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An inter- and transgenerational study on the effect of calving month of ancestors on dairy performances of Mediterranean buffalo

Nicolò P. P. Macciotta,[1] Mauro Fioretti,[2] Roberta Cimmino,[3] Mayra Gomez Carpio,[3] Gianluca Neglia,[4] Riccardo Negrini,[5] and Alberto Cesarani[1,6]*

[1]Dipartimento di Agraria, Università degli Studi di Sassari, 07100 Sassari, Italy
[2]Associazione Italiana Allevatori, 00187 Roma, Italy
[3]Associazione Nazionale Allevatori Specie Bufalina, 8100 Caserta, Italy
[4]Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II, 80137 Napoli, Italy
[5]Dipartimento di Scienze animali, della nutrizione e degli alimenti, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy
[6]Animal and Dairy Science, University of Georgia, Athens, GA 30602

ABSTRACT

In this work intergenerational and transgenerational effects of calving months on dairy performances of Italian Mediterranean river buffaloes were investigated. The EBVs for milk, fat, and protein yields, as well as fat and protein contents of 112,625 females (F_3) born between 1985 and 2022 were analyzed with a linear model that included the fixed effects of the calving month of their dams (F_2, 63,442 cows), granddams (F_1, 44,015 cows), and great-granddams (F_0, 33,452), the covariable of F_0 calving date, and the covariable of the F_1 EBV. For a subset of 53,706 F_3 buffaloes, further analyses were run including in the model the number of days with a temperature-humidity index larger than 70 during the last 100 d of their F_0 pregnancy. All the 5 considered traits were affected by the ancestor month of calving. The effect tended to decrease as the distance between generations increased. Buffaloes whose great-granddams calved in September showed the lowest LSM of EBV for dairy traits, whereas the largest values were exhibited by buffaloes whose F_0 had calvings in December. Similar pattern was observed for the F_1 calving month effect. An exception was found for the effect of F_2 calving month. The F_3 cows whose dams calved in December showed the largest average EBV for yield traits. This result seems to indicate that for adjacent generations, environmental conditions in the periconceptional period may be more important than in late pregnancy. Results of the present work confirmed previous reports of inter- and transgenerational effects of calving months of female ancestors in dairy cattle, suggesting the existence also for buffalo of mechanisms of epigenetic inheritance related to environmental conditions during pregnancy. A deeper understanding of the role of transgenerational and intergenerational epigenetic inheritance in the expression of phenotypes of economic interest would provide useful insights for the management and the breeding of river buffaloes.
Key words: heat stress, buffalo, transgenerational effect, epigenetic inheritance

INTRODUCTION

The water buffalo (*Bubalus bubalis*) is the most important livestock species of the genus *Bubalus* (Minervino et al., 2020), representing the third dairy species of the world, with a global stock of 205,141,830 animals in 2022 (FAOSTAT, 2024). Two main water buffalo subspecies exist: the river buffalo (*Bubalus bubalis bubalis*) and the swamp buffalo (*Bubalus bubalis carabanensis*), exhibiting morphological, karyotypical, and behavioral differences (Colli et al., 2018). The river buffalo is larger (weight between 450 and 1,000 kg), it has evolved as a dairy animal, and it is traditionally farmed in Asia and Europe, with a relatively recent introduction in Africa and South America. Swamp buffaloes are typical of rural areas of south and east Asia, are lighter (weight between 250 and 450 kg) than river (Minervino et al., 2020), and they are farmed mainly for providing draft power. Although the 2 subspecies have a different number of chromosomes (50 and 48 for river and swamp, respectively), they are interfertile, and several ancient and recent events of admixture have been reported (Colli et al., 2018).

One of the main reasons of the worldwide diffusion of the buffalo is represented by its adaptability to different environments (Stefani et al., 2022). This species is generally considered tolerant to hot and humid climates (Petrocchi Jazinski et al., 2023). However, buffaloes

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*Corresponding author: acesarani@uniss.it

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-26. Nonstandard abbreviations are available in the Notes.

