Effects of climate warming on the pine processionary moth at the southern edge of its range: a retrospective analysis on egg survival in Tunisia

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Abstract

In recent years, ectotherm species have largely been impacted by extreme climate events, essentially heatwaves. In Tunisia, the pine processionary moth (PPM), *Thaumetopoea pityocampa*, is a highly damaging pine defoliator, which typically lays eggs in summer. Its geographical range is expanding northwards in Europe while retracting from South Tunisia where summer temperatures can reach extremely high values. In this study, we aimed at exploring the effects of climate change on this species at its southern range edge. We investigated variations of fecundity and causes of egg mortality over time using historical and contemporary collections of egg masses from different Tunisian sites to seek relationships with regional climate change over three decades (1990-2019). Our results suggest negative effects of summer heat on egg survival, reflected in a decrease of hatching rate down to 0% in one site during a heatwave. Such a high hatching failure was found to result from both high egg sterility (our results did not allow distinguishing impeded mating success from failed egg maturation or early death of the embryo) and increased abortion of more developed embryos, but little effects of parasitism rate, thereby suggesting vulnerability to heat during embryonic development. We also observed decreasing female fecundity (*i.e.*, number of eggs laid per female) in regions where data were available both in the 1990s and the 2010s, which was associated with a decrease in parasitism rate, while the climatic variability increased. This study investigated direct hatching failure in nature that may be related to the magnitude of warming in summer. Previous studies have confirmed the thermal sensitivity of early instars of the PPM to temperatures observed in the present work, including one population from South Tunisia. However, further work is required to evaluate the relative importance of warming summers among populations because the risk of heat stress depends on the phenology of sensitive instars, and populations from the warmest areas may not necessarily be the most vulnerable to climate change if they already evolved phenological heat avoidance. In addition to heat-induced mortality, the ultimate fitness of individuals that survive challenging heat stresses during early developmental stages should also be explored to determine potential carry-over effects on subsequent life stages.

Keywords

Egg mass, heatwave, pine processionary moth, pinus, *Thaumetopoea pityocampa*, Tunisia

Introduction

During the period 1901-2010, land temperature has risen by 1.12 and 0.84°C in the Northern and Southern hemispheres, respectively (Jones et al., 2012). Climate warming~~This average climate warming already impacted phenology and distribution in many plant and ectotherm species~~ (Parmesan & Yohe, 2003) may induce heritable as well as plastic changes in a wide range of traits at multiple phenotypic levels (Pigliucci, 2001, 2005), such as (i) the timing of life cycle events such as flowering, migration and egg laying (Charmantier & Gienapp, 2014; Chuine, 2010; Chuine et al., 2013; Liu et al., 1995), (ii) the behaviour such as locomotor activity or movements to seek suitable microclimates (Pincebourde et al., 2021; Pincebourde & Woods, 2020; Woods et al., 2015), the morphology such as body size (Gardner et al., 2011; Ghosh et al., 2013; Sheridan & Bickford, 2011; Wu et al., 2019), or (iii) the physiology such as thermal acclimation or adaptation to thermal stressors. Those changes may ultimately translate into survival and in turn alter genetic frequencies, population density in given habitats, as well as distribution (Root et al., 2003). In the twentieth century, a wide range of taxa ranging from invertebrates to mammals and from grasses to trees have shifted their ranges poleward, upslope or both (Crozier, 2004; Hickling et al., 2005; Karban & Strauss, 2004; Parmesan et al., 1999; Parmesan & Yohe, 2003; Root et al., 2003; Walther et al., 2002).~~.~~ Climate change is also expected to increase temperature variability and, in turn, increase the magnitude and frequency of stochastic extreme thermal events, which is already increasingly observed over most parts of the world (Allen et al., 2012; Coumou & Rahmstorf, 2012; Fischer & Schär, 2010), particularly northern Africa (Fontaine et al., 2013; Nangombe et al., 2019; Zittis et al., 2021). In Tunisia, the mean average temperature has risen by about 1.4°C since 1901, with a remarkable increase in the last 30 years with an average warming of 0.4°C per decade, and most substantial warming has been observed during summer in southern regions where average maximum temperatures can often exceed 40°C (Verner et al., 2013). Together with average warming, increasing thermal fluctuations and extreme events may impact organismal fitness (Kingsolver et al., 2013; Thompson et al., 2013), and the~~their~~ combination of these is expected to dramatically impact survival, distribution and life cycles of many organisms.

The pine processionary moth (hereafter referred to as PPM), *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1776) (Lepidoptera, Notodontidae), is a highly damaging pest of pine forests across the circum-Mediterranean region (Carus, 2009; Démolin, 1969; Jacquet et al., 2013; Sbay & Zas, 2018). The geographic range of the PPM extends from northern Africa to southern Europe, from the Atlantic coast to the western part of Turkey (EPPO, 2004; Roques, 2015). The PPM is a well-documented~~ Lepidopteran~~ insect that has been acknowledged by the Intergovernmental Panel on Climate Change (IPCC) as one of the few species for which the causal relationship between climate warming and range expansion has been thoroughly proven (Battisti et al., 2005; Rosenzweig et al., 2007). The distribution range remained relatively steady until the late 1990s but then expanded towards higher latitudes and elevations in southern Europe. Indeed, warming winter temperatures have facilitated feeding in this winter-developing species and thus indirectly contributed to improving survival rate and growth rate in newly colonized areas (Battisti et al., 2005, Robinet et al., 2007).

Contrary to the beneficial effects of climate change demonstrated near the northern distribution edge of the PPM, adverse effects of climate change have been observed on the southern range edge (North Africa). Range retraction has been described in southern Tunisia and was found to result from increasing mortality rates of early life stages in a translocation experiment along a natural thermal gradient, which could be ascribed to local effects of climate warming (Bourougaaoui et al., 2021). Fecundity, hatching rate and predation at the egg stage (mostly from parasitoids) presumably play an important role in the PPM because this species is gregarious. Several studies have emphasized how larval performance depends on the realised group size, i.e. the number of neonates, and ultimately the survival of the whole colony until the end of larval growth (Clark & Faeth, 1997; Denno & Benrey, 1997; Ronnås et al., 2010). Colony density has been suggested to influence feeding activity and feeding efficiency of individual larvae, which is particularly critical in early stages when individuals have little desiccation and starvation resistances. The number of larvae was also shown to impact silk weaving activity to build and maintain the nests that shelter larvae during the day until their pupation in spring (Démolin, 1965; Martin, 2005). As a result, ~~Authors found that~~ the number of surviving tents and the average proportion of living larvae per tent were positively correlated to colony size (Pérez-Contreras et al., 2003; Roques et al., 2015). Focusing on the main drivers of colony size in early development is therefore of key importance to understand distribution changes and responses to climate change.

In Tunisia, the life cycle of PPM is generally univoltine, however it can extend over two years at high altitudes due to prolonged diapause in a fraction of the pupae (Roques, 2015). Flight periods are poorly documented, nonetheless a study conducted by Démolin and Rive in 1968 in high and medium latitudes, revealed that most individuals fly in the second half of July at high elevations and August to September at mid elevations (Ben Jamâa & Jerraya, 1999; Démolin & Rive, 1968). Due to the short lifespan of adults, egg laying occurs immediately after adult flights, and eggs and neonate larvae are presumably the instars that are most likely exposed to acute heat during the whole life cycle. Understanding the effects of warming on female fecundity, egg survival and egg parasitoids is crucial to explore the overall effects of climate change of this species at its southern range edge where warming is known to be of great magnitude.

In this study, we explored how climate warming over the last three decades may have impacted egg survival and hatching rate in Tunisia. To address this question, we combined historical and contemporary collections of egg masses originating from different Tunisian localities in the 1990s (1992, 1993, and 1995) and in the 2010s (2010, 2014, 2017, 2018, and 2019). Egg phenotypes and survival rate were investigated with regard to regional climatic features and contrasts analyzed from 30-year climatic data series across Tunisia. A cornerstone of this study is the identification of climate regions computed from multiple meteorological series, which allows comparing eggs from multiple sites within statistically consistent climates, instead of using arbitrary groups such as administrative regions. A grouping method was mandatory to analyze the long term data available on PPM eggs because exact sampling sites have changed over the years.

Materials and Methods

1 Historical data (1992-2014) and egg sampling done for this study (2017-2019)

A total of 755 egg masses from historical datasets and recent collections were analyzed in this study. Egg masses originated from 22 sites distributed across the PPM distribution in Tunisia (Table 1; Figure 1; Table SM1). Historical datasets on egg masses collected in 1992, 1993, 1995, 2010 and 2014 on Aleppo pine stands, *Pinus halepensis* Miller, were retrieved from institutional reports (unpublished data, INRGREF). These datasets report the length of egg masses, the number of eggs per egg mass, and the phenotype of individual eggs (parasitized, aborted, sterile, hatched). In addition, we collected egg masses in various locations in 2017, 2018, and 2019. All these egg masses were also collected on Aleppo pine stands, before hatching but as late as possible in each region to ensure eggs were exposed to natural conditions, and then and kept at ambient temperature (25 ± 2°C) at the INRGREF laboratory near Tunis where we followed a protocol similar to that used for historical collections of egg masses. Egg masses were kept individually in test tubes capped with cotton to allow ventilation. Egg hatching was checked daily. After a period of at least 40 days with no additional hatching, the protective scales that cover PPM egg masses were removed to observe individual eggs under a binocular magnifier and collect data similar to that available in historical datasets: length of egg masses, number of eggs per egg mass (fecundity), and egg phenotype. First, hatched eggs were distinguished from unhatched eggs based on the presence of the characteristic large jagged exit hole from which the neonate left the egg, and an empty transparent shell. Then, unhatched eggs were dissected to assess the cause of mortality (parasitized, *i.e.*, eggs with a small parasitoid exit hole and/or containing a dead parasitoid and/or containing parasitoid meconium; aborted, *i.e.*, dead embryo or dead PPM larva; and sterile, *i.e.*, undeveloped egg with dried-up yolk)

(Imbert, 2012). Parasitism rate was calculated taking into account both emerged parasitoids found in the test tubes and dead ones found inside unhatched eggs.

Table 1 . Collection of PPM egg masses in Tunisia (see Table SM1 for coordinates of the sites). Calculated climate clusters are indicated to represent the amount of data available per cluster.

Site	Year of collection	Number of egg masses	Cluster	Nearest meteorological station (within 100 km and 350 m in elevation); distance
Sejnane	1995	20	1	Béja; 51.0 km
Testour	2014	12	1	Béja; 26.3 km
Ain Jamala	2010	15	1	Béja; 26.5 km
El Krib	2010	18	1	Kef; 38.7 km
Henchir Naam	1992	53	1	Kef; 41.5 km
Jebel Kbouch	1993	56	1	Kef; 21.3 km
El Ayoun	1993	30	1	Kasserine; 73.2 km
Chaambi	1995	27	1	Kasserine; 30.3 km
	2014	11		
Thélepte	2017	43	1	Kasserine; 1.9 km
	2019	30		
Jebel Sidi Aich	2014	31	1	Kasserine; 32.7 km
Kesra	2010	18	1	Siliana; 20.8 km
Mghila	2014	51	1	Kasserine ; 71.6 km
Bir Lahfay	2014	12	2	Sidi Bouzid; 32.5 km
Jebel Motlag	2017	38	2	Sidi Bouzid; 20.6 km
	2018	29		
Jebel Rihane	2017	25	2	Sidi Bouzid; 25.7 km
El Fej	2017	18	2	Gafsa; 36.6 km
El Menzeh	1992	19	3	Carthage; 3.2 km
	1993	57		
	2014	10		
Cité Ettahrir	2014	21	3	Carthage; 7.9 km
Manouba	2010	15	3	Carthage; 10.5 km
Korbous	1992	30	3	Carthage; 32.5 km
	2010	15		
Dar Chichou	1995	20	4	Kelibia; 17.1 km
Oued Laabid	1995	31	4	Kelibia; 33.3 km

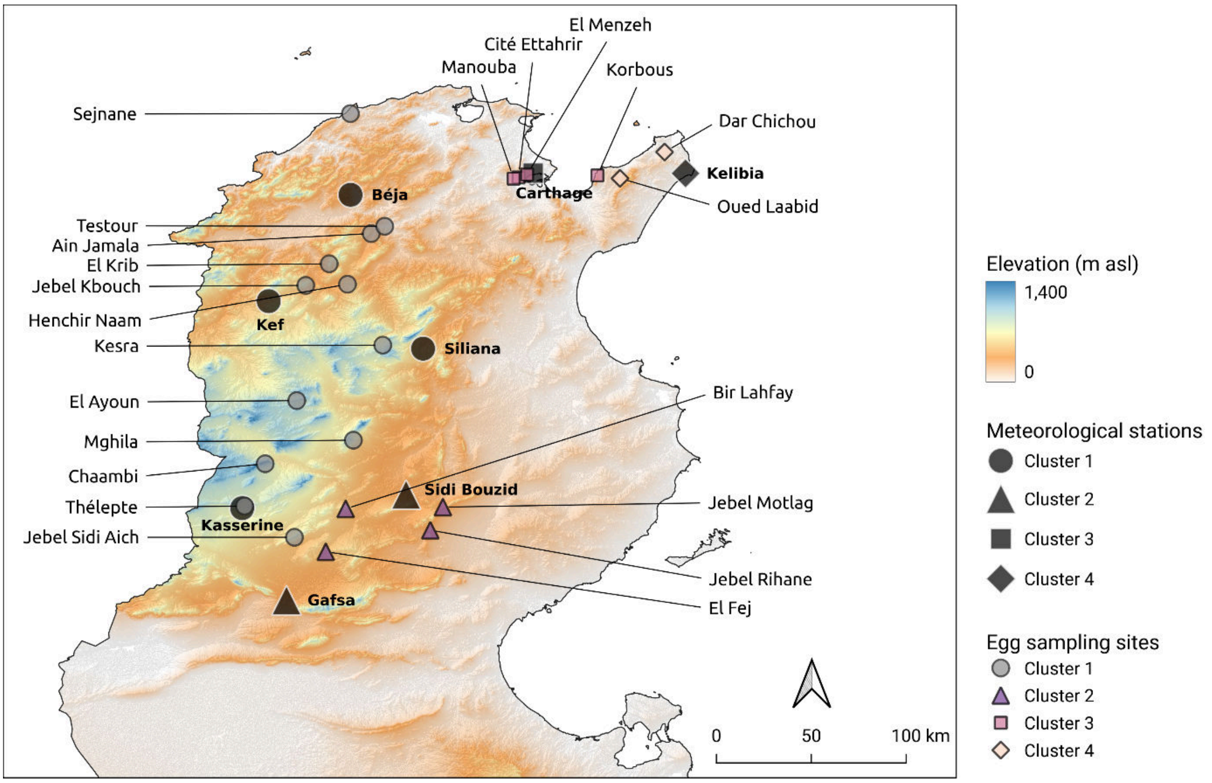


Figure 1. Location of egg sampling sites and meteorological stations, with associated calculated climate cluster.

