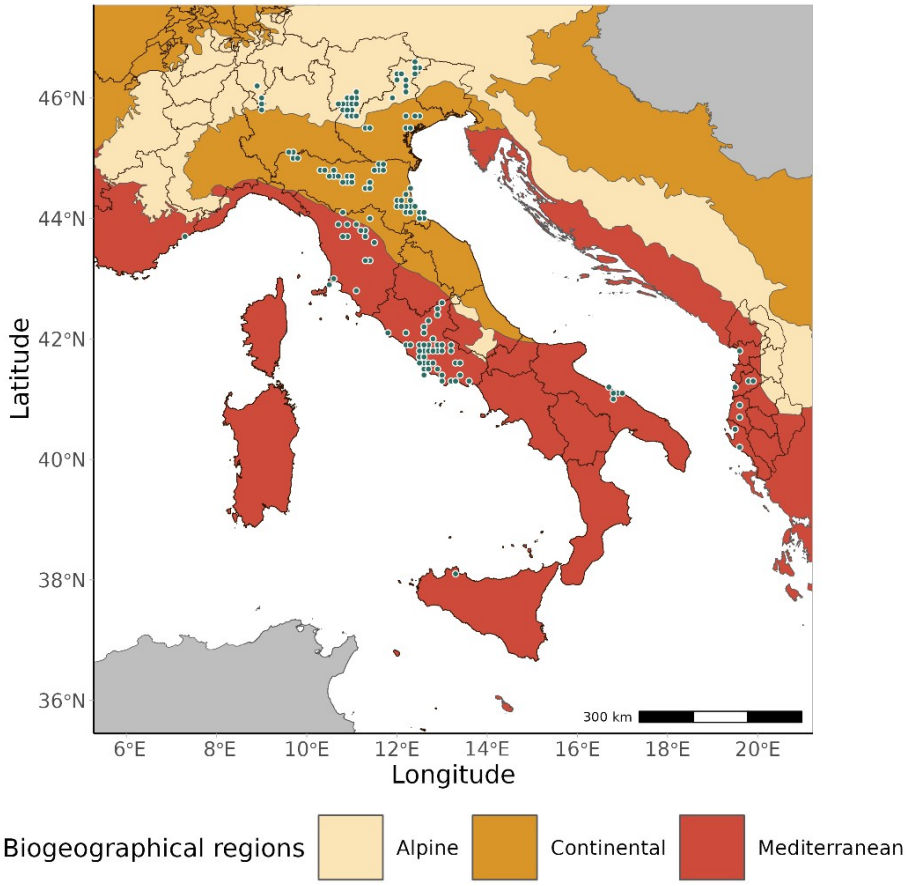


Fig. 1 Biogeographical regions of Europe according to Cervellini et al., (2020) and the location (green dots) of the aggregated egg observations at 9x9 km spatial resolution. The black lines represent the borders of the administrative areas of the countries of interest at the NUTS2 level.

2.2 Modelling

Stacked generalisation is a technique that combines predictions from multiple individual models, known as base learners or base models, to make a final prediction (Wolpert, 1992; Boehmke and Greenwell, 2019; Bonannella et al., 2022). In stacked generalisation, the outputs of individual base learners serve as inputs to a meta-learner, which is another model that learns from the predictions of the individual models. The meta-learner then generates the final prediction by combining and synthesising the information from the individual models (Fig. 2a). Stacking has the potential to improve the accuracy and robustness of ecological models by leveraging the strengths of different models and effectively capturing complex relationships in the data (Bonannella et al., 2022). However, it is important to remark that while stacking can reduce model variance and improve predictions, it comes with trade-offs as it increases model complexity, reduces interpretability, and augments the computational time compared to individual models (Zhou, 2012).

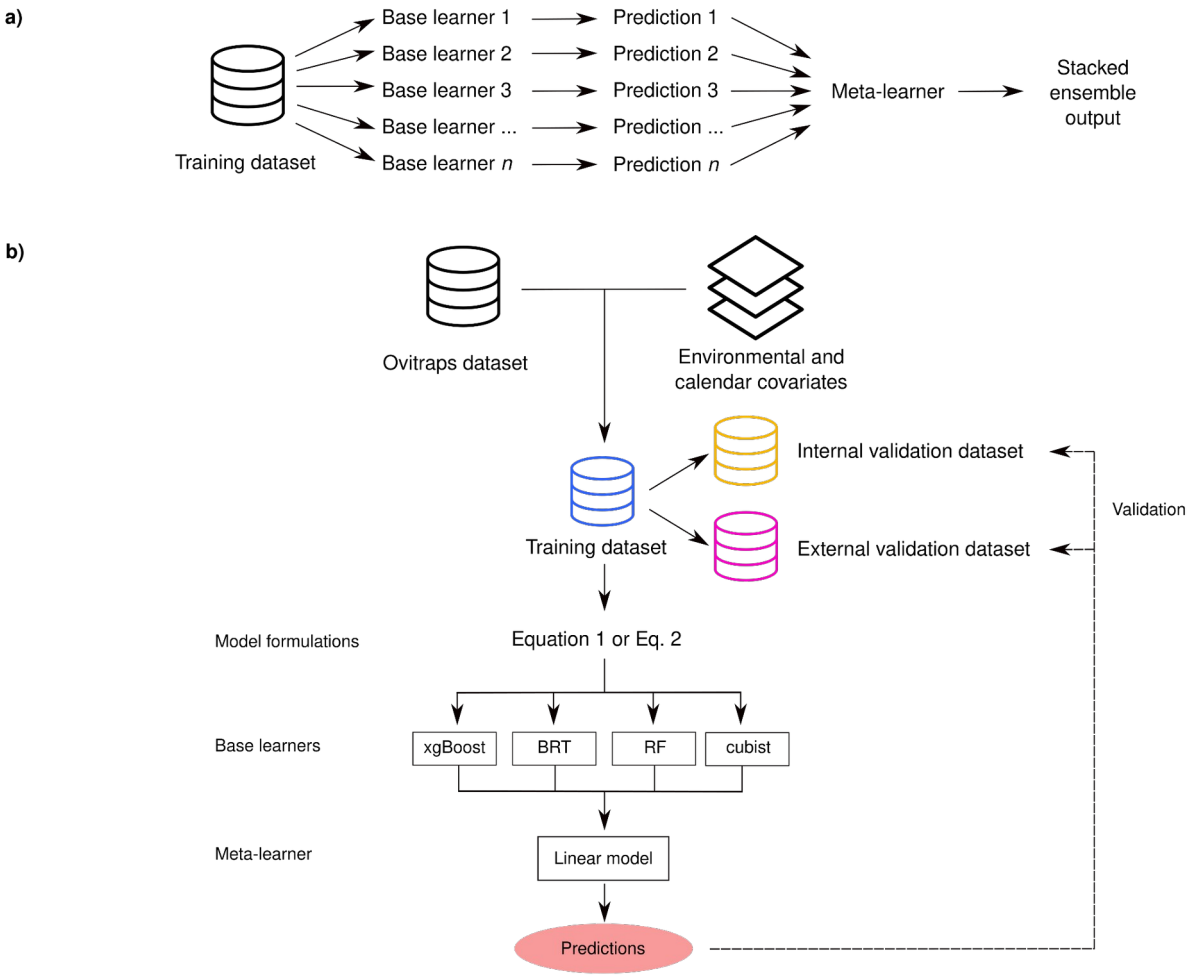


Fig 2. a) a conceptual representation of the stacking approach; b) framework of the modelling approach presented in the study.

2.2.1 Model formulation

As with all correlative models, stacked models require providing each base learner with a response variable and a set of covariates. We used as a response variable the spatially aggregated weekly median egg observations described in section 2.1. As covariates, we selected three main environmental drivers that significantly influence the behaviour and development of mosquitoes, namely temperature, photoperiod (i.e., duration of daylight in 24 hours) and precipitation (Toma et al., 2003; Becker et al., 2010; Roiz et al., 2010, 2011; Marini et al. 2020; Romiti et al., 2021; Carrieri et al., 2023; see SM1.1 for a detailed description of the covariates used, their ecological significance, and the preprocessing operations). For each of these three covariates, we considered also their lagged values, since the mosquito life cycle can take several days or weeks to complete (Becker et al., 2010; Roiz et al., 2010). The lagged temperatures and photoperiod were calculated as the median value between the observations recorded in the current week (i), the previous week (i-1), and the week before that (i-2), whilst the lagged precipitation was computed as the cumulative weekly value between the precipitation recorded in the current week (i), the previous week (i-1), and the week before that (i-2).