Table 1. Heritability estimates and EBV reliability statistics of F_3 buffalo cows for milk, fat, and protein yields, and fat and protein percentages provided by ANASB

		EBV reliability			
Trait	Heritability	Mean	SD	Minimum	Maximum
Milk yield	0.35	0.43	0.24	0.03	0.84
Fat yield	0.24	0.39	0.21	0.03	0.82
Protein yield	0.30	0.41	0.23	0.03	0.83
Fat percentage	0.25	0.39	0.22	0.03	0.82
Protein percentage	0.32	0.41	0.23	0.03	0.83

have been reported to be susceptible to heat stress (**HS**; Behera et al., 2023) due to the reduced development of sweat glands (Mishra, 2021) and to the dark skin that causes a large absorption of solar radiation (Marai and Haeeb, 2010). Studies carried out on Murrah buffaloes in India reported a reduction in dairy performances for temperature-humidy index (**THI**) values greater than 72 (Pawar et al., 2013) or 74 (Choudhary and Sirohi, 2019). Behera et al. (2023) observed a reduction in EBV for milk yield for increasing values of THI in Indian Murrah buffaloes. A study on Brazilian Murrah reported a decline in milk performances for THI values larger than 77.8 (Stefani et al., 2022) and an absence of correlation between the intercept and the slope of the genetic effect estimated by a reaction norm model, thus hypothesizing an absence of antagonism between HS tolerance and production in this species.

Most of the studies on the effect of HS on buffalo dairy performances have been carried out in tropical or subtropical climate areas. However, HS is becoming a serious concern also for livestock systems in temperate areas of the world due to the effects of climate change. Northern Mediterranean countries are considered an area particularly vulnerable to the effect of climate change (Carabaño et al., 2021). The Mediterranean Italian river buffaloes, a breed related to those of Romania and Mozambique (Colli et al., 2018), has been widely used for improving milk production in different countries. The high economic value of the milk, almost all destined to the production of the Protected Designation of Origin mozzarella Campana cheese, has resulted in an increase of the number of buffaloes farmed in Italy. In 10 yr, there has been an increase of the Italian buffalo stock of about +20%, passing from 348,861 in 2012 to 416,000 in 2022 (FAOSTAT, 2024). A depressive effect of high THI on bulk milk fat, protein, and lactose contents was reported in Italian Mediterranean water buffalo (Costa et al., 2020), whereas no effects were reported on individual daily milk yield for the same breed (Matera et al., 2022).

Studies on dairy cattle have reported that HS affects not only the animal directly exposed to high temperatures, but also its offspring. Heat stress occurrence in late pregnancy was reported to affect performances of female offspring after 1 (Laporta et al., 2020), 2 (Gudex et al., 2014), or 3 generations from the stress event (Weller et al., 2021; Macciotta et al., 2023; Laporta et al., 2024). In most of the studies, the calving month of female ancestors was regarded as an indicator of the period during which pregnancy occurs and, therefore, of the possible exposure to HS. An explanation of these phenotypic effects is the transmission of environmentally induced epigenetic variations during pregnancy in the female offspring through the germline (Khatib, 2021). Such a transmission of phenotypic variation, different from DNA transmission, is known as epigenetic inheritance (EI). In particular, the transmission between 2 adjacent generations is called intergenerational epigenetic inheritance (IEI), whereas when it happens across 3 or more generations (i.e., when effects are detected on animals not directly exposed to the environmental challenge), it is termed transgenerational epigenetic inheritance (TEI; Laporta et al., 2024). At the molecular level, TEI has been assessed in plants, nematodes, and insects whereas evidence for mammalians is limited, and its occurrence is still under debate (Khatib, 2021).

In this work, intergenerational and transgenerational effects of the calving months of Italian Mediterranean

Table 2. Type III sum of squares F statistics (corresponding *P*-values in parentheses) of the effect of calving month of great-granddams (F_0), granddams (F_1), and dams (F_2) on EBV for milk, fat, and protein yields, and fat and protein percentages of F_3 buffalo cows

Ancestor	Milk yield	Fat yield	Protein yield	Fat percentage	Protein percentage
F_0	3.51 (<0.0001)	4.01 (<0.0001)	3.66 (<0.0001)	3.97 (<0.001)	2.62 (<0.001)
F_1	9.70 (<0.0001)	10.38 (<0.0001)	9.73 (<0.0001)	4.87 (<0.0001)	3.93(<0.0001)
F_2	25.33 (<0.0001)	28.78 (<0.0001)	26.10 (<0.0001)	8.25 (<0.0001)	7.44 (<0.0001)