2 Climate data

We used series of daily temperatures recorded (by the Institut National de Météorologie, INM, Tunis, Tunisia) in eight meteorological stations distributed within the PPM range in Tunisia (Fig. 1; Table SM2). To fill missing data in INM time series, satellite measurement of daily temperatures were also retrieved from the NASA Prediction of Worldwide Energy Resources website (https://power.larc.nasa.gov/data-access-viewer/) on the grid cells of 0.5 degree × 0.625 degree (~ 50~~8~~ km × 60~~8~~ km) matching the location of INM weather stations (Table SM2). The similarity of both sources of data was evaluated using Pearson correlations tests for daily maximal and daily minimal temperatures in Tunis, where the data series from INM since 1990 was the most comprehensive. Daily maximal and minimal temperatures from both data sources were found to be strongly correlated (Pearson tests, r= 0.95, p < 0.001 and r = 0.94, p < 0.001, respectively). The two types of datasets where therefore combined in case of missing data in other INM series to reconstruct uninterrupted series for the period 1990-2019 (Table SM2).

Each site of egg sampling was assigned to the nearest meteorological station (< 100 km in all cases) among those situated at an elevation within 350 meters of the egg site, an arbitrary threshold we chose to mitigate potential climatic differences along elevation gradients (Table 1, Figure SM3).

To better understand climatic features in each of the eight meteorological series (Table SM2), (i) the normal daily temperatures with seasonal contrasts over the period, as well as (ii) the overall trend since 1990, were calculated. For (i), we averaged 30 years of daily maxima (TX) and minima (TN) by day of the year, and calculated the likelihood for each day of temperatures below 0 or above 32 and 40°C, which have been suggested by (Démolin, 1969) and Huchon & Démolin (1970) as pivotal thresholds for phenological strategies and survival in the PPM (see also discussion in Robinet et al.~~Huchon & Démolin (1969) as pivotal thresholds for phenological strategies and survival in the PPM (see also discussion in Robinet et al.~~ 2015). For (ii), daily TX and TN were averaged per year and represented along the 30 years of data, together with the total number of days below 0 or above 32 and 40°C. Those per-station climate summaries are provided in SM4.

3 Statistical analyses

Climate clusters. The unbalanced egg sampling design throughout historical data and recent collections prevents allochronic comparisons of egg phenotypes within individual sampling sites. Therefore, we investigated climatic similarities and dissimilarities among meteorological series in order to identify regional climate clusters within which multiple meteorological series and associated egg sampling sites could be statistically grouped together. Climate-based grouping appeared more relevant and less arbitrary than using administrative regions because of the heterogeneous landscape and overall size of some regions. To do so, the monthly averages of TN and TX were calculated in each meteorological series over the period 1990-2019, resulting in a set of 24 variables (2 × 12 months) and 30 values per series (30 years). A Principal Component Analysis (PCA) was used on the covariance matrix of those variables to project the 30 years of data from each of the eight meteorological locations and better visualize their intra- and inter-group variance on reduced dimensionality. The resulting multivariate object then fed a K-medoid clustering analysis using the PAM method (Partitioning Around Medoids, see Reynolds et al. 2006, Schubert and Rousseeuw 2019) to identify relevant climate clusters (listed in Table1). The PCA could be performed on unscaled temperature variables since they were all measured in the same unit (covariance PCA), thereby giving most weight to summer months due to generally higher temperature values, without neglecting other months in the overall variance structure. As a consequence, the climate clusters identified using all four seasons are mostly influenced by the season eggs are exposed to (roughly June to September). Details of cluster assignation to individual points in each meteorological series are detailed in SM5. Monthly means of TN and TX of the medoid of each cluster, *i.e.*, the individual point that best represents its cluster due to low average dissimilarity to all other points, are represented in SM6.

Interannual fluctuation of maximal summer temperature within clusters. To explore regional warming trends to which eggs are subjected within clusters over 1990-2019, the monthly means of daily maximal temperatures from meteorological series within each cluster were calculated from June to September. A linear model was then built for each cluster and each month to plot regressions over time and determine the slope for each cluster. The adequacy of residuals to Normality was checked using QQ plots. ~~Finally, the four months were averaged together per cluster and period to obtain the mean maximal summer temperature in each cluster and period. Then, a two-way ANOVA followed by a Tukey *post-hoc* procedure was performed to test the effects of cluster and period on the maximal summer temperature.~~

Egg phenotype comparisons. Egg phenotype variables did not meet assumptions of homoscedasticity and normality for parametric tests, therefore we used the non-parametric test (RANCOVA) proposed by Quade (1967) to compare eggs sampled within each of the four clusters in the 1990s or in the 2010s. First, the response variables (Fecundity, Hatching, Sterility, Abortion and Parasitism rates, Clutch length was discarded due to its high correlation and redundancy with Fecundity) and the covariate (monthly means of TX averaged from June to September per year per cluster) were ranked separately. Second, residuals from the respective linear regression of each ranked response variable on the ranked covariate were calculated. Third, the effects of grouping factor(s~~factors period (1990s vs 2010s) and Cluster (1, 2, 3, 4~~) on residuals were investigated for each response variable using the Quade's RANCOVA. Since~~When a significant interaction was found, we reported~~ the dataset is unbalanced, with both past and recent samples~~pairwise comparisons~~ in only two of the four climate~~dependent variable between~~ clusters, we conducted two separate Quade's RANCOVA analyses: ~~at each period level, as well as between period levels for each cluster using pairwise t-tests with Bonferroni corrections for multiple testing. When no significant interaction was found between period and cluster, a Tukey post hoc procedure was used to investigate pairwise differences between clusters.~~

- Analysis (1) was conducted on a subset of the data containing only clusters 1 and 3 (where past and recent samples exist), in which we included both Cluster and Period factors as well as their interaction. When the interaction was significant, pairwise comparisons were performed using pairwise t-tests and Bonferroni correction.
- Analysis (2) synchronically compared clusters within each period subset, i.e., all clusters were considered but compared only to other clusters during the period(s) they shared. A Tukey

post hoc procedure was used to investigate pairwise differences when a main effect was found.

Results

1 Climate clusters

The first plane (PC1 × PC2) of the PCA performed on climatic data from all eight meteorological series based on monthly averages of TN and TX each year (n = 8 × 30 = 240 data points) accounted for 71.28% of the total inertia (Fig. 2). PAM clustering on the PCA scores indicated four relevant groups with little overlapping (Fig. 2). Cluster 1 grouped Kef, Kasserine, Siliana and Béja together, cluster 2 grouped Sidi Bouzid and Gafsa together, while cluster 3 and cluster 4 corresponded to single meteorological series, Carthage and Kélibia, respectively. Depending on the meteorological series, between 76.67 and 100% of data points (years) were correctly assigned to their cluster (SM5).

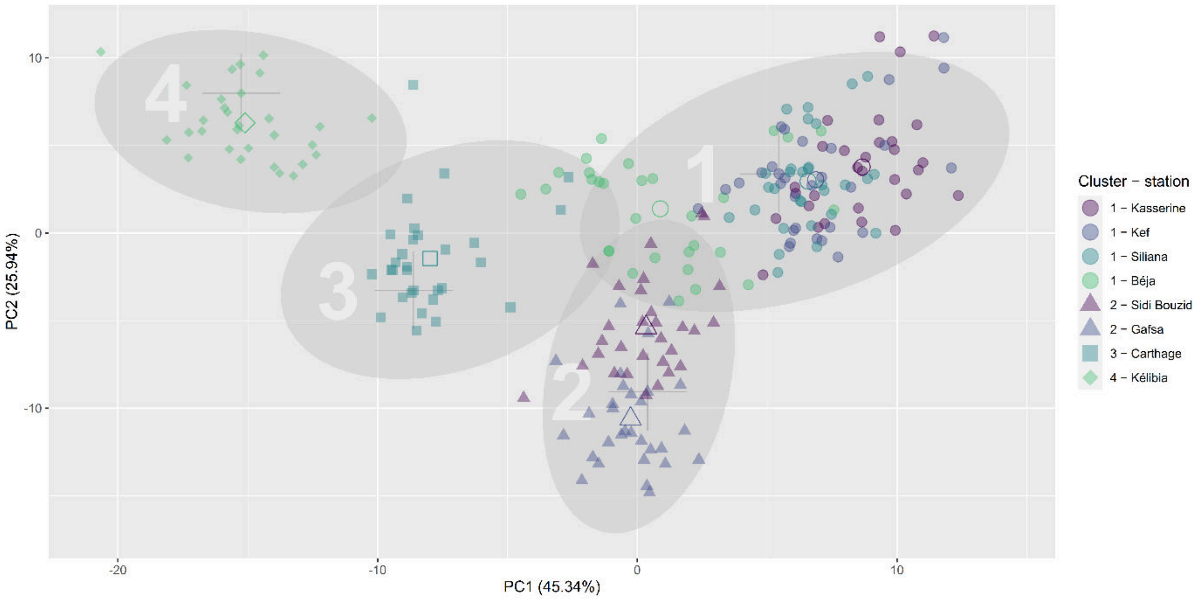
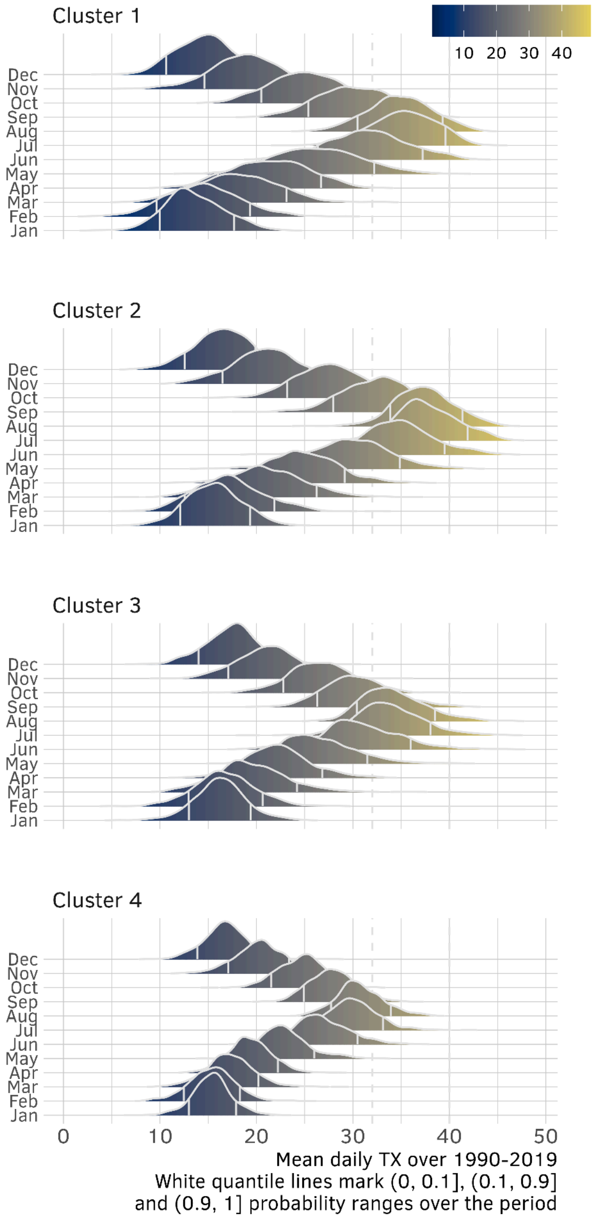


Figure 2. PCA scores for each year of data from the eight meteorological series (INM and NASA series, see table SM2) and 24 TN monthly average and TX monthly average variables. Each of the eight series are identified with different symbol and colour combinations. PAM clustering results are overlaid on the PCA scores with 95% confidence ellipses and different symbols for different clusters. Open points correspond to the centroids of each meteorological series, while large thin crosses mark the medoid point of each cluster.

Per-cluster climate reconstructions averaged from daily~~monthly~~ means of TX over years ~~in each cluster~~ (Fig. 3) indicated comparatively cold winters and hot summers with a high interseasonal variability in cluster 1, ~~a shift to~~ warmer winters and summers in cluster 2 with similar interseasonal variability, no extreme winters or summers and lower interseasonal variability in cluster 3, and the lowest interseasonal variability with comparatively mild summers in cluster 4. Within-month variability also appeared to be the highest over the last 30 years in clusters 1 and 2. The probability to overreach 40°C in summer was found to be the highest in cluster 2, while cluster 4 showed the lowest probability of overreaching 32°C, with clusters 1 and 3 sitting in between those extremes. July and August are the warmest months in all clusters (Fig 3, Fig 4). TN monthly average within each medoid appeared to roughly reflect TX monthly average across each months of the year (SM6~~, SM9~~), indicating that similar trends can be inferred for per-cluster TN monthly average.