In addition to the environmental covariates, which we include as distributed lags, we considered seasonal and cyclical components. Specifically, we used the Fourier series, with sine and cosine harmonic waves, to accommodate the yearly pattern and shorter-term seasonality. For these calendar effects the Fourier terms offer a more parsimonious representation than dummy variables, in particular when the frequency of the data is large (see Hyndman and Athanasopoulos 2021, Ch. 7 Sect. 4). We selected four relevant harmonic: one pair of harmonics is used for describing the yearly evolution, whereas another pair of harmonics is needed to capture some seasonal patterns. The four trigonometric waves were added to the other environmental predictors and provided a significant contribution to describing the cyclic patterns and fluctuations in the median weekly number of eggs.

Based on the results of the explorative modelling (SM1.2), we designed two different but complementary models. The regression model infers the number of eggs as a function of temperature, photoperiod, and precipitation, all lagged by -2 and -3 weeks, and the four Fourier's harmonics (Eq. 1).

(Eq. 1) Regression model: *Egg count ~ Temperature.lag2 + Temperature.lag3 + Photoperiod.lag2 + Photoperiod.lag3 + CumulativePrec.lag2 + CumulativePrec.lag3 + SineYear + CosineYear + SineSeasonal + CosineSeasonal*

The autoregressive model adds to the predictors considered in the Regression model (Eq. 1) an autoregressive component based on the number of eggs observed at week t-1 (Egg count.lag1).

(Eq. 2) Autoregressive model: *Egg count ~ Egg count.lag1 + Temperature.lag2 + Temperature.lag3 + Photoperiod.lag2 + Photoperiod.lag3 + CumulativePrec.lag2 + CumulativePrec.lag3 + SineYear + CosineYear + SineSeasonal + CosineSeasonal*

2.2.2 Stacked model

Each model formulation was applied to four individual base algorithms, namely extreme gradient boosting (xgBoost), boosted regression trees (BRT), random forest (RF) and cubist (Fig. 2b).

Extreme gradient boosting (xgBoost) is a powerful gradient boosting algorithm based on the concept of boosting, where weak models (typically decision trees) are sequentially trained to correct the mistakes made by the previous models (Friedman, 2001). The algorithm optimises an objective function by iteratively adding models to the ensemble, minimising the loss. xgBoost employs a gradient-based approach to improve the performance of the weak models and handle complex interactions among variables. Boosted regression trees (BRT) is a boosting algorithm that combines multiple decision trees to form an ensemble model (Elith et al., 2008). Similar to xgBoost, BRT sequentially trains decision trees, with each subsequent tree focusing on correcting the errors made by the previous trees. The algorithm optimises an objective function by iteratively adding trees, and the final prediction is a weighted sum of the predictions from all the trees. Random Forest is an ensemble learning method that constructs a collection of decision trees and combines their predictions to make accurate predictions (Breiman, 2001). Each tree in the RF is built on a randomly sampled subset of the data and a randomly selected subset of features. This

randomness helps to reduce overfitting and increase the diversity among the trees, and the final prediction is determined by averaging or voting the predictions from all the trees in the forest. Finally, cubist is a rule-based algorithm that combines decision trees with linear models. It creates a set of rules by recursively partitioning the data based on the predictor variables (Quinlan, 1992). Each rule corresponds to a specific region of the feature space and predicts the response variable using a linear model. The algorithm iteratively builds a series of decision trees and linear models, optimising an objective function that balances accuracy and complexity.

We tuned the hyperparameters of each of the four machine-learning algorithms for both model formulations (Eq. 1-2; SM2.1). Then, for each model formulation separately, we combined the predictions of each tuned algorithm into the meta-learner, defined as a linear regression of the egg count (response variable) and the four algorithms' predictions (covariates). Both meta-learners were used to predict the abundance of *Ae. albopictus* eggs over the period 2010-2022 on the training and validation datasets. The meta-learner trained with the four algorithms having as model formulation Eq. 1, i.e. the regression model, was also used to predict, and thus extrapolate, over the whole area of interest for the period 2010-2022.

All the analyses were performed in R 4.3 (R Core Team 2023). All the R scripts used for the analysis are available at the GitHub repository https://github.com/danddr/stackedML. The R scripts are shared with detailed comments to foster the methodology reproducibility and its application to case studies and species different to invasive mosquitoes.

2.3 Model validation

The aggregated egg observations were partitioned into one training and two testing datasets (Fig. 2b). To conduct external validation, we employed a random selection process, choosing two aggregated ovitraps within each distinct NUTS2 level for every biogeographical region. This selection was limited to aggregated ovitraps with a minimum of three years' worth of observations, guaranteeing the presence of a robust time series for validation. These observations were excluded from the training dataset allowing for an exhaustive coverage of the longitudinal and latitudinal gradient of the area of interest. We excluded Sicily from the external validation dataset because it hosts only one aggregated ovitrap and represents the southernmost observation in the area of interest (see Fig. S3.1 for the locations of the ovitraps used for the external validation). After excluding these stations, we defined the training dataset as all the observations spanning from 2010 to 2021 and the testing dataset as all the observations gathered in 2022. As an additional validation, we also performed a 10-fold cross-validation on the training dataset by retaining, for each fold, 70% of the observations to train the model. The class of models used is either regression or autoregression, so the standard k-fold cross-validation can be implemented, as suggested by Hyndman and Athanasopoulos (2021, Ch 5. Sect. 10). To estimate the model's predictive error we estimated, for each station and validation dataset, the root mean squared error (RMSE) and the mean absolute error (MAE).

2.4 Deriving pseudo-phenological indexes: introducing the period-over-threshold

In addition to utilising the stacked model for predicting the average number of eggs for each week within a given year across the area of interest, we also aimed to derive estimates about the predicted seasonality of *Ae. albopictus*. Different approaches have been proposed to compute seasonal indexes of mosquitoes like onset (i.e. beginning of the season), peak, and offset (i.e., end of the season; Rosà et al., 2014, Romiti et al., 2022). However, these approaches assume a repeated and even sampling of the species of interest, which, unfortunately, is not the case across the sampling locations of our dataset. Therefore, here we propose and define a pseudo-seasonal index that we call the period-over-threshold (POT).

The POT represents the period in which the variable of interest, i.e. the average number of eggs, is above a certain threshold. The POT might be of interest not only for spatial epidemiological applications but also for alien species monitoring or biological conservation, i.e. by identifying the areas and the period in which a given population is below or above a certain threshold and therefore requires local interventions. Here, we define the POT as the number of weeks in which the weekly average number of eggs is equal to or higher than 55 eggs, the spatially and weekly aggregated average median number of eggs (excluding zeros) observed over the whole area of interest during the period 2010-2022. We acknowledge that the POT, as defined here, is a heuristic approach, and therefore we performed a sensitivity analysis varying the threshold to 20 and 125, defined by the average interquartile range (IQR) of the observed distribution.

Finally, we investigated if the observed and predicted POT have varied in time and space among the different biogeographical regions over the 2010-2022 period. We tested whether the length of the observed and estimated POT is affected by the year (quantitative) and the biogeographical regions (qualitative: Alpine/Continental/Mediterranean) and their interaction, using Generalised Linear Mixed Models (GLMMs) with Poisson error distribution and log link function, considering the ID of the aggregated ovitraps as a random factor.

3. Results

3.1 Ovitraps dataset descriptive statistics

We collected observations from 2620 ovitraps in four European countries (Albania, France, Italy and Switzerland), resulting in 149 aggregated ovitraps stations after the aggregation at 9x9 km spatial resolution. Overall, 30 aggregated ovitraps were located in the Alpine biogeographical region, while 48 and 71 were located in the Continental and Mediterranean biogeographical regions, respectively. Most of the ovitraps were active during the period 2020-2022, with only a few stations that were monitored for more than three seasons (Da Re et al. 2023b). 120 aggregate ovitraps were used to train the models, whilst 19 were retained for the external validation.