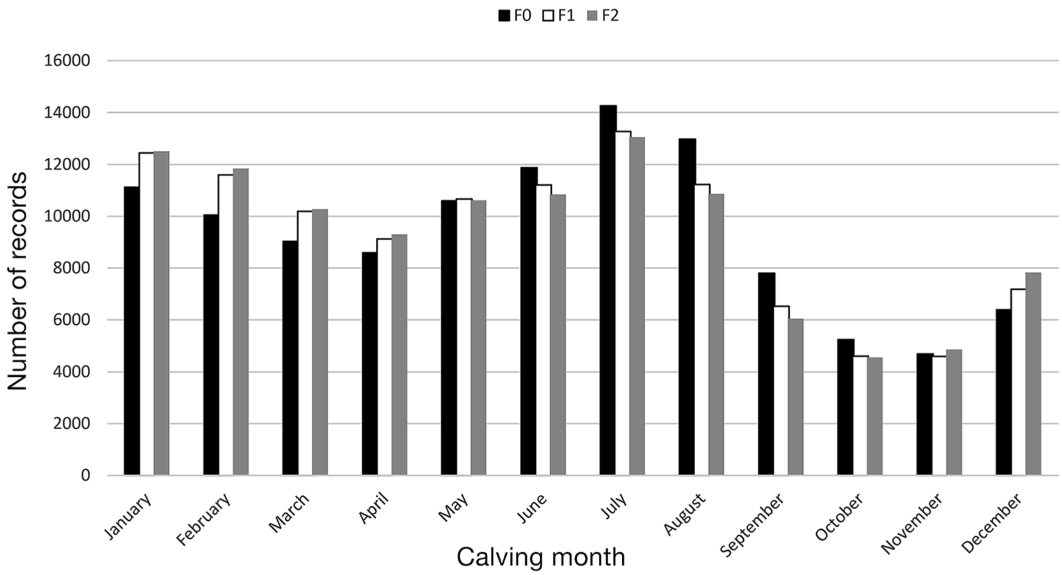

Figure 1. Distribution of F_3 buffalo cow records across different months of calving of their F_2 dams, F_1 granddams, and F_0 great-granddams.

buffaloes on their female offspring dairy performances are investigated.

MATERIALS AND METHODS

Data

Data were EBVs for milk, fat, and protein yields, and fat and protein contents of 112,625 buffaloes (F_3) born in the period 1985 to 2022, provided by the Italian Buffalo Association (**ANASB**). Animals were farmed in 629 commercial herds. The EBVs referred to a standardized lactation length of 270 d, and they were calculated by ANASB in the routine genetic evaluation round of the breed with a multiple trait animal model that included, as fixed effects, the interaction year of calving and number of milking, herd and province, age at calving, parity, and days open; random effects were the animal genetic effect, the permanent environmental effect, and the residual. Official heritability estimates and EBV reliabilities are reported in Table 1. For each buffalo cow, EBV for the dam (F_2, 63,442 cows), granddam (F_1, 44,015 cows), and great-granddam (F_0, 33,452) were available. Animal Care and Use Committee approval was not needed as data were obtained from preexisting databases.

Statistical Model

Data were analyzed with the following linear model:

$$y_{F3} = CMF_2 + CMF_1 + CMF_0 + bCDF_0 + bEBVF_1, [1]$$

where y_{F3} is the EBV for the considered trait of F_3 buffalo cows; CMF_2 is the fixed effect of the calving month of F_2 generation (dams); CMF_1 is the fixed effect of the calving month of F_1 generation (granddams); CMF_0 the fixed effect of the calving month of the F_0 generation (great-granddams); $bCDF_0$ is the fixed covariable of F_0 calving date; $bEBVF_1$ is the fixed covariable of the F_1 EBV. The $bCDF_0$ covariable was included in the model to account for the genetic trend, whereas the $bEBVF_1$ was included to avoid a confounding between the birth month and genetic value of the ancestor (Weller et al., 2021). The different reliability of EBV was accounted for by performing a weighted least squares method. Weights were proportional to the reciprocal of the error variances, whose structure was assumed to be a diagonal matrix in which the diagonal elements differed depending on the reliability for each record.