Cluster 1
Cluster 2
Cluster 3
Cluster 4
10
20
30
40
Dec
Nov
Oct
Sep
Aug
Jul
Jun
May
Apr
Mar
Feb
Jan
0
10
20
30
40
50
Mean daily TX over 1990-2019
White quantile lines mark (0, 0.1], (0.1, 0.9]
and (0.9, 1] probability ranges over the period

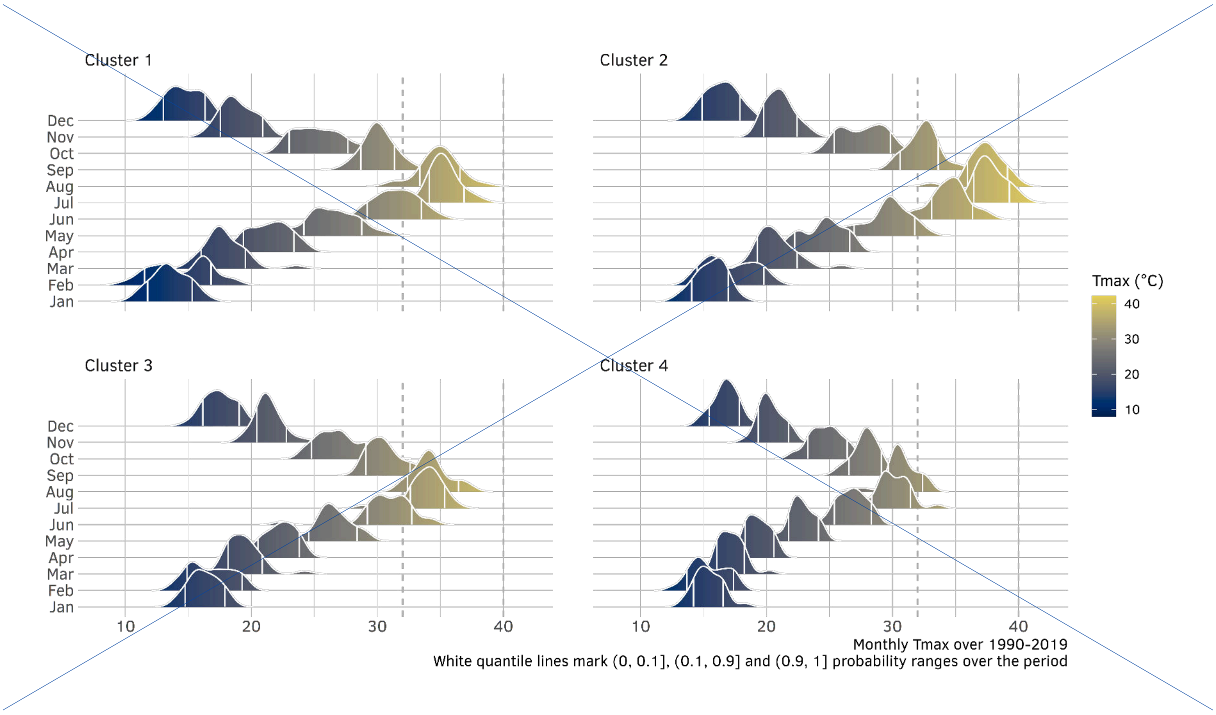
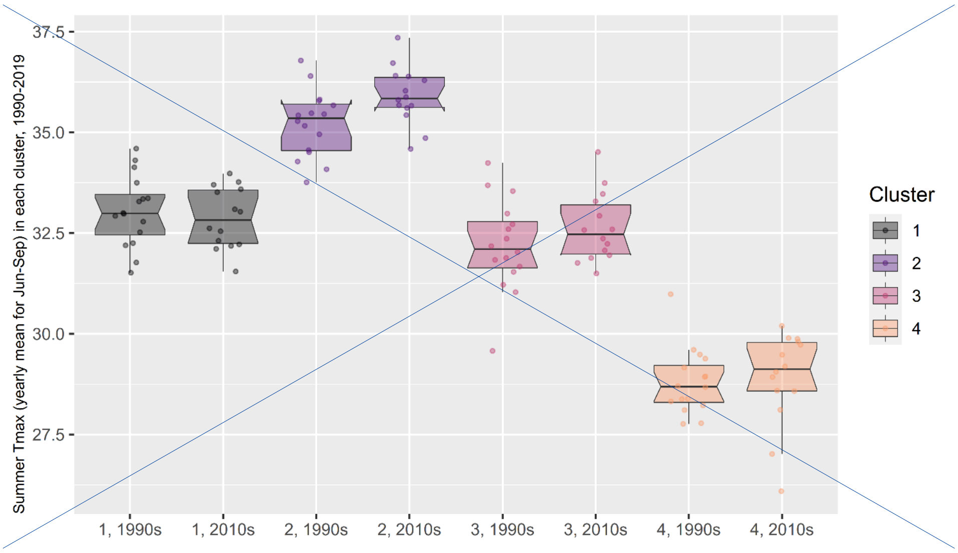


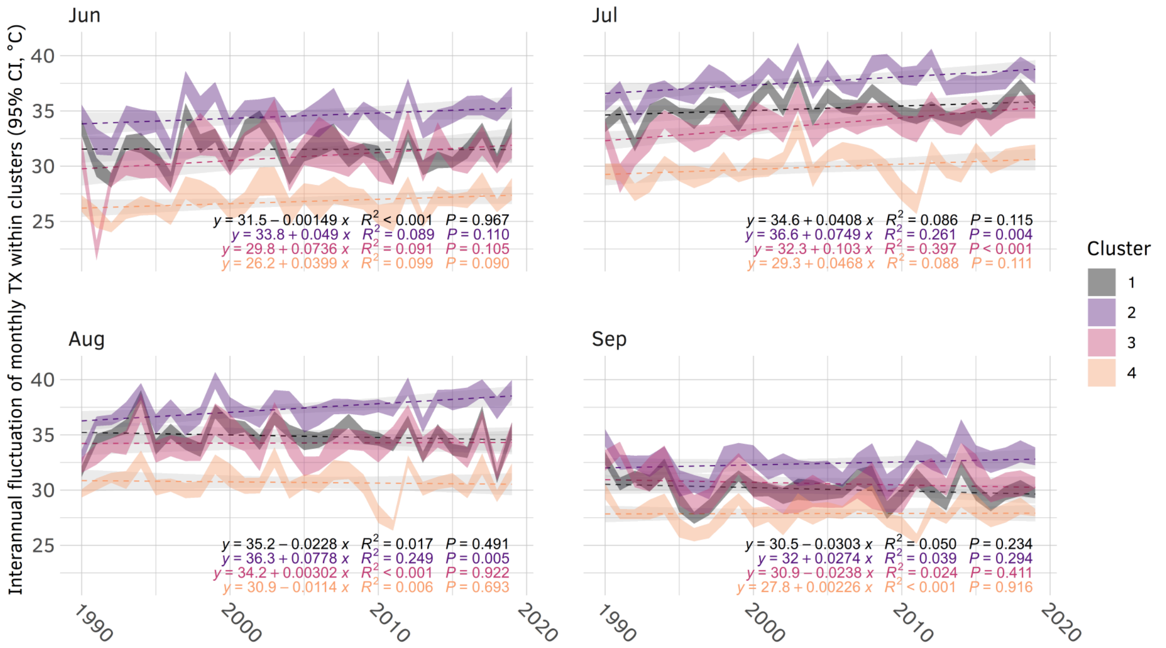
Figure 3. Ridge lines showing the distribution (kernel density) of daily~~monthly~~ means of TX over 1990-2019 for each cluster. The area of each distribution equals 1 and white vertical lines mark 0.1 and 0.9 quantiles.

~~Averaging TX monthly means (from June to September each year) within clusters and periods (Fig. 4) confirmed significant differences among clusters (ANOVA, Fdf = 275.2453, p < 0.001; significant differences in all pairs of clusters but 1 and 3 (Tukey post hoc, p < 0.0001)). Period did not influence this summary summer variable significantly despite an increasing trend in clusters 2, 3 and 4 (ANOVA, Fdf = 2.5111, Fig. 4), and the interaction term between cluster and period was not significant (ANOVA, Fdf = 2.5111, p = 0.116).~~



~~**Figure 4**. Maximal temperatures averaged from June to September each year, within each cluster and period. Year points in each group are slightly jittered along the x-axis to ease visualization. Cluster was the only significant factor, but period sub-groups are represented to reflect the two-way ANOVA we performed.~~

Fluctuations of monthly averaged TX during summer over the last three decades (Fig. 4~~5~~) in each cluster indicated that September has not warmed significantly over time (no apparent slopes, non-significant linear regressions). Contrariwise, July got significantly warmer over time, particularly in cluster 2 and 3 (positive slopes, significant linear regressions). Cluster 2 appeared as the cluster with the most striking summer warming, with positive increases of TX monthly average spanning not only July, but also June (non-significant trend (p =~~<~~ 0.11~~1~~), similar to cluster 3) and August (significant increase). Contrariwise, summers in clusters 1 and 4 appeared as the least subject to warming since 1990.



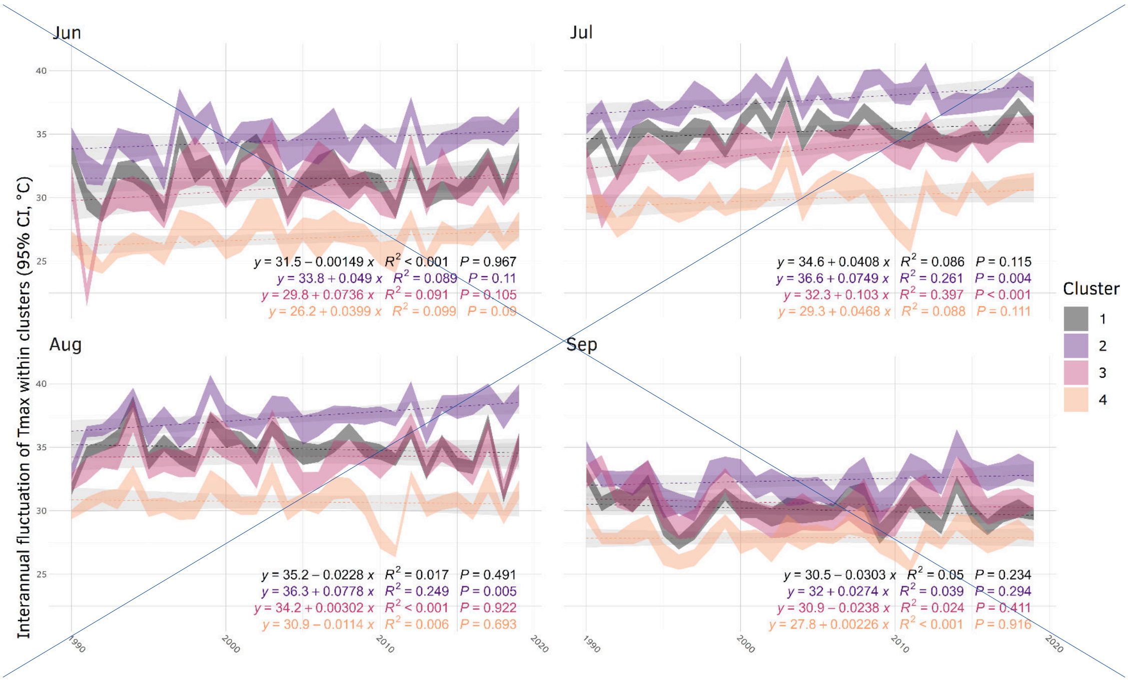
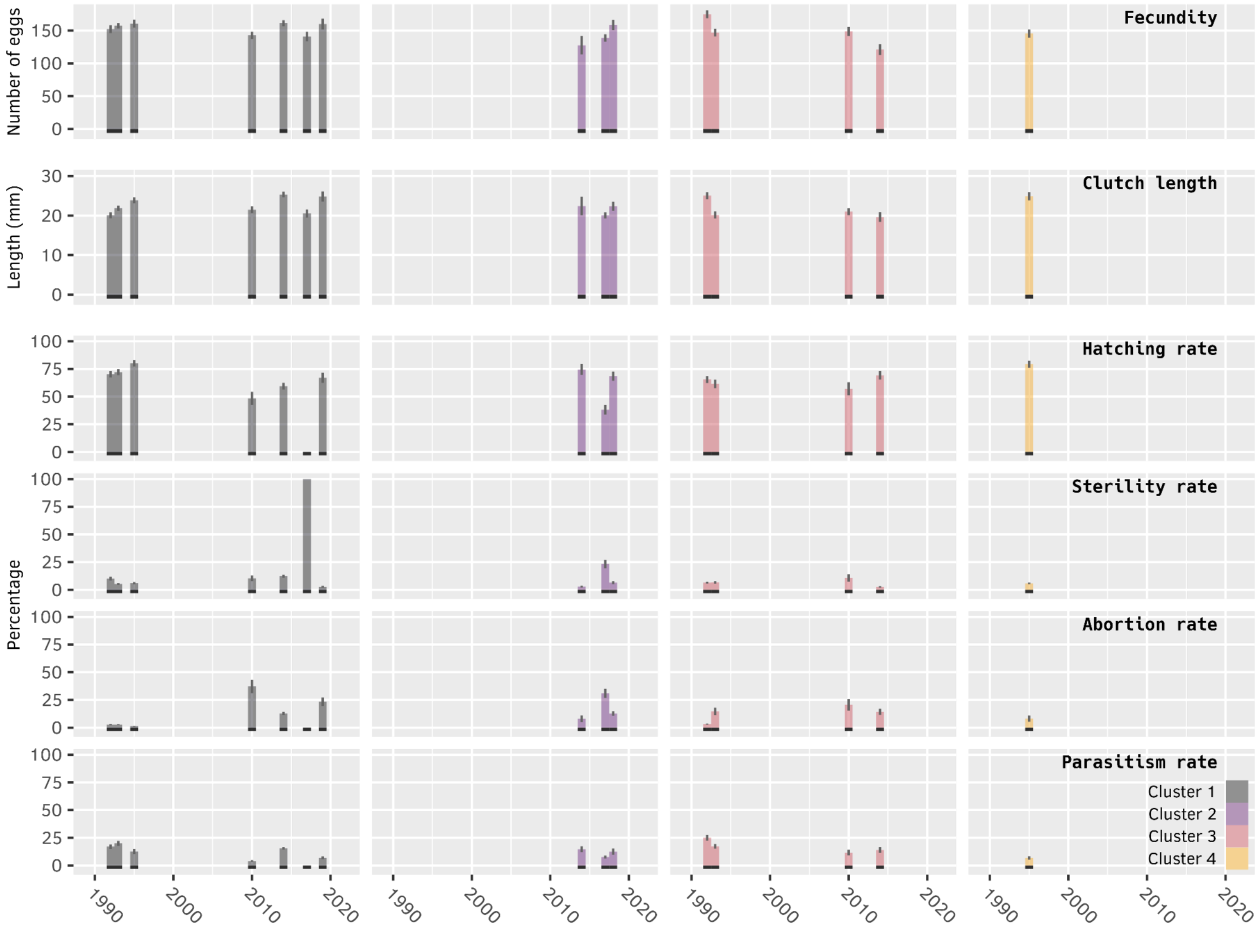