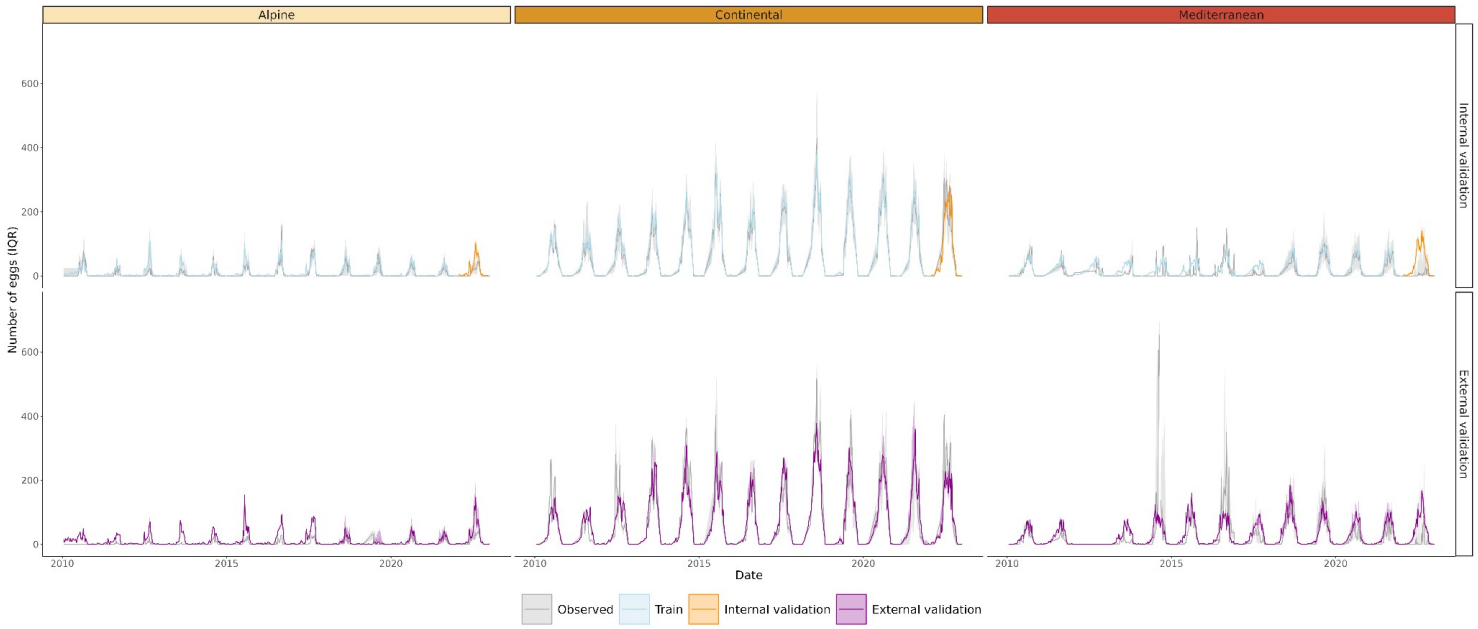
3.2 Model outputs

The random forest algorithm showed the highest regression coefficient in the regression stacked model and so resulted as the most important algorithm (Tab. S2.2), while the most important environmental predictors were the 3-week-lagged temperature and photoperiod (Fig. S2.3A). On the other hand, cubist was the most important algorithm for the stacked autoregressive model, with the 1-week-lagged value of observed eggs being the most important predictor (Fig. S.2.3B).

Both stacked models were able to capture the seasonal and interannual variability of the ovitraps time series in the training dataset and both validation datasets (Fig. 3, Fig. S3.2). A detailed representation of the external validations for both models, broken down at the location level, is available in SM3 in Fig. S3.3-S3.4 for the regression and autoregressive models respectively. The predicted values for both the internal and external validation matched the observation patterns in the three biogeographical regions, with the autoregressive model showing, in general, a closer association with the observations. The autoregressive model showed overall a higher R^2 and lower RMSE and MAE compared to the regression model in the training and both validation datasets (Tab. 1; Fig. S3.5).

Tab. 1 Stacked model validation metrics from the 10-fold cross-validation made on the training dataset.

Model	R^2	Residual standard error	10-fold CV RMSE	10-fold CV MAE
Regression	0.63	55.55	50.37	24.44
Autoregressive	0.85	35.38	34.39	12.66



364 **Fig. 3** Median and interquartile range of the number of eggs observed (grey lines) and
365 predicted by the regression model in both the internal and external validation. Both the
366 observed and predicted values were aggregated over the three biogeographical regions to
367 allow an easier representation.

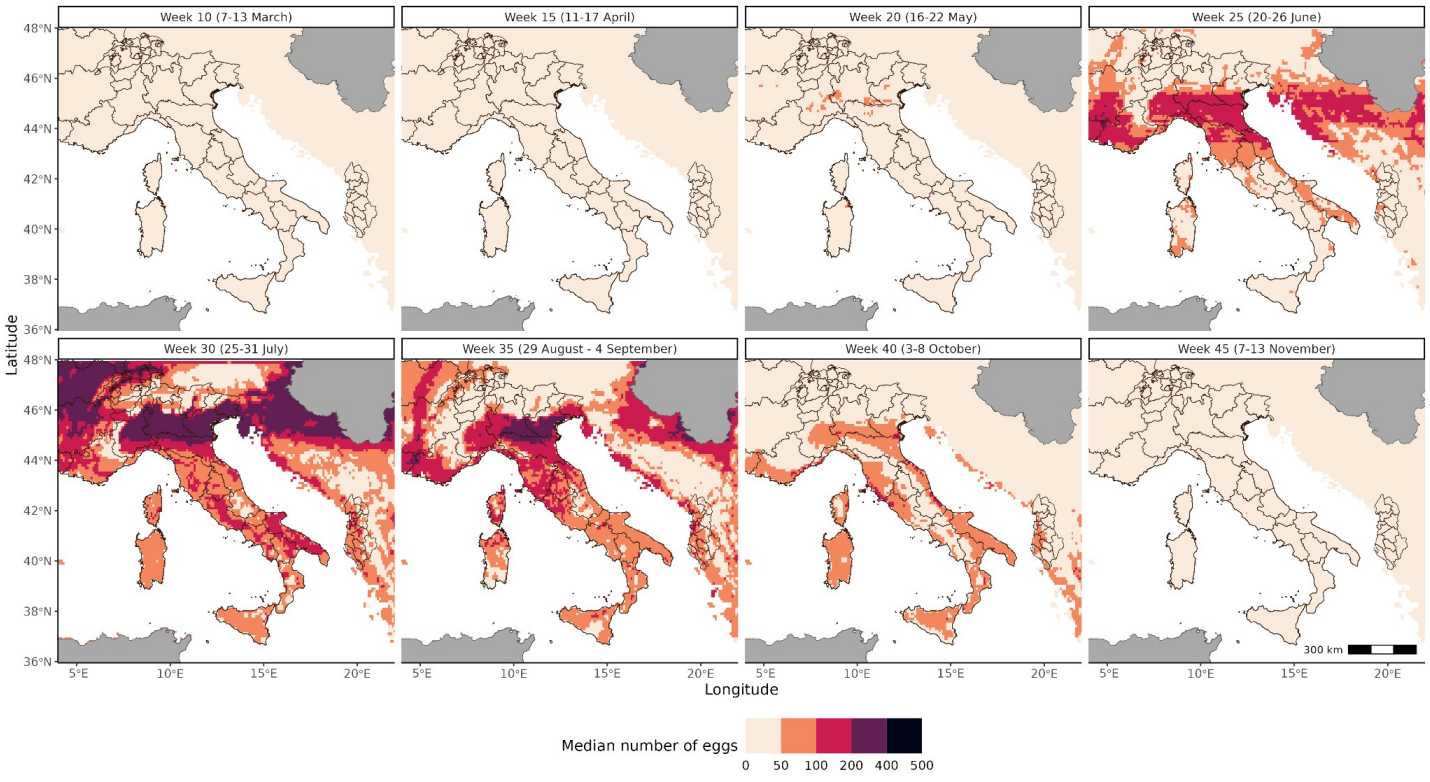


Fig. 4. Median number of eggs predicted weekly by the regression model in the area of interest for the year 2022. The black lines represent the borders of the administrative areas of the countries of interest at the NUTS2 level. The grey areas are outside the area of interest.

3.3 Spatial predictions

The spatio-temporal predictions of the stacked regression model for the year 2022 show seasonal and latitudinal variation in the area of interest (Fig. 4). Generally, we observe an average number of eggs higher than 50 in the entire study area from week 20 (mid-May) onwards. The peak of the season is estimated on week 30 (end of July), especially in the Po and Rhone valleys, as well as in the coastal areas of the Adriatic, Ionian and Tyrrhenian seas. The predicted average number of eggs decreases after week 35 (end of August). Still, it follows a latitudinal and geographical shift, with the Southernmost and coastal areas showing predicted values of eggs higher than 100 still in week 40 (beginning of October), decreasing below fifty only after week 45 (November) onwards.