Also in this study, the calving month was regarded as an indicator of the period during which pregnancy occurs. The rationale of the inter- and transgenerational model is the following: the F_0 generation is directly exposed to HS during pregnancy; their daughters (F_1) are also directly exposed to HS as embryos in uterus; F_1 primordial gem cells that will generate F_2 females, are also directly exposed to HS; F_3 are not directly exposed to HS. Effects on the adjacent or 2 generations apart are considered as intergenerational effects, whereas effect detected 3 generations far apart (i.e., without direct exposure to the environmental factor, are termed as transgenerational effects; Khatib, 2021) and can be interpreted as possible evidence of TEI.

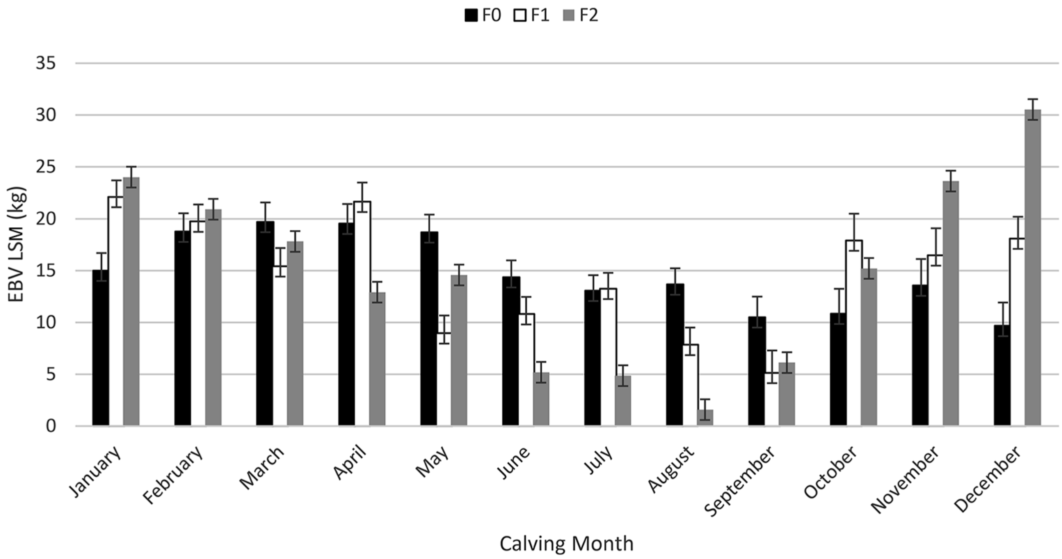

Figure 2. Least squares means and SE of the effect of calving months of great-granddams (F_0), granddams (F_1), and dams (F_2) on the EBVs for milk yield of cows (F_3).

Climate Data

The effect of THI experienced by F_0 cows during pregnancy on F_3 dairy EBV was investigated on a data subset of 54,706 F_3 buffaloes. In particular, the last 100 d of pregnancy were considered. The data of F_0 insemination was calculated by subtracting from the F_0 calving data the average pregnancy length in days (308) of the Italian water buffalo (ANASB, 2021). Climate data were retrieved using the "nasapower" R Package (Sparks, 2018).

The maximum temperature-humidity index (**THI_{MAX}**) of each day of F_0 pregnancy was calculated as (Kelly and Bond, 1971):

$$THI_{MAX} = (1.8 \times Tmax + 32) - (0.55 - 0.55 \times RHmin) \times [(1.8 \times Tmax + 32) - 58],$$

where Tmax is the daily maximum temperature expressed in Celsius degrees and RHmin is the minimum relative humidity expressed in percentage.

For each F_3 cow, a THI score (**SC_{THI}**) was calculated by counting the number of days of the last third (100 d) of her F_0 great-grandam pregnancy with a THI_{MAX} greater than 70. This value was set as a threshold of discomfort zone based on previous reports on buffalo (Marai and Haeeb, 2010; Chaudhary et al., 2022; Petrocchi Jasinski et al., 2023) and considering that higher thresholds were estimated on studies carried out in buffaloes farmed in hot and humid areas (Stefani et al., 2022). Data were then analyzed with a model similar to model [1] apart from the substitution of the fixed effect the calving month of the F_0 generation with the fixed covariate of the SC_{THI} referred to the F_0 pregnancy.