Figure 4~~5~~. Fluctuations over time of monthly maximal temperature within each cluster, from June to September. The upper and lower lines of each ribbon correspond to 95% confidence intervals.

2

~~3~~ Egg comparisons

Clutch size.~~Fecundity and hatching rate.~~ The length of egg masses was measured in case the distance between eggs of a clutch would differ among areas or periods, but this variable was found to be~~significantly and~~ highly positively correlated to fecundity (Spearman correlation test, rs = 0.72, *p* < 0.001), thereby leaving little room for variations in the fecundity/length ratio. Therefore,~~therefore~~ we focused further analyses on fecundity only, which~~. Fecundity across clusters and years~~ ranged from 121 ± 8.2 SE to 174.6 ± 5.9 SE among clusters and periods (Figure 5). Analysis (1) conducted on clusters where both past and present samples have been collected (clusters 1 and 3) showed no significant difference in fecundity between those two ~~6). Fecundity did not differ significantly between~~ clusters (Quade's RANCOVA, F_{df} = 2.88$_{1}$~~$_{363}$~~, p = 0.09) or ~~071) and between~~ periods (F_{df} = 3.01$_{1}$~~$_{521}$~~, p = 0.083~~61~~). However, ~~there was~~ a significant crossover interaction suggested non-parallel trends over time between them~~(*i.e.*, the effect of periods on the fecundity is opposite, depending on the value in each cluster)~~ (Quade's RANCOVA, F_{df} = 6.42$_{1}$~~$_{411}$~~, p = 0.012). Indeed, cluster 3 was the only cluster where fecundity changed over time (pairwise t-test, t_{df} = 3.1$_{1}$, p = 0.002 adjusted by Bonferroni correction), with an average decrease of 16 %. Analysis (2) performed on all clusters but synchronic periods revealed no ~~) suggested that significant~~ differences among clusters 1, 3 and 4 in the 1990s (Quade's RANCOVA, F_{dt} = 1.58$_{2}$, p = 0.208), whereas it confirmed significant differences among clusters 1, 2~~can be found in at least one of the two periods,~~ and 3 in the 2010s (F_{df} = 4.40$_{2}$, p = 0.013).~~evolved differentially over time.~~ Specifically, ~~no differences were found among clusters in the 1990s, whereas~~ fecundity was significantly higher in cluster 1 than cluster 3 (Tukey post hoc, p = 0.009).~~in the 2010s (pairwise t-test, t_{df} = 3.2$_{2}$, p = 0.005 adjusted by Bonferroni correction). Cluster 3 was the only cluster where fecundity significantly changed over time (t_{df} = -3.1$_{1}$, p = 0.002), with a decrease of 16 %.~~



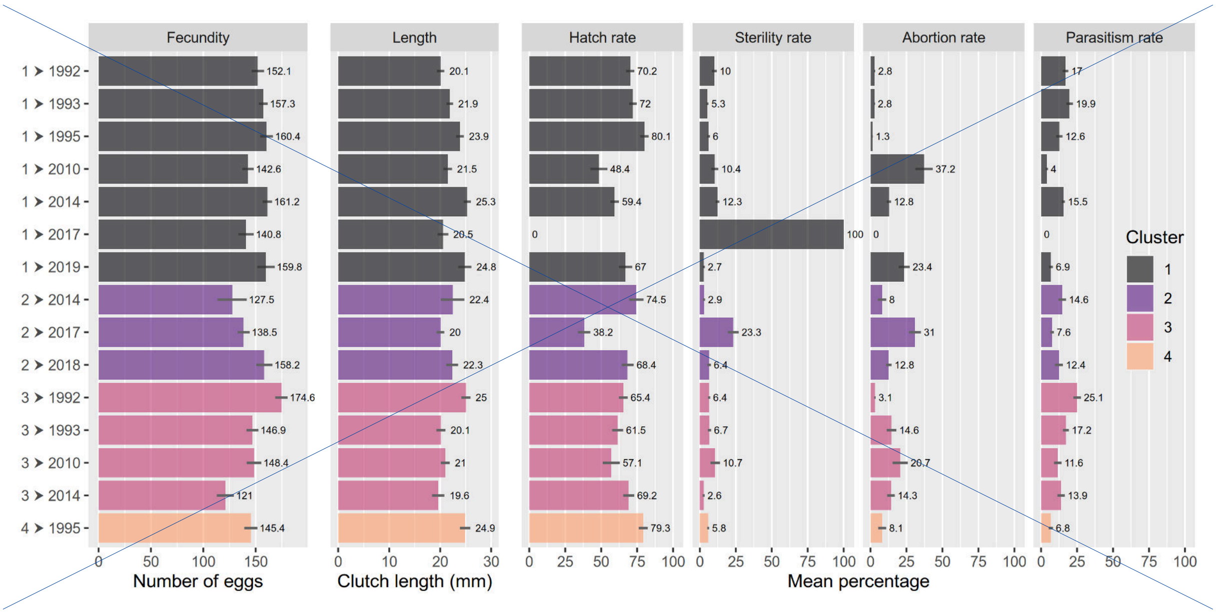


Figure 56. Fecundity (raw count of eggs), clutch length, hatching rate, sterility rate, abortion rate and parasitism rate per cluster and sampling year. Black markers at the bottom show years when samples have been collected, to distinguish true zeros from missing values.~~in each egg sampling site and sampling year.~~ Error bars: SEM.

~~Hatching rate significantly differed between clusters (Quade's RANCOVA, F_{df} = 3.8$_3$, p=0.01) and between periods (F_{df} = 24.9$_1$, p <0.001). As found for fecundity, differences in hatching rate among clusters varied between the 1990s and the 2010s (Quade's RANCOVA, F $_{df}$ = 21.7$_1$, *p* < 0.001). Hatching rate was significantly lower in cluster 3 than cluster 1 (pairwise t-test, t_{df} = -4.7$_2$, p < 0.001 adjusted by Bonferroni correction) and cluster 4 (t_{df} = -3.6$_2$, p = 0.001) in the 1990s, and higher in cluster 2 than cluster 1 in the 2010s (t_{df} = 2.4$_2$, p = 0.05) (Table 2). The mean difference between periods showed that hatching rate in cluster 1 was significantly higher in the past (t_{df} = 6.7$_1$, p < 0.001) (Table 2). Particularly, in 2017, hatching rate dropped to zero in cluster 1, and largely decreased in cluster 2 compared to other years (Figure 6).~~

~~**Table 2.** Descriptive statistics: observed mean (M), Quade's adjusted mean (Madj) and associated standard error (SE) for the different response variables.~~

Egg mortality factors. Base abortion and parasitism rates differed significantly between clusters 1 and 3 (Analysis (1), Quade's RANCOVA, abortion rate: F_{df} = 17.13$_1$, p < 0.001; parasitism rate: F_{df} = 4.53$_1$, p = 0.034) and periods (abortion rate: F_{df} = 50.92$_1$, p <0.001; parasitism rate: F_{df} = 20.36$_1$, p < 0.001). However, the temporal increase in abortion and decrease in parasitism were similar in the two clusters since no significant interaction was observed (abortion rate: Fdf = 0.08$_1$, p = 0.776;

parasitism rate: $F_{df} = 1.34_1$, $p = 0.248$). Yet, in 2017, hatching rate dropped to zero in cluster 1 with all eggs visually scored as sterile. Consequently, abortion and parasitism rates were also the lowest (0 %) in this cluster that year since those mortality factors can only occur at a later development stage (Figure 5, Table SM7). Cluster 1 was the only cluster where such a dramatic change was observed, and where hatching rate changed significantly between the past and recent periods (Analysis (1), pairwise t-test, $t_{df} = 7.5_1$, $p < 0.001$ adjusted by Bonferroni correction). This average decrease over time can be attributed not only to the striking drop in 2017, but also to decreases of lower magnitude in other recent years (Figure 5). Those changes correlate negatively with the aforementioned trends in abortion rate and sterility rate. Of the two cluster, the increase in abortion rate was most prominent in cluster 1 (Figure 5), although it may be underestimated in recent years due to its null value in 2017 when eggs did not develop enough to score abortion rate. Analysis (2) showed synchronic differences among clusters in the rates of hatched, sterile, aborted and parasitized eggs (see Tables 2 and 3 for details).

Finally, to evaluate the influence of the 2017 peculiarity on overall trends in cluster 1 and investigate other changes that may have been concealed by this heatwave, similar analyses (1) and (2) have been performed on a subset of the data without cluster 1 in 2017 (see descriptive statistics in SM8). Analysis (1) revealed that hatching and abortion rates still changed over time in the two clusters, to a lower extent than when considering data from 2017 in cluster 1 (hatching rate: $F_{df} = 8.2_1$, $p = 0.004$; abortion rate: $F_{df} = 7.6_1$, $p = 0.006$), whereas sterility and parasitism did not change in any of the two clusters (sterility: $F_{df} = 1_1$, $p = 0.315$; parasitism: $F_{df} = 0.3_1$, $p = 0.563$). Analysis (2) revealed different patterns than those found when considering data from cluster 1 in 2017, since no difference among clusters appeared in the 2010s in any of the variables (hatching rate: $F_{df} = 0.9_2$, $p = 0.412$; sterility rate: $F_{df} = 2.8_2$, $p = 0.063$; abortion rate: $F_{df} = 0.01_2$, $p = 0.993$; parasitism rate: $F_{df} = 1.5_2$, $p = 0.218$).

Table 2: Results of Tukey's post hoc tests in the 1990s.

Variable	Clusters~~Period~~	Mean difference (I-J)~~Cluster~~	Standard error~~M (SE)~~	*p* value~~Madj (SE)~~
~~Fecundity~~	~~1990s~~	~~1~~	~~156.6 (3)~~	~~19.9 (1.1)~~
		~~2~~	~~NA~~	~~NA~~
		~~3~~	~~159.7 (4.3)~~	~~24.6 (2.1)~~
		~~4~~	~~145.4 (6.3)~~	~~-55.3 (4.3)~~
	~~2010s~~	~~1~~	~~153 (3)~~	~~14 (1)~~
		~~2~~	~~142.1 (4.5)~~	~~-12.8 (1.8)~~
		~~3~~	~~134.5 (5.6)~~	~~-84.2 (3.5)~~
		~~4~~	~~NA~~	~~NA~~
Hatching rate	1 vs 3~~1990s~~	43.5*~~1~~	11.6~~73.5 (1.7)~~	0.001~~76.8 (1)~~
		~~2~~	~~NA~~	~~NA~~

		~~3~~	~~63.3 (2.5)~~	~~-42.5 (1.7)~~
		~~4~~	~~79.3 (3.1)~~	~~82.2 (3.7)~~
	~~2010s~~	~~1~~	~~46.8 (2.5)~~	~~-59.8 (1)~~
	1 vs 4	-43.9*~~2~~	15~~48.9 (3.4)~~	0.01~~-4.9 (1.8)~~
	3 vs 4	-87.4*~~3~~	16~~63~~.2 ~~(3.6)~~	<0.001~~2.8 (3.3)~~
		~~4~~	~~NA~~	~~NA~~
Sterility rate	1 vs 3~~1990s~~	9.8~~1~~	11~~6~~.8 ~~(0.6)~~	0.68~~-30.8 (1)~~
		~~2~~	~~NA~~	~~NA~~
	1 vs 4	32.1~~3~~	15.3~~6.6 (0.7)~~	0.09~~-22.5 (1.7)~~
	3 vs 4	22.3~~4~~	16.5~~.8 (0.7)~~	0.37~~-21.8 (3.2)~~
	~~2010s~~	~~1~~	~~27.1 (2.5)~~	~~46.7 (1.1)~~
		~~2~~	~~17.3 (2.6)~~	~~21.8 (1.9)~~
		~~3~~	~~6.6 (1.7)~~	~~-67.6 (3.2)~~
		~~4~~	~~NA~~	~~NA~~
Abortion rate	1 vs 3~~1990s~~	-42.8*~~1~~	11.8~~2.4 (0.2)~~	~~-96.2 (~~0.001~~8)~~
		~~2~~	~~NA~~	~~NA~~
	1 vs 4	-13.9~~3~~	15~~9~~.3 ~~(1.9)~~	0.64~~22.2 (1.8)~~
	3 vs 4	29~~4~~	16.5~~8.1 (2.8)~~	0.19~~-2.6 (4.1)~~
	~~2010s~~	~~1~~	~~17.2 (1.8)~~	~~18.1 (1)~~
		~~2~~	~~24.4 (2.9)~~	~~36.2 (1.8)~~
		~~3~~	~~17.4 (2.9)~~	~~116.6 (3.3)~~
		~~4~~	~~NA~~	~~NA~~
Parasitism rate	1 vs 3~~1990s~~	-29.8*~~1~~	11.4~~17.2 (1.3)~~	0.03~~60.2 (1)~~
		~~2~~	~~NA~~	~~NA~~
	1 vs 4	72*~~3~~	14~~20~~.8 ~~(1.7)~~	<0.001~~89.5 (1.8)~~
	3 vs 4	101.4*	16~~6.8 (1.5)~~	<0.001~~-173.4 (4.2)~~
	~~2010s~~	~~1~~	~~8.9 (0.7)~~	~~-55.3 (1)~~
		~~2~~	~~9.4 (1.1)~~	~~-1 (1.6)~~
		~~3~~	~~12.8 (1.8)~~	~~15.4 (3.3)~~
		~~4~~	~~NA~~	~~NA~~

~~***Egg mortality factors.***~~ *. The mean difference is significant at the .05 level.