3.4 Period-over-threshold

On average, the observed POT spans 7 (6-9 IQR) weeks for the Alpine biogeographical region, 20 (19-22 IQR) and 15 (14-16 IQR) weeks for the Continental and Mediterranean biogeographical regions, respectively. The predicted POT shows generally a shorter length, with 4 (3-5 IQR) weeks for the Alpine biogeographical region, and 17 (16-18 IQR) and 11 (9-12 IQR) weeks for the Continental and Mediterranean biogeographical regions respectively. The spatial representation of the POT for the year 2022 (Fig. 5) shows a longer length of the POT in the Po Valley and coastal zones of the area of interest (POT > 20 weeks). Mountainous and foothills areas show, on average, short (POT <= 5) or absent POT.

The Poisson GLMMs investigating the effect of the interaction between the year and the biogeographical regions on the POT show significant effects for all the explanatory variables and their interactions, except for the Alpine biogeographical region in the Observed (Tab. S3.7). The predicted values of the models trained on the observed and estimated POT showed a positive increase, independent from the reference year, for all the biogeographical regions but the Alpine in the observed dataset over the period 2010-2022 (Fig. 6).

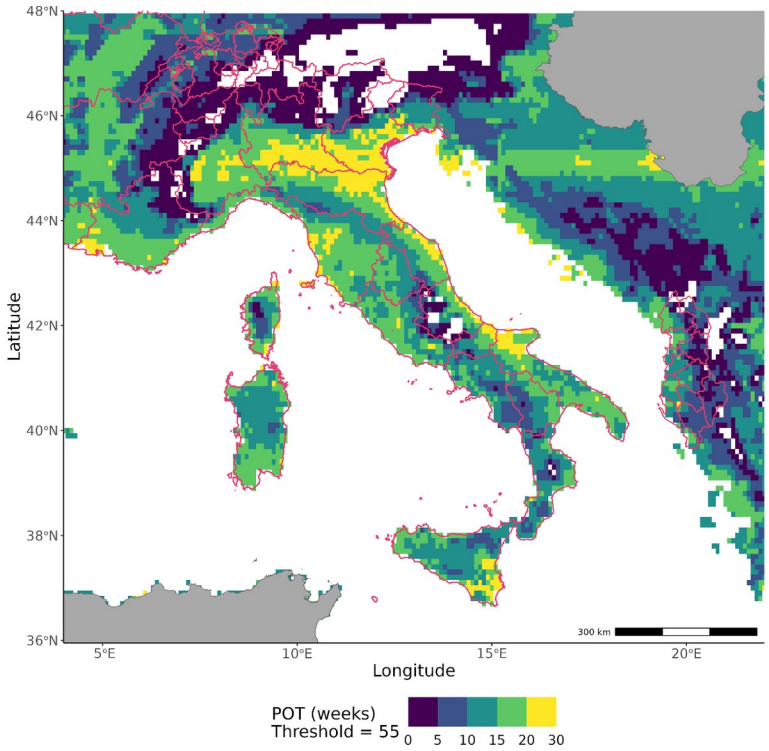


Fig. 5. Spatial representation of the critical period-over-threshold (POT) for the year 2022 in the area of interest. White pixels are characterised by an average median weekly number of eggs always lower than 55. The pink lines represent the borders of the administrative areas of the countries of interest at the NUTS2 level.

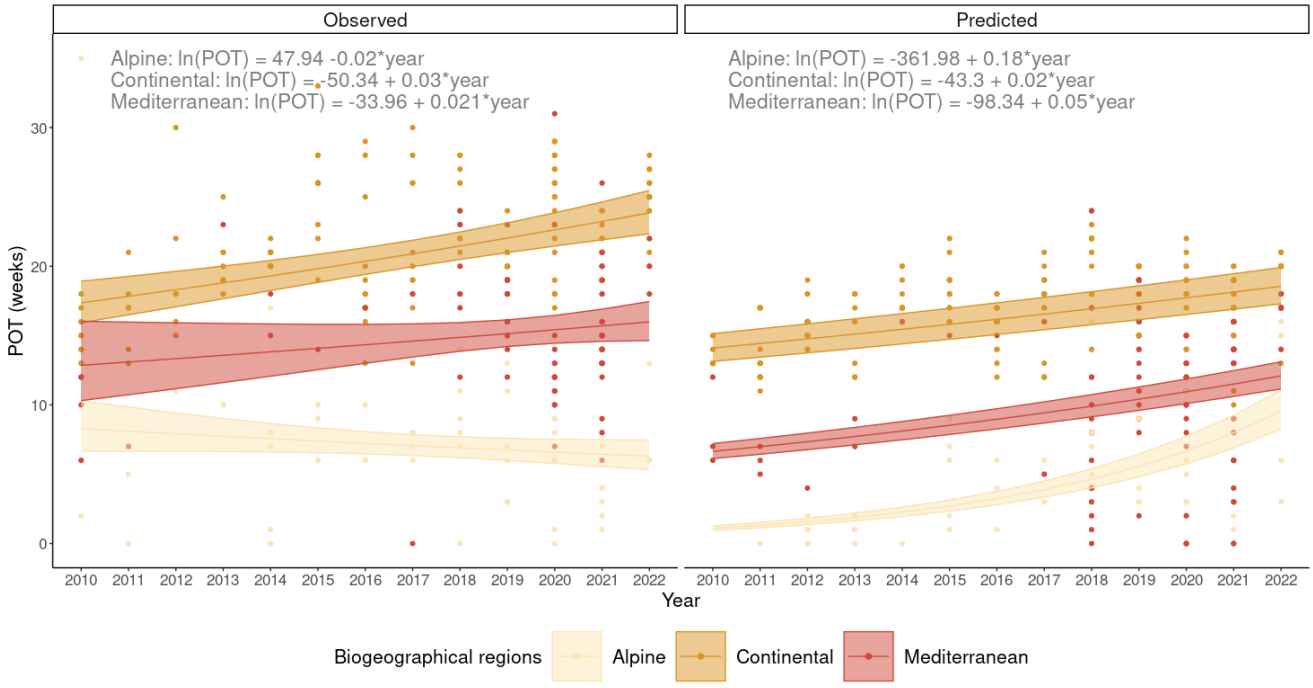


Fig. 6. Modelled relationships between the Period-over-threshold and the interaction between the year and biogeographical regions using a Poisson GLMM.

4. Discussion

Ensemble modelling is a popular technique to mitigate the artefacts or errors that may arise from individual algorithm predictions. Among the different ensemble techniques available, stacking has recently risen as one of the approaches leading to a more robust and accurate final prediction (Bonannella et al., 2022; 2023). In this study, we proposed a reproducible application of a stacked model to infer the spatio-temporal abundance of a species of interest, the Tiger mosquito *Ae. albopictus*. We stress that our approach can be replicated and applied to different species, not only those of medical interest if longitudinal observations on their abundance and phenology are available. The application of a stacked model on both regression and autoregressive model formulations resulted in reliable estimates of mosquito egg abundance. Such results can contribute to supporting local public health authorities' efforts in mosquito management and control: the regression model in particular allowed us to rapidly infer the weekly egg abundance in areas not covered by monitoring activities. This is extremely important to support local public health authorities to better allocate monitoring and surveillance resources and to simulate scenarios for the next mosquito season under different climatic conditions.

4.1 Predictive accuracy of the models

The performance metrics of the regression and autoregressive models indicated a consistently strong predictive accuracy throughout the entire time series. Furthermore, the values of RMSE and MAE displayed similarities between the internal and external validation datasets. The autoregressive model showed generally higher predictive performance than the regression model, having the lagged number of eggs observed as the most important predictive variable. This was not unexpected, since the preliminary exploratory analysis (SM 1.2), made with genuine regression models, displayed strong empirical autocorrelation, of order one, in the residuals. However, the high predictive accuracy of the autoregressive model has the drawback of not being able to spatially extrapolate its predictions outside the training dataset. Whilst this can be seen as a limitation, it offers the opportunity of having accurate estimates and forecasts in specific locations, allowing the model to be informed with local and high-quality environmental information, using e.g. weather station observations. On the contrary, the regression model allowed us to spatially extrapolate the predictions in areas that were not previously sampled. The median number of eggs predicted over the study area for the year 2022 matches the expected seasonal dynamic of the species. Though egg-laying activity might occur in March and April, it increases around week 20 (mid-May) and ends in early October following elevational and latitudinal gradients (Romiti et al., 2022; Carrieri et al., 2023; Lencioni et al., 2023). In the alpine areas, our spatial estimates resembled those obtained using different modelling techniques and training datasets (e.g. Ravasi et al., 2022). Previous dynamical distribution modelling approach forecasting *Ae. albopictus* eggs abundance at high spatial (0.01 latitudinal and longitudinal degrees) and temporal (weekly) resolution over ten Balkan countries projected annual peaks in egg abundance between the summer months of August and September i.e. approximately from weeks 32 to 38 (Tisseuil et al., 2018). The field investigation in Albania in 2023 has shown the peak of the season in July (weeks 28-29-30) and another peak at the end of August and beginning of September (weeks 35-36; personal communication of E. Velo). For the coastal areas of the Tyrrhenian Sea, the spatio-temporal prediction of the stacked

regression model confirms the results of previous studies carried out in central Italy, where the peak of activity and the end of the season is expected at the end of August and November, respectively (Fig. 4; Romiti et al., 2021; 2022). Interestingly, these estimates based on ovitrap data (i.e. collection of eggs) comply with independent estimates obtained from data acquired collecting a different life stage of the mosquito (i.e., host-seeking females) at the two opposite ends of Italy (Trentino and Sicily) that identified the beginning of the season in mid-June and mid-March and the peak of the season in early August and August-September, respectively (Guzzetta et al., 2016; Torina et al., 2023).