RESULTS

Figure 1 reports the distribution of F_3 records across calving months of their F_0, F_1, and F_2 ancestors, respectively. A consistent distribution of records across the 3 generations can be observed. Heritability and EBV reliability values are reported in Table 1. The EBV reliabilities are lower than common values for dairy cows, due to the different structure of the Buffalo population characterized by a limited use of artificial insemination and incomplete pedigrees (Gómez et al., 2021). However, 75% of the reliability values were between 0.18 and 0.83. Ancestor month of calving affected all the 5 considered traits (Table 2). The largest type III sum of squares of the effect was observed for the F_2 calving month, and it tended to decrease as distance between generations increased.

Least squares means of F_3 yield traits EBV across calving months exhibited a partially similar pattern in the 3 generations of female ancestors, although with different magnitude (Figures 2, 3, and 4). In general, winter and spring months exhibited largest LSM, whereas the smallest were observed in summer. September was the calving month with lowest LSM in F_0 and F_1, August in F_2, and they were statistically lower (Tukey-Kramer corrected P-value <0.01) than spring and winter months. Largest LSM were observed in December, January, and April for F_2, F_1, and F_0 calving months, respectively, for all of the 3 yield traits. The effect of calving month of the F_2

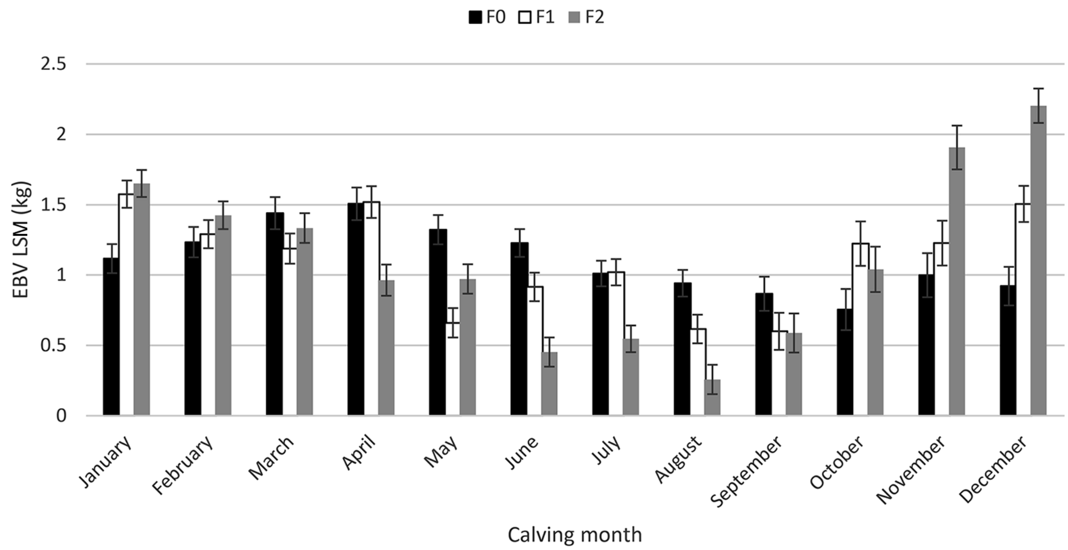

Figure 3. Least squares means and SE of the effect of calving months of great-granddams (F_0), granddams (F_1), and dams (F_2) on the EBVs for fat yield of cows (F_3).

generation showed the largest magnitude and variability, whereas the lowest were observed for F_0, respectively.

Milk composition traits exhibited a quite opposite pattern (Figures 5 and 6). Effects of ancestor calving months were larger for fat percentage compared with protein. For both traits, largest LSM of F_3 EBV were observed in December, September, and July for F_0, F_1, and F_2 calving months, respectively. Lowest LSM were estimated for February, for F_0 and F_1, and January for F_2, respectively.

The average THI_{MAX} of the different F_0 calving months averaged for the period 1974 to 2018 is reported in Figure 7. It can be observed that from June to September it exceeded the threshold of mild discomfort zone (Marai and Haeeb, 2010; Chaudhary et al., 2022; Petrocchi Jasinski et al., 2023). The SC_{THI} was on average 32.9 but markedly right skewed (Figure 8) with a large SD. (34.61). The pattern of the average F_3 milk yield EBV across increasing SC_{THI} values (Figure 9) highlight a tendency to decrease, even if with large variation. Table 3 reports the Pearson correlations between SC_{THI} and the F_3 EBV for the 5 considered traits. SC_{THI} showed a weak negative correlation with milk, fat, and protein yield EBVs, positive for fat percentage, whereas the value for protein content was not statistically different from zero.