~~Abortion and parasitism rates were the lowest (0%) in cluster 1 in 2017, while sterility was the highest, reaching 100% that year (Figure 6). The latter varied significantly between clusters (Quade's RANCOVA, F$_{df}$ = 2.8$_3$, p = 0.037) and periods (F$_{df}$ = 5.9$_1$, p = 0.016). Sterility differences among clusters varied between the 1990s and 2010s (Quade's RANCOVA, F$_{df}$ = 9.1$_1$, *p* = 0.003). No differences were found between clusters in the 1990s, however, sterility was significantly lower in cluster 3 than cluster 1 (Pairwise t-test, t$_{df}$ = -3.7$_2$, p = 0.001 adjusted by Bonferroni correction) and cluster 2 (t$_{df}$ = -2.6$_2$, p = 0.025) in the 2010s. Cluster 1 was the only cluster where sterility significantly increased over time (t$_{df}$ = 3.6$_1$, p <0.001) (Table 2).~~

~~Abortion and parasitism rates significantly differed between clusters (abortion rate: $F_{df} = 9.2_3$, $p < 0.001$; parasitism rate: $F_{df} = 17.6_3$, $p < 0.001$) and periods (abortion rate: $F_{df} = 40.2_1$, $p < 0.001$; parasitism rate: $F_{df} = 36.8_1$, $p < 0.001$). However, unlike the other variables, no significant interactions were found between the main effects (abortion rate: $F_{df} = 0.3_1$, $p = 0.607$; parasitism rate: $F_{df} = 1.2_1$, $p = 0.283$). Pairwise differences for these variables between clusters are summarized in Table 3.~~

~~**Table 3.**~~

Table 3: Results of Tukey’s~~Turkey’s~~ post hoc tests in the 2010s~~at a threshold of 0.05 for abortion and parasitism rates~~.

Variable	Clusters~~Cluster~~	Mean difference (I-J)~~Observed mean (M)~~	Standard error~~-Quade’s adjusted mean (Madj)~~	*p* value~~Statistical significance for alpha = 0.05~~
Abortion Hatching rate	1 vs 2	-18.7~~10.6~~	13~~-33~~.1	0.33~~a~~
	1 vs 3	-46.7*	16.8	0.02
	2 vs 3	-28	18.3	0.28
Sterility rate	1 vs 2	16.5~~24.4~~	13~~36~~.2	0.42~~bc~~
	1 vs 3	59.2*~~12.3~~	16.9~~56.7~~	<0.001~~bd~~
	2 vs 3~~4~~	42.6~~8.1~~	18.4~~-2.6~~	0.054~~ab~~
Abortion rate	1 vs 2	-34.3*	13.2	0.03
	1 vs 3	-35.2	17	0.10
	2 vs 3	-0.9	18.5	0.99
Parasitism rate	1 vs 2	-21.3~~13~~	13.1~~-3.5~~	0.24~~b~~
	1 vs 3~~2~~	-48.1*9	16.9~~-1~~	0.01~~bc~~
	2 vs 3	-26.8~~18~~	18.4~~62.5~~	0.31~~d~~
	~~4~~ ~~7~~	~~-173.4~~		~~a~~

*. The mean difference is significant at the .05 level.

Discussion

Climate change has been recognized to be one of the major phenomena that may affect forest insect populations (Ramsfield et al., 2016). While many studies reported~~report~~ the occurrence of more frequent and larger insect outbreaks (Raffa et al., 2008; Robinet & Roques, 2010), the opposite~~diminishments in outbreak severity~~ have also been observed (Pureswaran et al., 2018;

Rozenberg et al., 2020). Ongoing~~ The ongoing~~ global warming may exert mixed effects on population dynamics (Dreyer & Baumgärtner, 1996; Huang et al., 2008), and ultimately have an impact on species distributions, as has been observed with the PPM northern range expansion ~~–~~in Europe (Battisti et al. 2005) and the southern range retraction in Tunisia (Bourougaaoui et al. 2021). A report by the German Technical Cooperation Agency (GTZ et al. (2007)) has predicted more intense and longer heatwaves in Tunisia, with temperatures tending to rise even further in the coming century. To better understand potential adverse effects of climate change at the southern edge of the PPM range, the present study sought to explore variations in egg survival and hatching and their potential relationship with climate variations, based on a set of historical and recent field samplings across Tunisia.

~~Fluctuations of monthly averaged TX during summer months over the period 1990-2019 showed that July and August are the warmest months overall (Fig 3, 5).~~ The ~~fastest warming was observed in June and July with an increasing trend (although not always significant) in clusters 2, 3 and 4 for June, and in all the clusters for July. In August, positive increase trends were noted in clusters 2 and 3, but the trend was significant in cluster 2 only (Fig. 5).~~

~~Hatching rate tended to~~ decrease ~~between the 1990s and the 2010s in cluster 1 (for which the number of egg samples were the highest). Particularly, in 2017, a sharp decrease to 0%~~ in hatching rate ~~was~~ observed ~~(and reflected~~ in ~~an equally steep increase in sterility rate), with no PPM larva or parasitoids (Fig. 6). The decrease in hatching rate observed during other years of~~ the 2010s period was ~~less critical and~~ caused by a mixture of increases in the rate of sterile eggs, parasitized eggs, and ~~most importantly,~~ a clear increase in~~ the~~ abortion rate (i.e., fertile eggs with failed embryo development), the latter being possibly related to warmer conditions during embryonic development. The strikingly high sterility rate observed in 2017 could be related to an unusually long series of 10 consecutive days above 40 °C recorded that year (see Fig. 6 and Fig SM~~8 ~~9for meteorological data from the closest station of the site sampled that year). While extreme compared to the last 30 years, this anomaly reflects the global increase in the total number of acute heat days recorded in August in this station (Fig. 6). This overall trend in turn corroborates the assumption that the likelihood of such stochastic events should increase with future climate change and cannot be neglected since they might represent a prime cause of mortality in the PPM, before the average warming. We found that July is the most rapidly warming month in Tunisia, but egg masses in sites within cluster 1 are mostly laid after July and occur in August. Since all 43 egg masses from cluster 1 in 2017 were collected in late August, after this long heatwave, egg development may have been directly impacted before any sign of embryogenesis could be detected (noted as "sterile" from visual inspections). Such acute heat may also have accelerated pheromone decay due to higher evaporation rate, hence affecting mating success and egg fecundation in the first place (Groot & Zizzari, 2019; Linn et al., 1988), or adult gametes~~The unusually high sterility rate observed in 2017 could be explained by extreme temperatures during that particular summer, suggesting that the trend is more than a simple correlation and might be causal. Maximal temperature overreached 40°C during approximately 10 consecutive days in August, which matches the usual timing of egg occurrence in sites within cluster 1. Such acute heat may have directly damaged newly laid eggs during that period before any embryo could be visible (*i.e.*, not sterile per se, but aborted very early), or alternatively, the heat stress may have impacted adult gametes and fertility~~ (Sales et al., 2018). These results bear a close resemblance to those shown by Rocha et al. (2017), which revealed that negative effects appeared on Tunisian egg masses at 42°C after only 3 days of heatwaves, and no survivorship was noted at 44°C.~~ Caution must be taken when interpreting the hatching rate drop in 2017, however, because only one site (Thélepte) was sampled in cluster 1 during that peculiar year, and it remains unknown whether populations in the vicinity and similarly exposed to the heatwave have also incurred a severe drop in hatching rate.~~

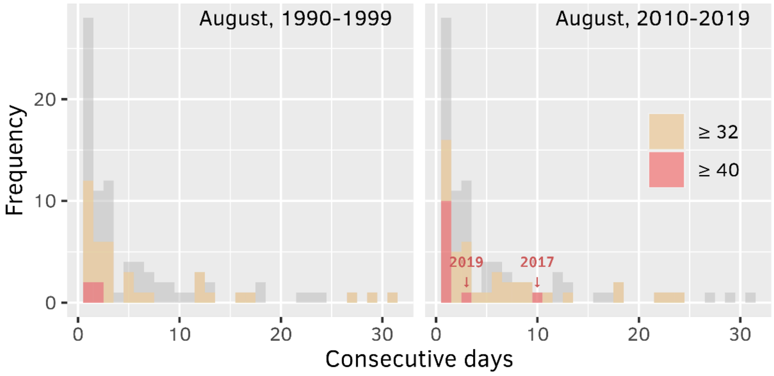


Figure 6. Frequency of consecutive days in August overreaching 32 (orange) and 40°C (red) over 1990-1999 and 2010-2019 in the Kasserine station. This station is situated less than two kilometers away from Thélepte, the egg sampling site where 43 egg clutches (100 %) were sterile in 2017. Grey histograms correspond to the total distribution across both periods.

Hatching rate was slightly higher in cluster 2 than cluster 1 in the 2010s (Table SM7), which can be attributed to the drop in 2017 in cluster 1 (Figure 5). Despite being true for all egg masses in cluster 1 that year, caution must be taken before generalizing the low egg survival recorded in 2017 to a temporal trend in the whole cluster 1. First, all eggs of cluster 1 in 2017 originated from the single site of Thélepte, however egg masses were collected on multiple trees scattered across the area, thereby reducing the risk of biased sampling, and the sample size was one of the largest of the whole data series (43 eggs masses; see Table 1). Second, inter-annual variability in climate and other factors not measured in this study cannot be neglected, meaning that the recent years cannot be summarized to the year of 2017 which has been shown to be extreme. However, global climatic models as well as the data presented in this manuscript suggest that these types of events are likely to increase in frequency with climate change, together with average warming, and therefore should be explicitly taken into account when analyzing PPM dynamics because they represent prime causes of lethality. By putting together long data series, the present study provides a broader view on the spatial and temporal variations in the fecundity penalty that may result from these extremely high temperatures, as well as how the timing of these heatwaves may differentially impact populations from different areas. Beyond the dramatic drop in egg survival observed in 2017 in cluster 1, smaller penalties on hatching rate have also been observed during other years of the 2010s. Those smaller decreases of egg survival may have a snowball effect on whole colony success, as shown in Spain by Pérez-Contreras et al. (2003) who found that 32 individuals is a threshold above which larval growth reaches its maximum and mortality drops substantially. A similar result was observed in an exploratory experiment in a French population during winter, where survival was null for colonies of less than 50 individuals (Roques et al., 2015).

The probability to overreach biological thresholds was found to be the highest in cluster 2 and to significantly increase over time, but no historical egg data from this cluster are available to confirm whether egg survival used to be higher in the 1990s. Our finding showed that August conditions in 2017 have likely been lethal in Thélepte (and possibly to a largest extent in cluster 1) may indicate that similar dramatic effects associated with stochastic meteorological events are to be expected in cluster 2 where summers are already both the hottest and the most rapidly warming. However, eggs occur later in cluster 2 (hatching starting in mid-October for recent samples) than in cluster 1 (hatching starting in early September), and may therefore mitigate summer heatwave threats thanks to phenological avoidance. This contrasting situation warrants the high relevance of future research in populations from cluster 1 and 2 to observe in real-time and test how climate change may cause the retraction of the PPM at its southern edge (see Bourougaaoui et al. 2021).

Temperature thresholds of 32°C and 40°C have been frequently used in the literature as pivotal for range limits of the PPM. They have been inferred from observations of survival in nature, but also appear consistent with more recent experiments in multiple populations of the PPM. Eggs from a French population were able to withstand a short transient exposure to a daily maximal temperature of 40°C during several consecutive days with no mortality impact (Robinet et al., 2013), while eggs from a nearby population could survive a single 6-hour-long exposure to up to 44°C (Poitou, 2021). However, mortality appeared on egg masses from a Tunisian population at 42°C after only three days of 4-hour daily exposures (Rocha et al., 2017). While slightly different methods have been used and make it difficult to compare populations, those results help narrowing down the tipping point at which PPM egg survival is impacted, depending on the duration of exposure. Regarding larvae, the survivorship of L1 and L2 from Portuguese populations started to drop after 4-hour exposures to 36°C and 40°C, respectively (Santos et al., 2011), showing higher susceptibility in early larvae compared to eggs. Recently, Poitou et al. (2022) determined experimentally the thermal performance curves in development rate in the first four larval instars in a French population, highlighting that the 32°C threshold is above the optimal development temperature, within the decreasing performance phase. The 32°C and 40°C thresholds proposed by Démolin (1969) and (Huchon & Démolin, 1970) thus appear as conservative but consistent integrators of whether a population is facing stressful conditions regardless of the exact duration of exposure, which our results corroborate with full mortality after the 2017 heatwave but not after the intense yet less stringent 2019 heatwave (Figure SM~~8~~9).