4.2 Period-over-threshold

The pseudo-phenological index POT computed for *Ae. albopictus* during the year 2022 also showed latitudinal and elevational gradients, with the Po Valley and coastal areas having generally longer POT than mountainous areas. In the coastal areas of the Tyrrhenian Sea, the estimated POT is between 15 and 20, decreasing to 5 weeks in low mountain areas and to zero in high mountain peaks, similar to what was described in previous studies (Romiti et al., 2021; 2022). The spatial pattern depicted in Fig. 5 resembles those representing the estimates *Ae. albopictus* seasonal length presented by Petric et al. (2021). However, the estimates of Petric et al. (2021), based on multiple conditional statements of temperature and photoperiod, show a generally longer period of activity of the species compared to the estimated POT. This is not unexpected, because the two outputs are intrinsically different: the POT measures the length of the period over a user-defined threshold, whilst Petric et al. (2021) have estimated the length of the period of activity of the mosquito considering the environmental conditions triggering eggs hatching, which in Mediterranean areas can begin in early March though at low density (Petric et al., 2021).

In both the Continental and Mediterranean biogeographical regions, both observed and estimated POT durations have been increasing by approximately one week every year. This trend suggests a potential direct impact of global warming on the abundance of this species, as discussed in previous studies (Kramer et al., 2021; Oliveira et al., 2021; Del Lesto et al., 2022; Romiti et al., 2022; Lührsen et al., 2023). However, it is also essential to consider that the prolonged POT duration might be influenced by other factors, such as the increased monitoring efforts and the expanding range of the insect, leading to higher observed counts and longer POT periods.

Other threshold-based indexes have been proposed specifically for *Ae. albopictus,* but those are mostly epidemiological indexes (e.g. Carrieri et al., 2012; Aryaprema et al., 2023). We believe that the strength of the POT method lies in its broad interpretability and applicability. This approach can be employed not only in spatial epidemiology applications, as demonstrated in our case study but also in monitoring alien species or supporting biological conservation efforts. It enables the identification of locations and periods when a particular population falls below or surpasses a defined threshold, signalling the need for targeted local interventions.

4.3 Limitations and future perspectives

As for most ecological models, one of the main limitations of these results relies on the quantity and quality of the training dataset (Cayuela et al., 2009). First of all, egg observations were pooled from ovitraps having different volumes, shapes, oviposition substrates, liquid solutions, revisit times, etc. (Da Re et al., 2023b). This, unfortunately, is a limitation related to the different sampling and monitoring schemes employed by the different

institutions (Da Re et al., 2023b). Despite the preprocessing operation on the observations collected by the ovitraps, some of these sources of variability have likely influenced our results and therefore should be taken into account while interpreting the results. Therefore, we want to highlight the importance of carrying out reproducible and comparable sampling schemes following the most updated standards for ovitraps monitoring, as those presented as the outcome of the AIM-COST cost action by Miranda et al. (2022).

Our dataset is also spatially biased because most of the observations are spatially clustered in north-central Italy, especially in the Emilia-Romagna region, where one of the most consistent and long-lasting surveillance programs has been carried out since 2010. The observations coming from this region had likely the highest quality, having been sampled continuously every two weeks from 2010 onwards. Spatial clustering is known to bias the models' estimates and predictions (Sillero and Barbosa, 2020) and therefore has likely produced sub-optimal predictions in the southern part of the area of interest. This detrimental aspect of our outputs can only be resolved by increasing the sampling effort in the southern part of the study area.

Another potential source of variability in the training dataset is the effect of vector control practices affecting the abundance of collected eggs. Pest control agencies act to limit the abundance of the species and reduce the nuisance the bites are causing to the population (Ravasi et al., 2021). Unfortunately, this is an effect that we cannot control, as we do not have access to the location and period of each pest containment treatment carried out in the area and period of interest.

Despite these limitations, the proposed framework seems feasible to be implemented to produce both local and continental scale predictions and forecasts, contributing to supporting the stakeholders in their effort against *Ae. albopictus*. Using e.g. regional circulation models and/or weather generators, the methodology presented here can be used to produce estimates for the next seasons under different climatic scenarios. Interestingly, the presented methodology can be implemented and corrected during the season by including the results of the monitoring activities in the training dataset. The estimates of these models can also be compared to estimates produced by other correlative models (e.g. Georgiades et al 2023), or mechanistic models such as `albopictus` (Erguler et al., 2016), and `dynamAedes` (Da Re et al., 2022), producing a plethora of models' estimates accounting for different aspects of the biological system studied.

By employing the results of our modelling approach, public health authorities can make informed decisions regarding the implementation of control measures, allocation of resources, and targeted interventions to mitigate the risks posed by invasive mosquitoes and safeguard human and animal health. The latter aspect has gained particular interest during the past two decades when the impact of invasive species on public health has become more evident (e.g., Zink et al., 2012; Schaffner et al., 2020). Apart from the public health aspect, we believe our work has a broader scope, providing a tool that can be adapted to infer the spatio-temporal abundance and seasonality of different species of interest.

5. References

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Supplementary materials 1

1.1. Environmental Covariates

We selected three main environmental drivers that can significantly influence the behaviour and development of mosquitoes, namely temperature, photoperiod and precipitation (Toma et al., 2003; Becker et al., 2010; Roiz et al., 2010, 2011; Romiti et al., 2021; Carrieri et al., 2023).

As ectothermic organisms, warmer temperatures generally promote faster development and increase the overall metabolic rate of mosquitoes, leading to shorter life cycles and higher population growth rates (Delatte et al., 2009; Pumpuni et al., 1992; Waldock et al., 2013; Marini et al., 2020). We downloaded the ERA5Land (Muñoz-Sabater et al., 2021) dataset representing the hourly gridded estimate of mean temperature over Europe for the period 2010-2022 and computed the weekly median temperature for each grid cell using the R function `terra::app()`(Hijmans 2023). By aggregating weekly, we observed that the aggregated values tended to be relatively high during the European colder months (November-February). To better capture the seasonality of mosquito phenology and avoid predicting oviposition during the winter season, a threshold-like variable was created by setting all temperature values below 15 °C to zero. By applying this modification, temperatures below 15 °C were essentially associated with zero or low egg abundance. This manipulation helps ensure that the model focuses on the temperature conditions more relevant to mosquito activity and development, which are typically associated with warmer periods. Applying this modification to temperature might appear as a strong assumption, given that *Ae. albopictus* is capable of completing its life cycle and surviving within a wide temperature range (Reinhold, Lazzari and Lahondère, 2018). However, several studies suggest that the optimal temperature range to complete the life cycle lies between 15 °C and 35 °C, even though the lower developmental zero temperature for this species is reported to be around 10.4 °C (Reinhold, Lazzari and Lahondère, 2018 and reference therein; Marini et al., 2020; Petrić et al., 2021 and reference therein). Considering this biological evidence, setting a temperature threshold of 15 °C in the analysis aligns with the findings that *Ae. albopictus* optimal development and activity are more favourable within this temperature range (Marini et al., 2020).