The effect of the number of days with THI_{MAX} greater than 70 in the last part of F_0 pregnancy was estimated with the modified model [1], where the fixed effect the calving month of the F_0 generation was replaced with the fixed effect (covariate) of the SC_{THI}. All the yield traits were significantly affected by SC_{THI}, especially milk and protein yields, but not protein percentage (Table 4). The sign of the covariate coefficient was negative for yield traits, indicating a decrease in F_3 EBV as the SC_{THI} increases.

DISCUSSION

Results of the present study highlighted transgenerational and intergenerational effects of the calving month of ancestors on the dairy performance of their female progeny in Italian Mediterranean river buffalo. In agreement with previous reports on TEI in dairy cattle, F_3 buffaloes whose F_0 great-granddams calved in summer months, especially September, showed the lowest LSM of EBV for dairy traits, whereas the largest values were exhibited by buffaloes whose F_0 had calvings in December, respectively. Results for IE highlighted a different pattern between adjacent and nonadjacent generations. Trans- and intergenerational effects detected at phenotypic level in livestock are interpreted as suggestions for the existence of EI. Main epigenetic mechanisms are represented by DNA methylation, histone modification, chromatin remodeling, and gene silencing by noncoding RNA (van Otterdijk and Michels, 2016; Laporta et al., 2024). Epigenetic modifications during an individual's life can be determined by environmental exposure to nutritional, physical, and chemical factors (Jirtle and Skinner, 2007). In the case of HS, the exposure of the dam during pregnancy to high temperatures results in a suboptimal intrauterine environment for the developing embryo that can modify the epigenome (Laporta et al., 2020). This mechanism of adaptation prepares the fetus to survive in a potentially difficult postnatal environment (Rhoads, 2020). Such a developmental

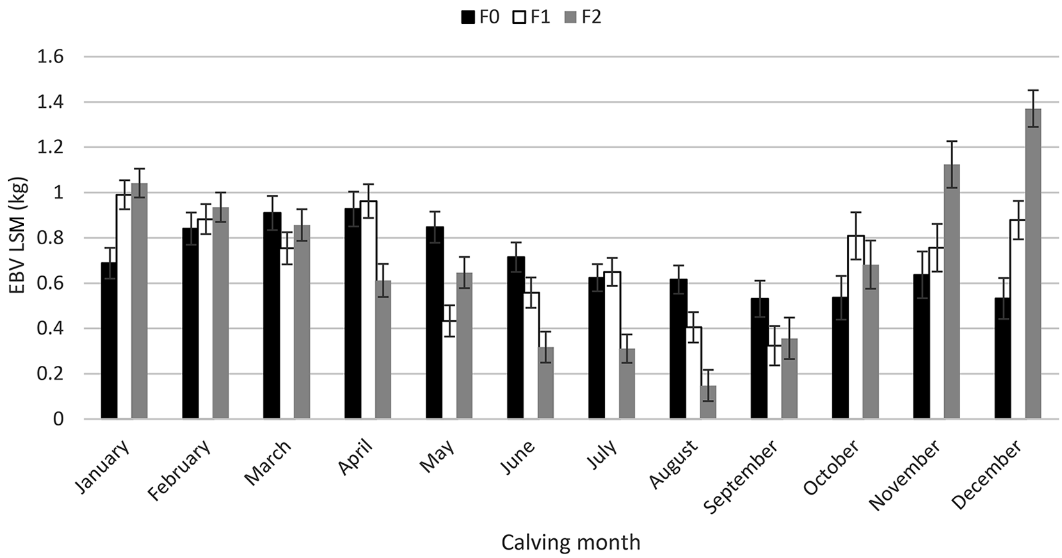

Figure 4. Least squares means and SE of the effect of calving months of great-granddams (F_0), granddams (F_1), and dams (F_2) on the EBVs for protein yield of cows (F_3).