~~Hatching rate was slightly higher in cluster 2 than cluster 1 in the 2010s (Table 2), which can be explained by the lowest hatching rate that dropped to 0% in 2017 in cluster 1 (Figure 6). This dramatic decline contributed the most to the greater decrease in hatching observed in cluster 1 than cluster 2 over the entire 2010s. Although the probability to overreach 32°C (proposed by Démolin (1969) as a biological threshold above which physiological processes in eggs and/or larvae may be altered and epizootic may be favoured, although it remains unclear whether this threshold was experimentally verified and compared among populations) and 40°C in summer since the 1990s was found to be the highest in cluster 2, no historical egg data from cluster 2 are available to confirm whether hatching rate was lower in cluster 2 than cluster 1 in the 1990s. During uneven years with unusually hot summers, the biological thresholds of the PPM summer instars may be overreached in an unpredictable way and in turn negatively affect egg survival, as already demonstrated in southern Tunisia (Bourougaaoui et al. 2021). According to this study, temperatures exceeded 35°C on more than half of July and August months (nearly each year during the period 1980-2019), even 40°C for few days in the southern regions. In September, temperatures above 35°C and 40°C were found to be frequent in Tataouine over 1980-2019, which suggested the contribution of these high temperatures to population declines and to the disappearance of the PPM from southernmost areas (Bourougaaoui et al., 2021). The present study provides a broader view on the spatial and temporal variations in the fecundity penalty that may result from these extremely high temperatures. Beyond striking drops in fecundity observed in 2017 in cluster 1, lower fecundity penalties may have a snowball effect on whole colony survival. In Spain, Pérez-Contreras et al. (2003) found that 32 individuals is a threshold above which larval growth reaches its maximum and mortality drops substantially. A similar result was observed in an exploratory experiment in a French population during winter, where survival was null for colonies of 25 or 50 individuals (Roques et al., 2015).~~

Temperature is a crucial abiotic factor that can lead to local adaptations in insects and shape their geographic range (Bush et al., 2016; Hoffmann et al., 2002; Kellermann et al., 2012; Sinclair et al., 2012). Various thermal environments may therefore be associated with differences in behaviours and even physiological tolerance (Calosi et al., 2010). Numerous studies have focused on geographical variation in thermal tolerance in the fruit fly *Drosophila melanogaster*, considered as one of the most widely distributed insect species, highlighting local variation in the thermal tolerance and performance of distinct populations (see e.g., Sinclair et al., 2012). In this species, Hoffmann et al. (2002) found opposing clines in resistance to temperature extremes when comparing numerous Australian populations along a gradient from tropical to temperate latitudes, which suggests that thermal tolerance traits are under direct climatic selection.

Likewise, summer temperature has been hypothesized as being a strong selection pressure in the PPM due to the mortality observed in eggs (Rocha et al. 2017) and neonate larvae (Santos et al. 2011) after experimental heat exposure, combined with the wide range of climates under which the PPM occurs. Variations in environmental parameters may induce phenological shifts in all stages of this species either as a consequence of altered developmental time or responses to stressors (Berardi et al., 2015; Robinet et al., 2015). In areas where summers~~summer~~ are the warmest, early mortality caused by heat stress may favour late-emerging individuals through natural selection, especially as adult females are shortlived and lay eggs only once (Rocha et al. 2017). A striking example of phenological differentiation among PPM populations was recently discovered in Portugal, where two sympatric forms exhibit contrasted life cycles: one with the typical overwinter larval development, and one with a summer larval development (Santos et al., 2011). While little is known on the causal factors that promoted the emergence of both forms in the same area, Godefroid et al. (2016) demonstrated that the range of the summer population is restricted to central-coastal Portugal due to climatic constraints, while winter populations expands northward. This may be due to the comparatively low thermal resistance found in eggs of the summer form, which develop into mature larvae before the peak of summer, as opposed to eggs and early instar larvae of the winter-developing form which usually occur in the middle of summer and are more likely to be exposed to heats (Rocha et al. 2017). The increase in climatic variability is expected to exert effects on insect species that differ from those caused by gradual global warming (Schreven et al., 2017). Large-scale heatwaves are known to have contrasted effects on different populations of the PPM depending on local climates and phenologies (Robinet et al., 2015). In Europe, the summer heatwave in 2003 led to a collapse of PPM populations in northern areas in France (Robinet et al., 2013), while it benefited to adult dispersal and altitudinal expansion in the Italian Alps, likely due to flight thermal thresholds more easily met than usual near the elevation edge~~)~~ (Battisti et al., 2006).~~ .~~ These findings are congruent with the available data in Tunisia where phenology in cluster 2 is delayed compared to that in cluster 1, most likely due to the climatic and altitudinal differences found between those clusters. This fact could contribute to explain the lower hatching rate found in cluster 1 than in cluster 2 in 2017 (Figure 5~~6~~), since eggs and neonate larvae occur after most summer heats in cluster 2 as a result of~~due to~~ later adult emergences. This suggests that populations from the warmest areas of Tunisia may not be the most vulnerable to climate change thanks to phenological adaptation favouring heat avoidance, as opposed to populations from areas where individuals are close to their physiological limits but did not evolve differentiated phenology in response to heat lethality.

Despite the impacts of the PPM on Aleppo pine forests in Tunisia, little is known about how climate change can alter the phenology of Tunisian population and whether it is is spatially structured by climate heterogeneity. According to Robinet et al. (2015), predictable unfavourable conditions to which PPM populations are exposed can be alleviated by differentiated phenologies (phenological plasticity and/or adaptations), which is supported by later adult emergences in cluster 2 on average. By contrast, unpredictable adverse conditions may be mitigated by bet-hedging strategies such as prolonged diapause, as it creates heterogeneity within populations and siblings by expanding the life cycle of only a fraction of individuals that stay sheltered in the ground at the pupal stage and evade climate stochasticity (Salman et al., 2019). Diapausing individuals may therefore contribute to reconstituting local population collapses after extreme events, but the cost is that survival decreases with the total duration of diapause due to other factors of mortality (fungi, pupal predation, etc.) (Salman et al. 2019). A significant relationship was found between the rate of prolonged diapause and both cold and warm winter temperatures, presumably because they serve as cues of the likelihood of either cold or hot lethal temperatures caused by inter-annual climate uncertainty (Salman et al. 2019). The acute heat stress exerted on PPM egg masses in Tunisia may help explain the retraction of this pest from southernmost regions, and further investigations on phenological differentiations and prolonged diapause strategies among populations incurring different levels climate harshness are now necessary to predict further distribution changes.

A growing body of literature reveals that parasitoids are often more sensitive to climate warming than other trophic levels because of their higher position in the food web (Jeffs & Lewis, 2013; Rosenblatt & Schmitz, 2016). Climate change can lead to phenological asynchrony between parasitoids and their hosts in cases where the phenology of the interacting species respond variously to the same climatic cue (Visser & Holleman, 2001), or when the interacting species use different cues to initiate emergence or development (Jeffs & Lewis, 2013; Walther, 2010). Phenological asynchrony may also appear if one of the interacting partners rapidly develops or has a seasonal diapause in response to warming (Forrest, 2016). Parasitism rates therefore tend to decrease with increasing climatic variability that impedes parasitoids from tracking host populations (Chidawanyika et al., 2019). Alternatively, but not exclusively, eggs may escape parasitic attacks thanks to various counter-adaptations whose relative weight may differ among regions and populations. The identification of eggs by natural enemies may be hindered making egg masses inconspicuous, as PPM female covering eggs by greyish-brown scales similar to the colour of pine twigs (Battisti et al., 2015). These scales function not only as visual protection but also as factors impacting the microclimate of eggs and therefore their development rate (Milani, 1990), and as physical barriers limiting parasitoids from locating individual eggs in the clutch and greatly reducing their success (Pérez-Contreras & Soler, 2004). The chief defence against parasitoids, widely discussed in insects and in particular Drosophila spp., is encapsulation, an immune responses which sequesters and kills foreign body (Cavigliasso et al., 2021; Wertheim et al., 2005). Such immune system with specific cells (hemocytes) is well-developed in larval stages as already observed in PPM larvae (Boudjahem et al., 2019), however, a study conducted by Reed et al. (2007) showed that hosts in the egg stage can mount a cellular immune response against parasitoid eggs and larvae (Reed et al., 2007). Research on a wide range of species reveals that small changes in temperature can significantly shape insect immunity as well as parasitoid fitness (Murdock et al., 2012). Increases in temperature can consequently promote or repress the encapsulation process, which in turn may influence the outcome of parasitic success, (Cavigliasso et al., 2021). The outbreaks of phytophagous insects are expected to increase in the future as parasitism decreases~~As a result, outbreaks of phytophagous insects are expected to increase in the future~~ (Stireman et al., 2005). At the southern edge of PPM distribution (North Africa), some studies showed that extremely high temperatures could both disrupt population regulation mechanisms and decrease the severity of outbreaks (Bouzar.Essaidi et al., 2021; Pureswaran et al., 2018). The lower PPM fecundity in this area (when compared to that at the northern edge, in southern Europe) due to lower plant quality (Bouzar.Essaidi et al., 2021; Pimentel et al., 2010) may explain why egg parasitism is also lower with climate warming. Our results showed that the mean number of eggs per egg mass (155 ± 2.1 in cluster 1, 142 ± 4.5 in cluster 2, 150 ± 3.5 in cluster 3, 145 ± 6.3 in cluster 4; 151 ± 1.7 when merging all clusters) was considerably lower than that found in Bulgaria (226 ± 43.2) and France (194.3 ± 50.1) (Georgiev et al., 2020). The average rate of parasitism also showed a different pattern than what was found in northern parts of PPM distribution, with increases in coastal regions namely in cluster 1 in Tunisia, while it decreases in France and Spain from core to front populations and along an altitudinal gradient, respectively (Georgiev et al., 2020; Hódar et al., 2021). Although parasitoids account in egg mortality, their influence here was low compared to other factors (sterility and abortion) (Figure 5), and is therefore unlikely to be the main driver of PPM collapses at the southern edge of the distribution.~~Similarly, the average rate of parasitism in the four clusters was low (13 ± 0.7% in cluster 1, 9 ± 1.1% in cluster 2, 18 ± 1.3% in cluster 3, 7 ± 1.5% in~~

~~cluster 4; 13 ±0.5% when merging all clusters) compared to northern parts of PPM distribution (24.4 % in France and 29% in Bulgaria) (Georgiev et al., 2020), and is therefore unlikely to be the main driver of PPM collapses at the southern edge of the distribution. Our findings confirm Bouzar et al. (2021)'s results showing that fecundity and parasitism were lower in Algeria than in southern Europe~~. Significant local warming in Tunisia appears as a prime candidate factor contributing to the sharp decrease of PPM populations (Bourougaaoui et al., 2021).

Factors other than climate warming may putatively affect the survival and persistence of the PPM at its southern range edge. Embryonic mortality can be impacted by excessive exposure to intense solar radiation, particularly in southern parts of the distribution, as PPM females tend to lay their eggs exposed to the sun (Démolin, 1969). Another factor often modulating the spatial occurrence of insects is food availability. Nevertheless, it is rarely a limiting factor in the PPM because larvae feed on evergreen trees that are well distributed in the environment, from natural or semi-natural stands to urban areas where they often occur in relatively high numbers as ornamental trees (Martin, 2005). Natural enemies such as pathogens or predators (mostly insect parasitoids) at early larval stages have been suspected to cascade into increasing mortality during larval development because of the impact on the colony size and silk weaving effort to build a tent (Auger Rozenberg et al., 2015; Roques et al., 2015), however (1) there is no evidence that the enemy pressure would differ among areas investigated here, and (2) temperature, particularly summer heat waves or early autumnal cold snaps, are often put forward as a major cause of early mortality (Robinet et al., 2015).

Heat tolerance has received close attention in insects, however its fluctuation throughout ontogeny and effects persisting from one developmental stage to another are still poorly documented. Besides the PPM, few case studies showed that the effects of acute heat stress received early in life cycle may be carried over to later instars. This was demonstrated in holometabo~~a~~lous insects such as the tropical butterfly, *Bicyclus anynana* (Klockmann et al., 2017). Beside consequences of heat on immediate mortality investigated in experimental work (e.g., Rocha et al 2017) or inferred in the present study by putting together long time series, the ultimate fitness of individuals that survive challenging heats at the egg stage or first larval instar would therefore be of great interest to understand the impacts of climate warming at the southern edge of the PPM. This insect remains one of the ideal models to study these questions owing to (~~I~~i) the availability of historical data, (ii) ~~and~~ its already demonstrated spatial and phenotypic causal response to climate change (Battisti et al., 2005; Robinet et al., 2007; Poitou et al., 2022), and (iii) ongoing processes at play in its southernmost distribution affecting population persistence (Bourougaaoui et al. 2021; this study). ~~(Battisti et al., 2005; Robinet et al., 2007).~~

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Conflict of interest disclosure

The authors declare no conflict of interest.