Photoperiod refers to the duration of daylight in 24 hours. It plays a crucial role in regulating mosquito behaviour, particularly concerning their activity, feeding patterns, the timing of mating and egg-laying behaviours (Pumpuni et al., 1992). In fact, in the temperate strain of *Ae. albopictus,* changes in the photoperiod length trigger the laying of cold-resistant eggs commonly referred to as “diapausing eggs” (Thomas et al., 2012; Urbanski et al., 2012; Lacour et al., 2015; Diniz et al., 2017). Diapause refers to an insect species' evolutionary adaptation to overcome poor environmental conditions by passing through an alternate and inactive physiological stage. In the case of *Ae. albopictus*, the maternal photoperiod experienced at pupal and adult stage is the environmental stimulus implied to induce oviposition of “diapausing eggs” (Lacour et al., 2015). To account for the yearly variation in the weekly photoperiod length, we computed the daily photoperiod for each 9 x 9 km grid cell between 2010 and 2022 using the R function `geosphere::photoperiod()`(Hijmans 2021) and then computed the weekly median.

Precipitation is another environmental factor that can influence mosquito populations and their behaviour, as mosquitoes require standing water for their larvae to develop (Becker

et al., 2010; Roiz et al., 2010). Precipitation events can create or replenish breeding sites for mosquitoes, while heavy rain or storms can flush out eggs and larvae from the breeding sites or temporarily disrupt mosquito flying activity due to the impact of raindrops and unfavourable flight conditions (Koenraadt and Harrington, 2008; Caldwell et al., 2021). We downloaded the ERA5Land (Muñoz-Sabater et al., 2021) dataset representing the hourly gridded estimate of precipitation over Europe for the period 2010-2022 and computed the weekly cumulative sum for each grid cell using the R function `terra::app()`(Hijmans 2023).

For each of these three variables, we considered lagged values of these factors as well, since the mosquito life cycle can take several days or weeks to complete (Becker et al., 2010; Roiz et al., 2010). The lagged temperatures and photoperiod were calculated as the median value between the temperatures recorded in the current week (i), the previous week (i-1), and the week before that (i-2), whilst the lagged precipitation was computed as the cumulative value between the precipitation recorded in the current week (i), the previous week (i-1), and the week before that (i-2).

1.2 Explorative analysis and model building

We carried out an explorative analysis on a subset of the aggregated ovitraps to understand which are the main predictors to include in the stacked model. The subset of the aggregated ovitraps (n = 20) was randomly selected but with the constraint of having at least three years of observations.

The graphical inspection of the aggregated observations showed a regular pattern repeating approximately every year. Additionally, within any year, there are two sub-periods, disentangling the cold period (where no eggs are generally reported), from the warmer period, spanning from spring to fall, where the number of recorded eggs has a magnitude that seems affected by other factors than the seasonality. Therefore, we tested for environmental drivers associated with the species' biology (temperature, photoperiod, and precipitation), that influenced the time evolution of the observed eggs.

We included the seasonal and environmental variables in a regression model with autoregressive errors of order one, i.e. for the fitting we use the ordinary least squares (OLS) with AR(1) errors. We have attempted to capture the seasonality by employing Fourier's harmonics sine and cosine waves, with four waves in total. One pair of sine and cosine waves is responsible for capturing the annual pattern, while the other pair is utilized to account for the seasonality within a given year. Concerning the environmental predictors, we tested the inclusion of both weekly and weekly-lagged values (e.g. the median temperature recorded two and three weeks ago).

The model's structure with different predictors and lags was fitted on different aggregated ovitraps, and the best model formulation, i.e. that one displaying overall goodness of fit (in sample) R2adjusted ~0.75 and equally good forecast performance (out of sample), was selected as that one inferring the number of eggs as a function of temperature, photoperiod, and precipitation, all lagged by -2 and -3 weeks, the four Fourier's harmonics, and an autoregressive component based on the number of eggs observed at week t-1. The residuals from model fitting were approximately Gaussian in all cases.

The benefit of selecting the error term as AR(1) was two-fold: the residuals are mostly uncorrelated and with such parameterization, it is possible to rewrite the model as a genuine regression model, so the cross-validation requires minimum adjustments, and stacking

883 computing routines are readily available. Further generalisation to more involved ARMA
884 errors seemed to provide no extra benefit in terms of goodness of fit, and do not share the
885 same representation as the OLS-AR(1), making the cross-validation harder to implement
886 and trust.

Supplementary materials 2

2.1 Hyperparameters tuning

In machine learning, hyperparameters tuning is the process of selecting the optimal settings for configuration parameters that are not learned from the data. It is a crucial task because it improves model performance, avoids overfitting or underfitting, adaptability to different datasets, and generalisation to new data. For each algorithm, a hyperparameter space was defined and the tuning was conducted using a random search strategy with a 10-fold cross-validation. Due to computational constraints, we chose only specific hyperparameters to conduct the tuning, while for the remaining ones, the values are set to default. The hyperparameter space by algorithms is shown in Tab. S2.1.

Table S2.1: Hyperparameter space for the analysed algorithms

Algorithm	Hyperparameter	Type	Lower	Upper
Boosted Regression Trees (BRT)				
	n.trees	integer	200	10000
	interaction depth	integer	1	5
	cv folds	integer	5	15
Cubist				
	committees	integer	1	10
Extreme Gradient Boosting (XGBoost)				
	nrounds	integer	10	30
	max depth	integer	4	10
	eta	numeric	0.2	0.4
	subsample	numeric	0.9	1
	min child weight	integer	1	4
	colsample by tree	numeric	0.5	0.6
Random Forest (RF)				
	n.trees	integer	200	500
	mtry	integer	4	7

	min node size	integer	5	10

2.2 Stacked models parameters and summary results

Tab. S2.2 Parameters of the ensemble models

	Regression model		**Autoregressive model**	
	Estimate (SE)	**P-value**	**Estimate (SE)**	**P-value**
Intercept	0.854 (0.401)	p < 0.05	1.14 (0.239)	p < 0.05
xgBoost	-0.002 (0.014)	p = 0.874	-0.055 (0.014)	p < 0.05
RF	0.722 (0.016)	p < 0.05	0.056 (0.017)	p < 0.05
GBM	-0.068 (0.011)	p < 0.05	0.226 (0.009)	p < 0.05
Cubist	0.371 (0.017)	p < 0.05	0.782 (0.015)	p < 0.05

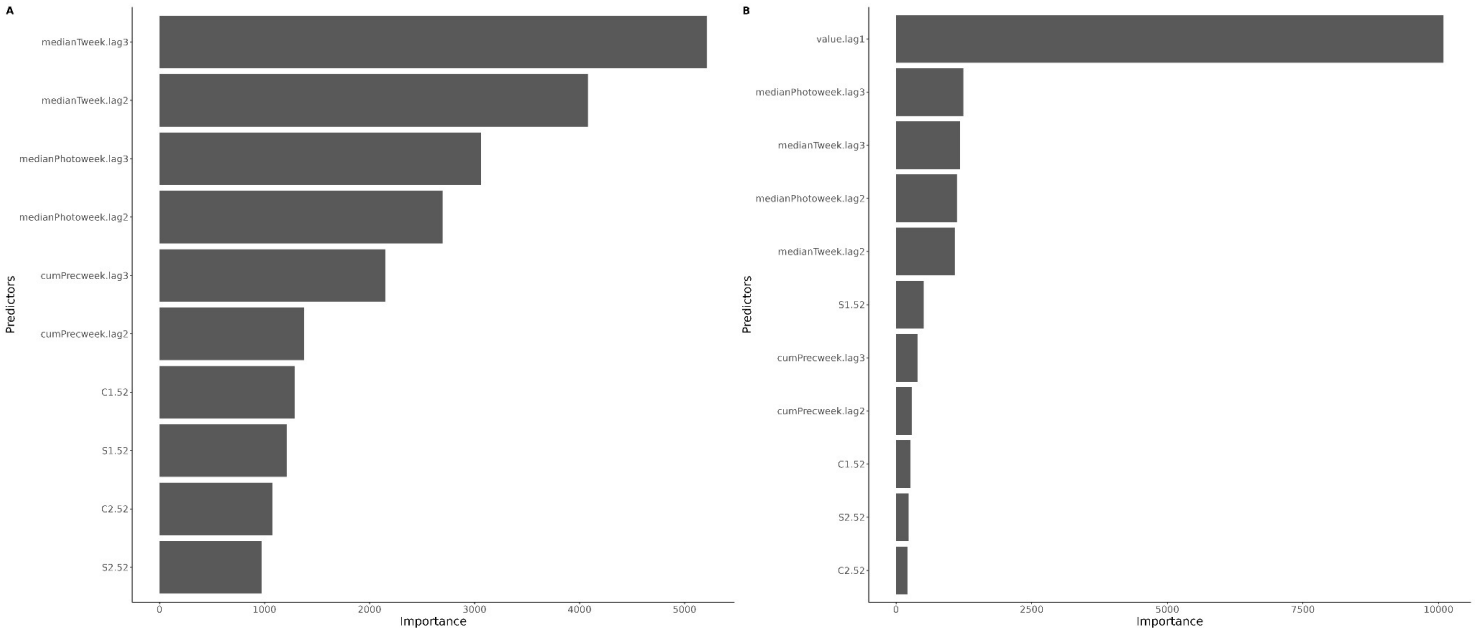

Fig. S2.3 Variable importance of the random forest base learner for the two model formulations: A) regression model and B) autoregressive model.