plasticity could, however, result in severe problems for the offspring in case of a mismatch between prenatal and adulthood environment (Jirtle and Skinner, 2007; Rhoads, 2020). Most of these epigenetic marks are erased by 2 waves of epigenetic reprogramming that occur during gamete formation and early embryo stages after fertilization (Burton and Metcalfe, 2014), that aims at restoring totipotency of specialized germ cells (Khatib, 2021). The molecular explanation of TEI relies on the fact that a part of epigenetic marks generated by environmental stress during F_0 pregnancy partially escape the epigenetic reprogramming. In human and mice, for example, regions that contain imprinted genes and transposable elements escape the epigenetic reprogramming in early embryo development (van Otterdijk and Michels, 2016; Laporta et al., 2024). Thus, effect detected in the third generation after the mother exposure first generation not triggered by the environmental challenge (F_3 generation in this case) can be considered as expression of TEI.

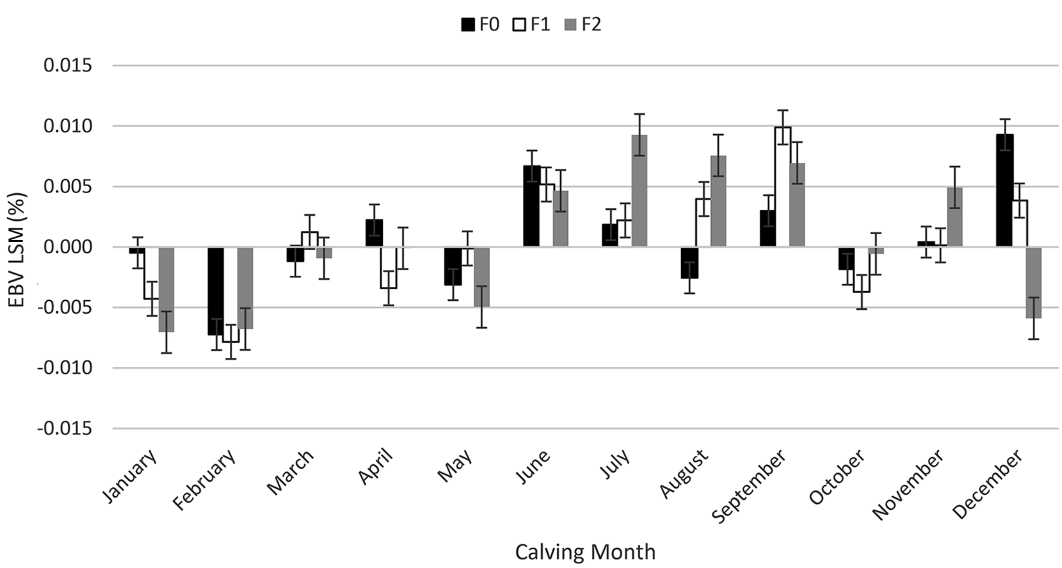

Figure 5. Least squares means and SE of the effect of calving months of great-granddams (F_0), granddams (F_1), and dams (F_2) on the EBVs for fat percentage of cows (F_3).