Author contributions

Conceptualization: AB, CR, MLBJ, ML; Data curation: AB; Formal analysis: AB, ML; Funding acquisition: CR, MLBJ; Investigation: AB; Methodology: AB, CR, ML; Project administration: CR, MLBJ; Supervision: CR, MLBJ, ML; Writing – original draft: AB, CR, ML; Writing – review and editing: AB, CR, MLBJ, ML.

Data, script and code availability

Data and R scripts are publicly available at https://doi.org/10.15454/RUEIOA.

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Supplementary information

~~Available temperature data from INM and NASA sources, GPS coordinates of meteorological stations and sites of egg sampling.~~

Table SM1. Coordinates of sampling sites.

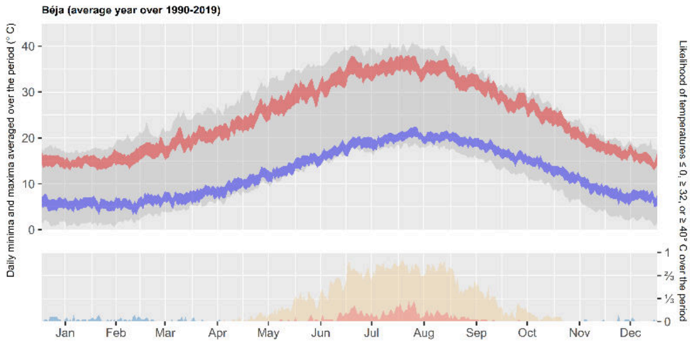
Field egg masses sampling sites	Latitude (°N)	Longitude (°E)
Chaambi	35.200000	8.700000
El Ayoun	35.557653	8.879097
Mghila	35.333333	9.200000
Thélepte	34.960611	8.583472
Bir Lahfay	34.944697	9.156361
El Fej	34.701582	9.043508
Jebel Motlag	34.954417	9.707389
Jebel Rihane	34.823708	9.636170
cité Ettahrir	36.821750	10.135778
Dar Chichou	36.965594	10.964017
Korbous	36.833333	10.583333
Sejnane	37.183333	9.183333
Oued Laabid	36.816542	10.711303
El Menzeh	36.837850	10.184692
Manouba	36.814722	10.108361
Ain Jamala	36.503839	9.301167
Testour	36.544944	9.377444
Henchir Naam	36.216667	9.166667
Jebel Kbouch (Kef)	36.210000	8.930000
El Krib (Siliana)	36.332544	9.063128
Kesra (Siliana)	35.871833	9.366583
Jebel Sidi Aich (Gafsa)	34.783292	8.865861

Table SM2. Temperature datasets (combination of data from the Institut National de Météorologie, INM, and the NASA data in corresponding 8 km × 8 km grid) and coordinates of meteorological stations.

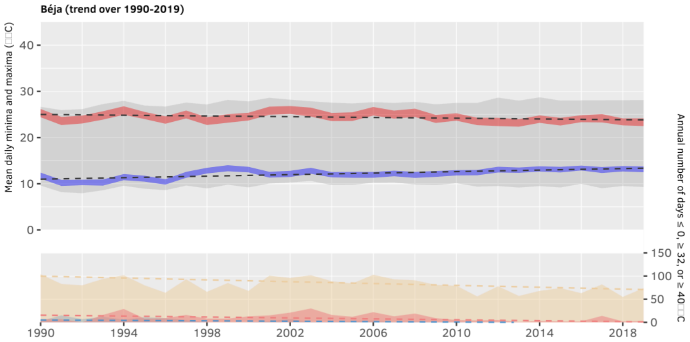
INM meteorological station	Latitude (°N)	Longitude (°E)	Available data (INM)	Data from NASA used to complete the INM datasets
Kélibia	36.844855	11.082701	2001-2011	1990-2000 2012-2019
Carthage	36.846081	10.219053	1990-2014	2015-2019
Béja	36.723338	9.184013	1990-1997 2001-2011	1998-2000 2012-2019
Siliana	35.851853	9.595147	1990-1997	1998-2019
Kef	36.120862	8.720267	1990-1997 2001-2011	1998-2000 2012-2019
Kasserine	34.948369	8.569550	2001-2011	1990-2000 2012-1019
Sidi Bouzid	35.025685	9.498840	1990-2014	2015-2019
Gafsa	34.427352	8.820959	1990-2014	2015-2019

Figure SM3. Geographic and elevational distances between meteorological stations and egg sampling sites (lower opacity if >100 km and >350 m, respectively).

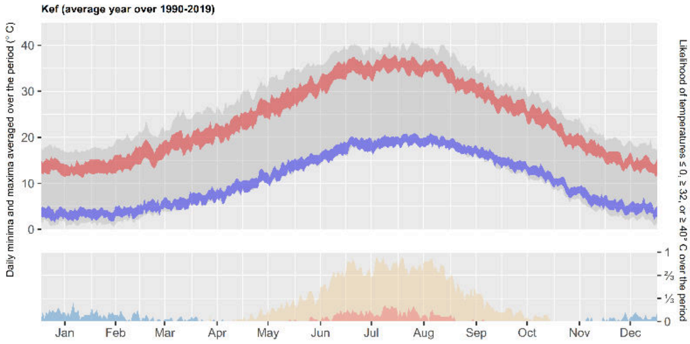
Béja (average year over 1990-2019)



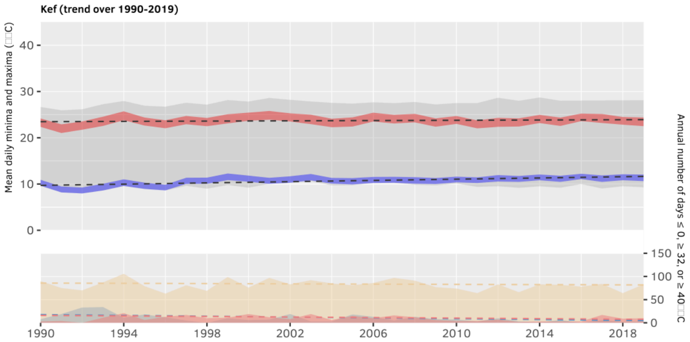
Béja (trend over 1990-2019)



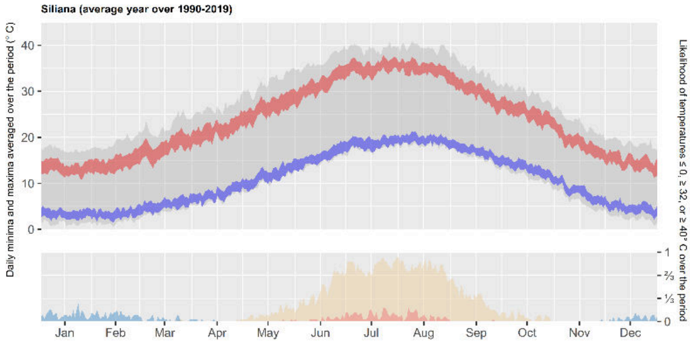
Kef (average year over 1990-2019)



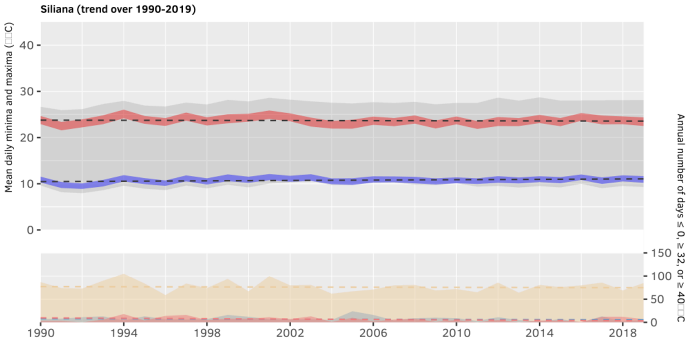
Kef (trend over 1990-2019)



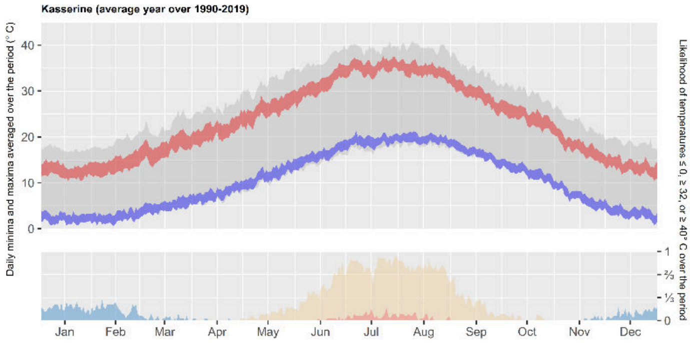
Siliana (average year over 1990-2019)



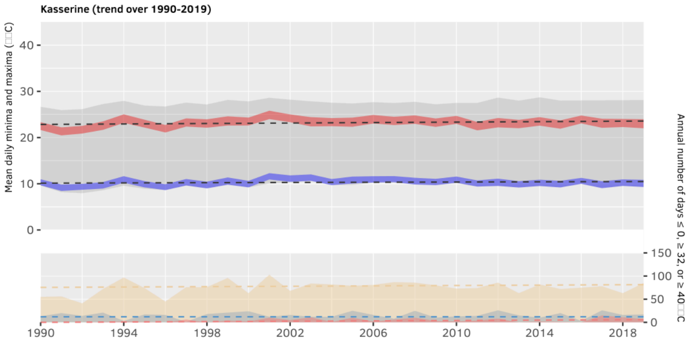
Siliana (trend over 1990-2019)



Kasserine (average year over 1990-2019)



Kasserine (trend over 1990-2019)



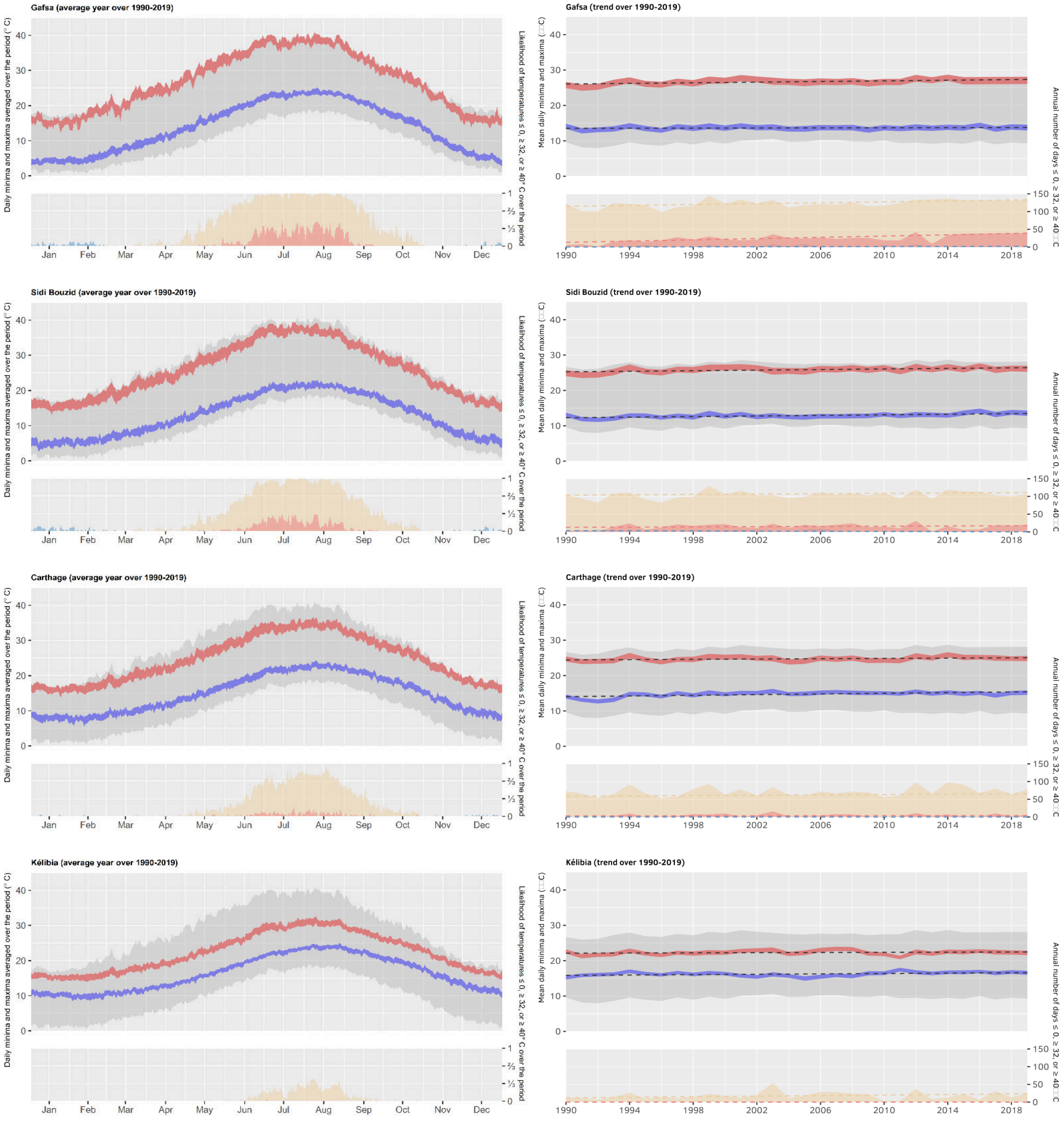


Figure SM4. Climate data in eight Tunisian regions between 1990 and 2019 (data source: INM and NASA, see Table SM2). Left charts show the mean year in each region by averaging daily maxima (red) and minima (blue) by day of the year over the period, represented as 95% CI ribbons. Right charts show the yearly average of daily maxima (red) and minima (blue), represented as 95% CI ribbons, and corresponding Theil-Sen estimators. Thick grey ribbons in the background show the maximal thermal range across all nine regions depending on day of the year (left) or year (right). The bottom part of left charts shows the likelihood of temperatures ≤ 0 (blue), ≥ 32 (beige) or ≥ 40 °C (red), while the bottom part of right charts shows the annual number of days below or above those thresholds. The 366th day during leap years was discarded due to its lower sample size.