Supplementary materials 3

Internal and external validations

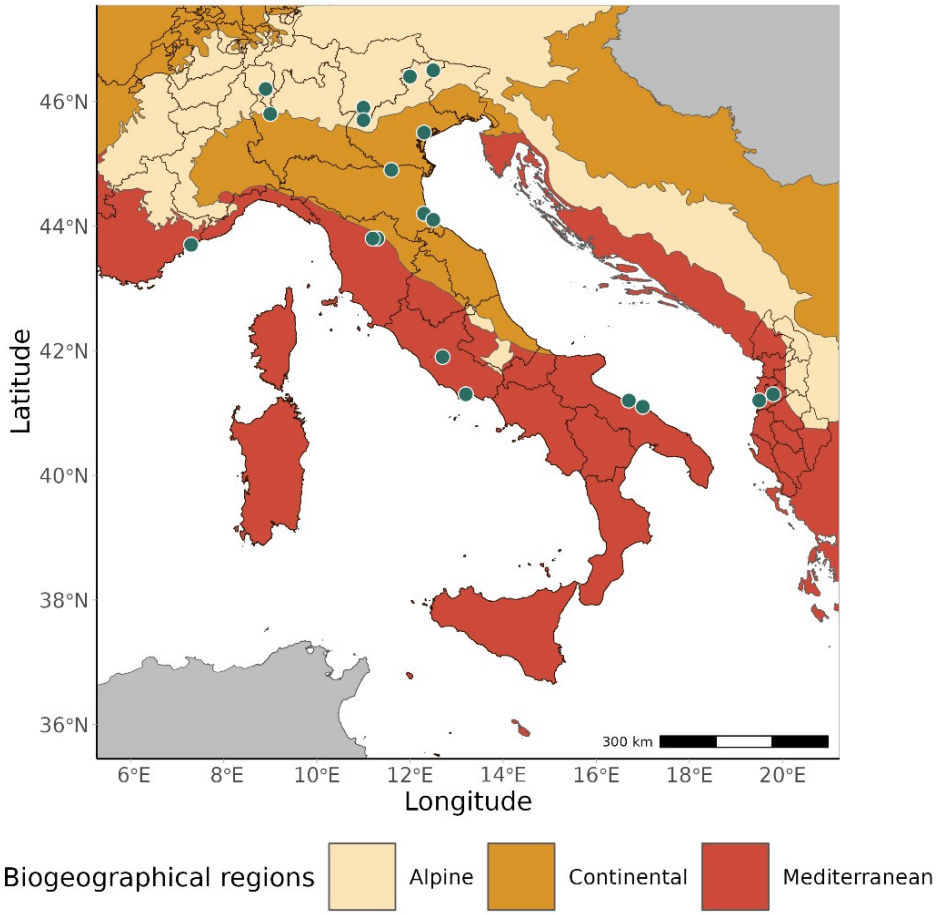

Fig. S3.1 Location of the aggregated ovitraps employed for the external validation.

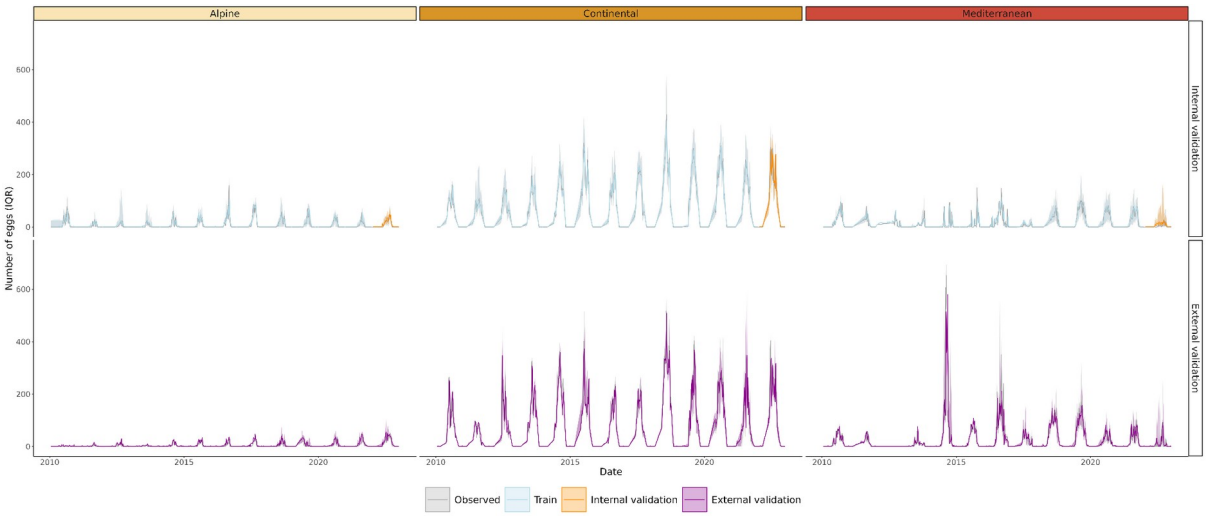

Fig. S3.2. The median and interquartile range of the number of eggs observed (grey lines), and predicted by the autoregressive model in both the internal and external validation. Both the observed and predicted values were aggregated over the three biogeographical regions to allow an easier representation.

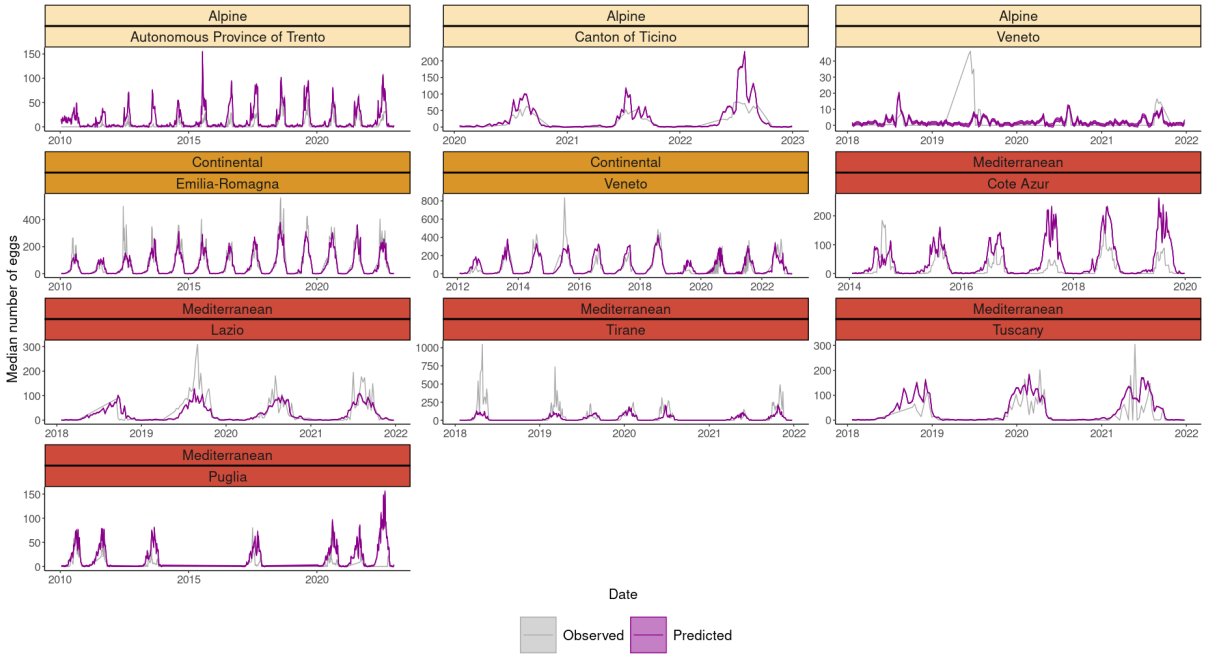

Fig. S3.3 The median and interquartile range of the number of eggs observed (grey lines) and predicted by the regression model in external validation. Both the observed and predicted values were aggregated over the NUTS2 levels to allow an easier representation.