~~B. Multivariate analyses on climate data and K-medoids clustering.~~

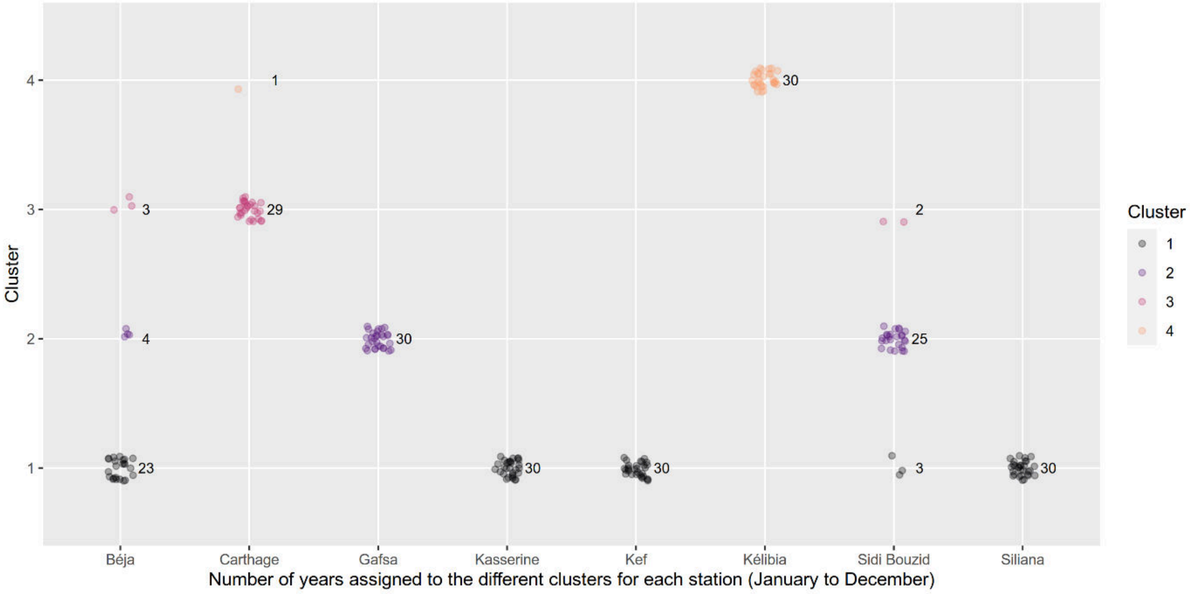


Figure SM5. Number of years assigned to the different clusters for each meteorological series (PAM clustering on data from January to December).

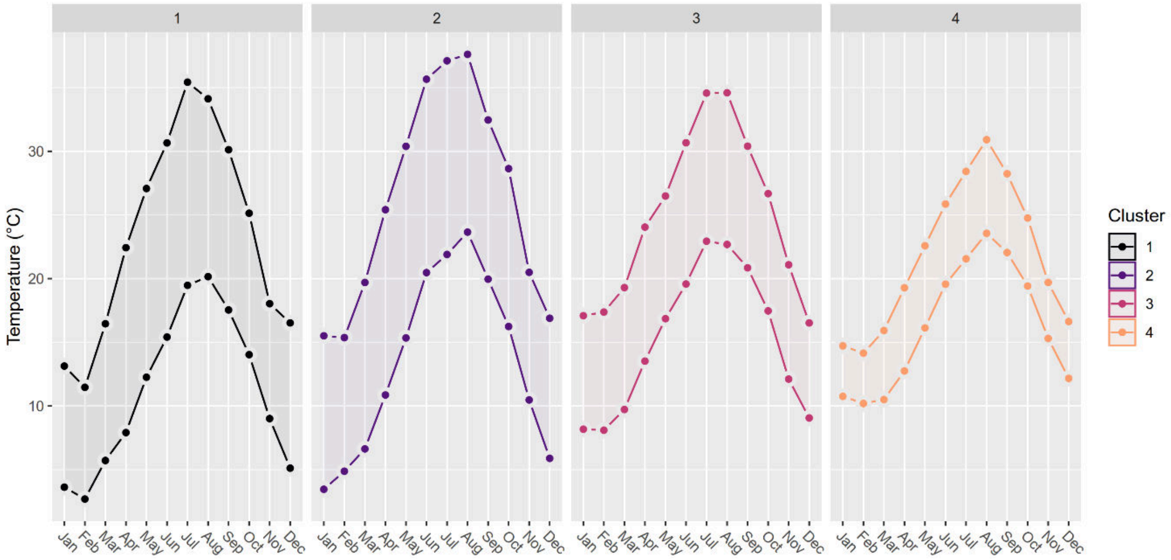


Figure SM6. T~~min~~X and TN~~max~~ from January to December in each cluster medoid (PAM clustering on data from January to December).

Table SM7. Descriptive statistics with all data including cluster 1 in 2017: observed mean (M), Quade's adjusted mean (Madj) and associated standard error (SE) for the different response variables.

Variable	Period	Cluster	M (SE)	Madj
Fecundity	1990s	1	156.6 (3)	-0.2(6.9)
		2	NA	NA
		3	159.7 (4.3)	10 (10.2)
		4	145.4(6.3)	-20 (14.5)
	2010s	1	153 (3)	10.7 (7.8)
		2	142.1 (4.5)	-0.3 (10.8)
		3	134.5 (5.6)	-39.7 (14.7)
		4	NA	NA
Hatching rate	1990s	1	73.5 (1.7)	6.9 (7.1)
		2	NA	NA
		3	63.3 (2.5)	-36.6 (8.7)
		4	79.3 (3.1)	50.8 (14.1)
	2010s	1	46.8 (2.5)	-12.4 (8)
		2	48.9 (3.4)	6.2 (10.6)
		3	63.2 (3.6)	34.2 (12.6)
		4	NA	NA
Sterility rate	1990s	1	6.8 (0.6)	7.8 (7.3)
		2	NA	NA
		3	6.6 (0.7)	-2 (9.3)
		4	5.8 (0.7)	-24.3 (12.8)
	2010s	1	27.1 (2.5)	13.6 (8.4)
		2	17.3 (2.6)	-2.9 (9.9)
		3	6.6 (1.7)	-45.5 (11.7)
		4	NA	NA
Abortion rate	1990s	1	2.4 (0.2)	-15.3 (6.6)
		2	NA	NA
		3	9.3 (1.9)	27.5 (9.9)
		4	8.1 (2.8)	-1.4 (15.4)
	2010s	1	17.2 (1.8)	-15.4 (8)
		2	24.4 (2.9)	18.9 (10.7)
		3	17.4 (2.9)	19.8 (12.8)
		4	NA	NA
Parasitism rate	1990s	1	17.2 (1.3)	1.4 (6.8)
		2	NA	NA
		3	20.8 (1.7)	31.2 (9.4)
		4	6.8 (1.5)	-70.1 (13)
	2010s	1	8.9 (0.7)	-13.4 (8.3)
		2	9.4 (1.1)	7.9 (9.8)
		3	12.8 (1.8)	34.7 (13.3)
		4	NA	NA

Table SM8. Descriptive statistics without data from cluster 1 in 2017: observed mean (M), Quade's adjusted mean (Madj) and associated standard error (SE) for the different response variables.

Variable	Period	Cluster	M (SE)	Madj (SE)
Fecundity	1990s	1	156.6 (3)	-0.2(6.9)
		2	NA	NA
		3	159.7 (4.3)	10 (10.2)
		4	145.4 (6.3)	-20 (14.5)
	2010s	1	155.9 (3.3)	13.6 (7.7)
		2	142.1 (4.5)	-2.5 (9.6)
		3	134.5 (5.6)	-36.6 (13.2)
		4	NA	NA
Hatching rate	1990s	1	73.5 (1.7)	6.9 (7.1)
		2	NA	NA
		3	63.3 (2.5)	-36.6 (8.7)
		4	79.3 (3.1)	50.8 (14.1)
	2010s	1	57.6 (2.5)	1.3 (7.8)
		2	48.9 (3.4)	-8.6 (9.9)
		3	63.2 (3.6)	13.2 (12.2)
		4	NA	NA
Sterility rate	1990s	1	6.8 (0.6)	7.8 (7.3)
		2	NA	NA
		3	6.6 (0.7)	-2 (9.3)
		4	5.8 (0.7)	-24.3 (12.8)
	2010s	1	10.2 (1.1)	-2.5 (8)
		2	17.3 (2.6)	15.4 (9.7)
		3	6.6 (1.7)	-23.1 (11.7)
		4	NA	NA
Abortion rate	1990s	1	2.4 (0.2)	-15.3 (6.6)
		2	NA	NA
		3	9.3 (1.9)	27.5 (9.9)
		4	8.1 (2.8)	-1.4 (15.4)
	2010s	1	21.2 (2.1)	-0.6 (7.8)
		2	24.4 (2.9)	0.8 (10.3)
		3	17.4 (2.9)	0.5 (12.3)
		4	NA	NA
Parasitism rate	1990s	1	17.2 (1.3)	1.4 (6.8)
		2	NA	NA
		3	20.8 (1.7)	31.2 (9.4)
		4	6.8 (1.5)	-70.1 (13)
	2010s	1	11 (0.8)	3 (8.1)
		2	9.4 (1.1)	-12.3 (9.3)
		3	12.8 (1.8)	15.4 (12.9)
		4	NA	NA

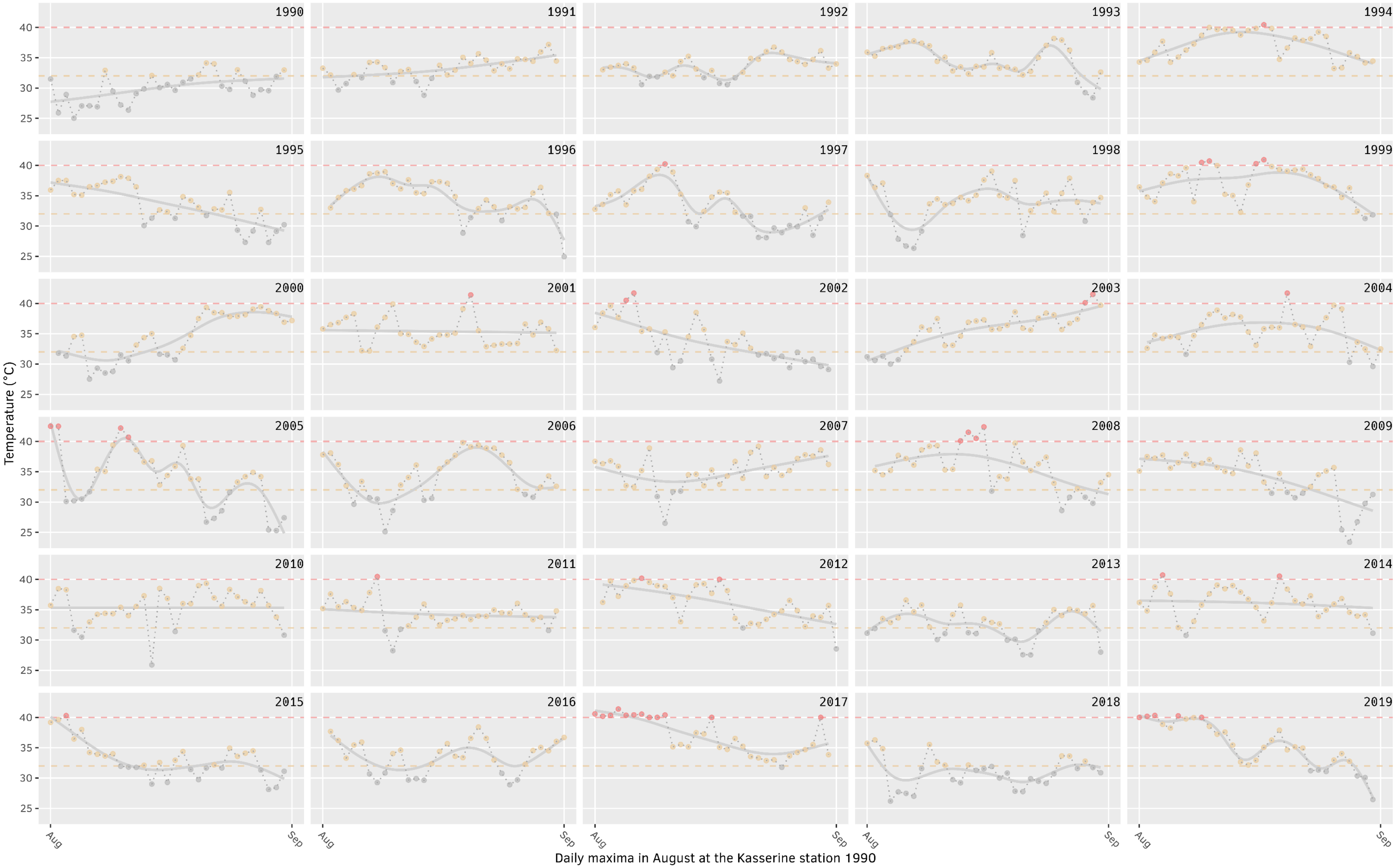


Figure SM9: August daily maximal temperature recorded in 1990-2019 at the Kasserine station, near the sampling site of Thélepte (cluster 1). Yellow points correspond to daily maxima ≥ 32 °C, red points correspond to daily maxima ≥ 40 °C. Smooth lines are fitted with the “gam” modelling method.