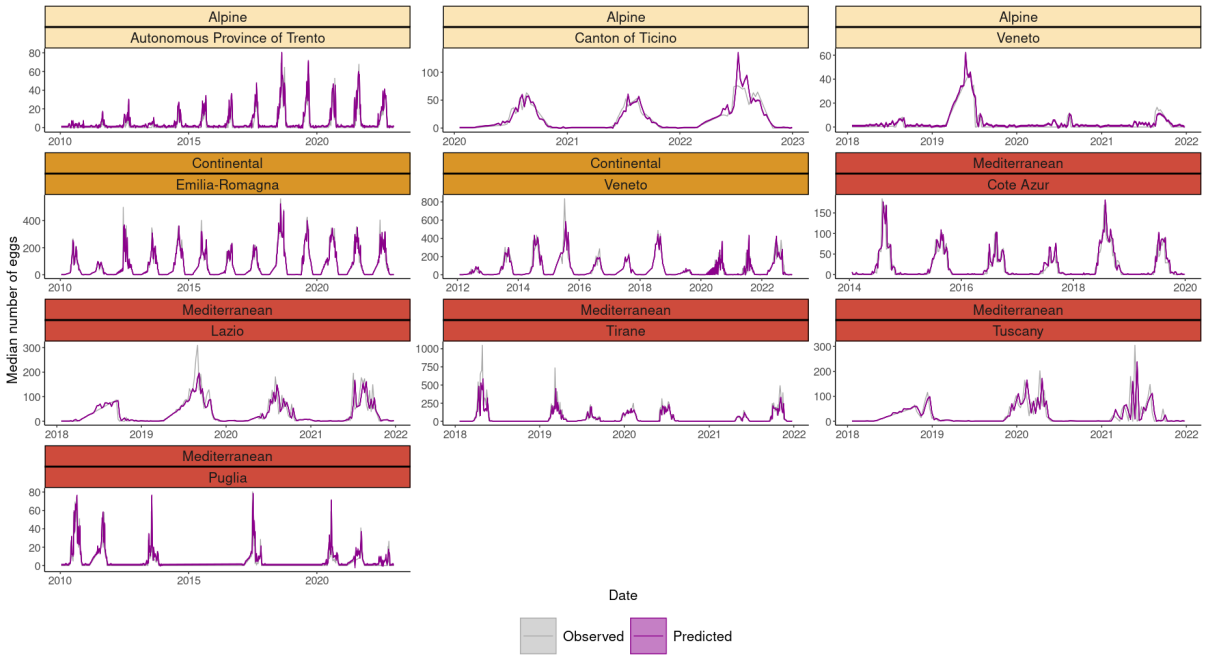

Fig. S3.4 The median and interquartile range of the number of eggs observed (grey lines) and predicted by the autoregressive model in external validation. Both the observed and predicted values were aggregated over the NUTS2 levels to allow an easier representation.

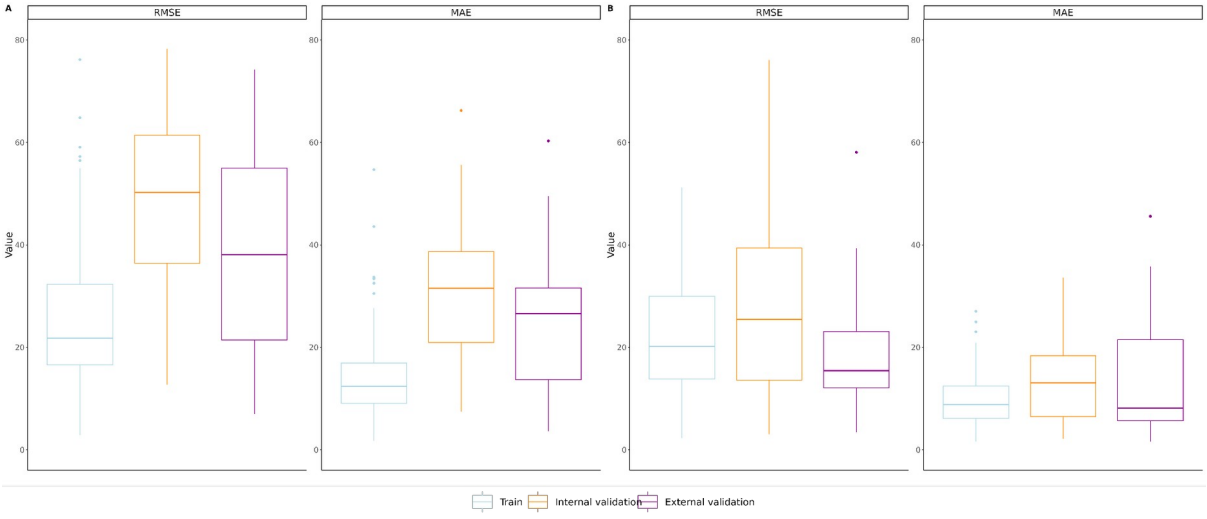

Fig. S3.5 Root mean squared error (RMSE) and mean absolute error (MAE) for the A) regression model (Eq. 1) and B) autoregressive model formulation (Eq. 2).

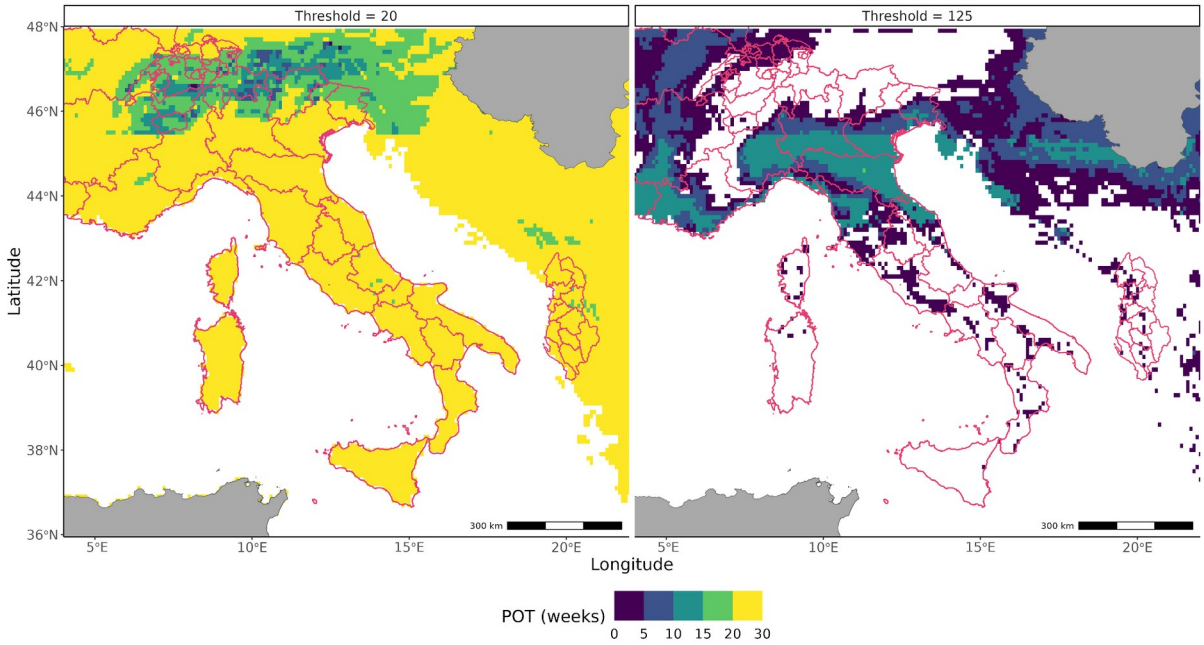

Fig. S3.6. Spatial representation of the interquartile range of period-over-threshold (POT) length for the year 2022 over the area of interest.

Tab. S3.7 Estimates coefficients and statistics of the period-over-threshold model (GLM with Poisson error).

	Observed		**Predicted**	
	Estimate (SE)	**P-value**	**Estimate (SE)**	**P-value**
Intercept	47.943 (27.718)	(p = 0.084)	-361.983 (1.018)	(p < 0.05)
year	-0.023 (0.014)	(p = 0.097)	0.18 (0.001)	(p < 0.05)
bgrContinental	-98.28 (29.559)	(p < 0.05)	318.68 (1.268)	(p < 0.05)
bgrMediterranean	-81.904 (36.298)	(p < 0.05)	263.636 (1.2)	(p < 0.05)
year:bgrContinental	0.049 (0.015)	(p < 0.05)	-0.157 (0.001)	(p < 0.05)
year:bgrMediterranean	0.041 (0.018)	(p < 0.05)	-0.13 (0.001)	(p < 0.05)

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