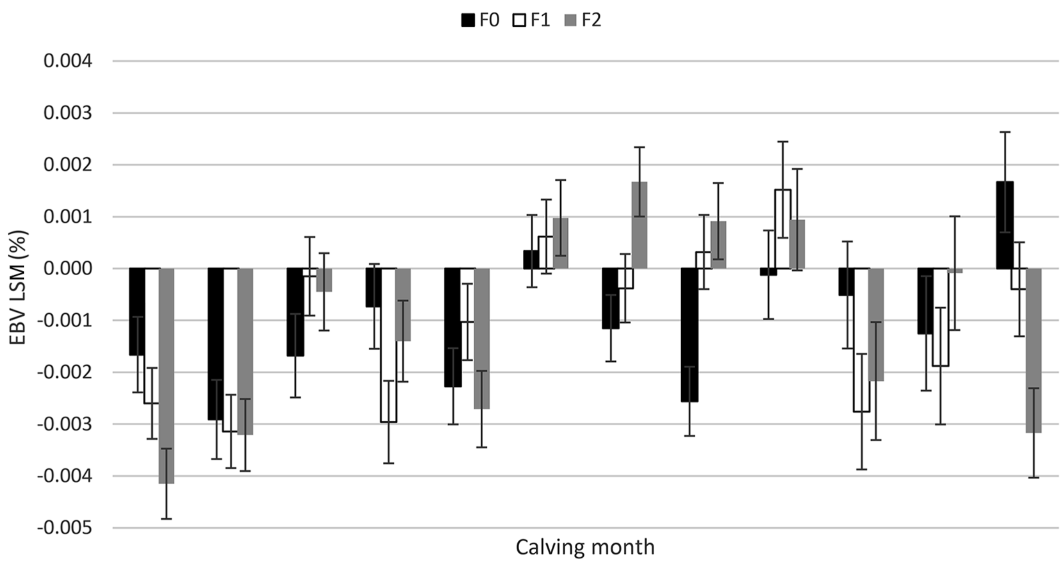

Figure 6. Least squares means and SE of the effect of calving months of great-granddams (F_0), granddams (F_1), and dams (F_2) on the EBVs for protein percentage of cows (F_3).

Dairy breeds represent an interesting model for inter- and transgenerational observational studies on thermal stress effect, due to the intensive recording of phenotypes and pedigree data at large scale by (DHI) programs (Rhoads, 2020; Weller et al., 2021) and to easy access to meteorological data. The ancestor month of calving has been included in these studies to account for the effect of climate conditions during pregnancy (Weller et al., 2021; Macciotta et al., 2023). This is undoubtedly a strong assumption because not necessarily all the females that calved in a specific month in different years or places are subjected to the same environmental conditions during gestation, and no specific measurements of HS (e.g., rectal or vaginal temperature) are available at large scale. It should be, however, pointed out that the THI_{MAX} averages of summer months recorded in the present study (Figure 7) were above the threshold of mild thermal discomfort reported for buffalo (Petrocchi Jasinski et al., 2023). Other approaches have been used to find suitable proxies of environmental conditions, as in

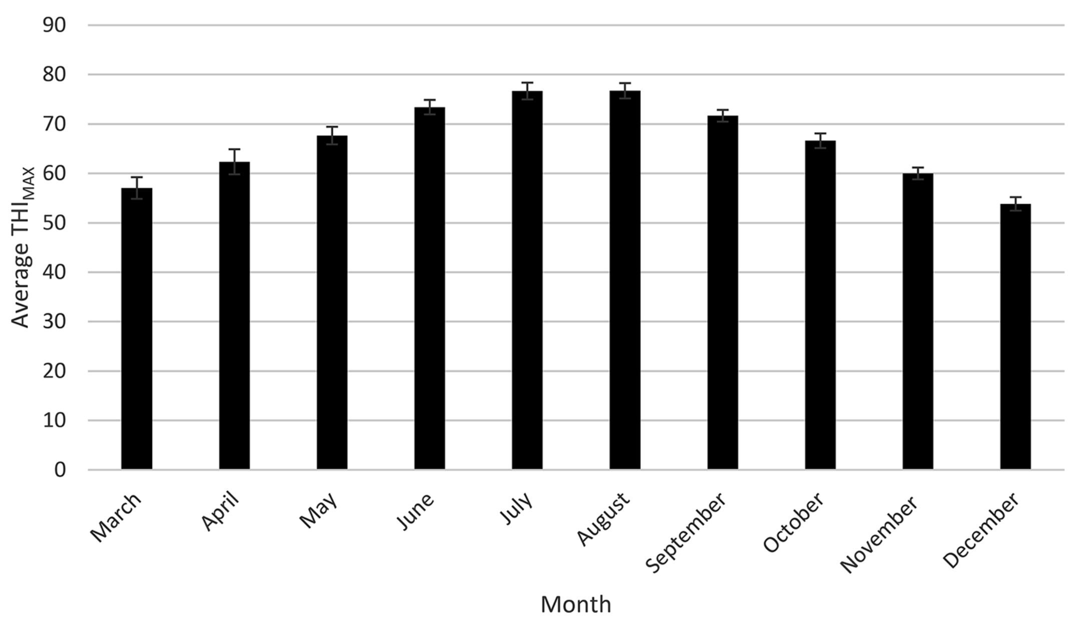

Figure 7. Monthly average maximum (±SD) temperature-humidity index (THI_{MAX}) values in the great-grandam calving years 1974 to 2018.

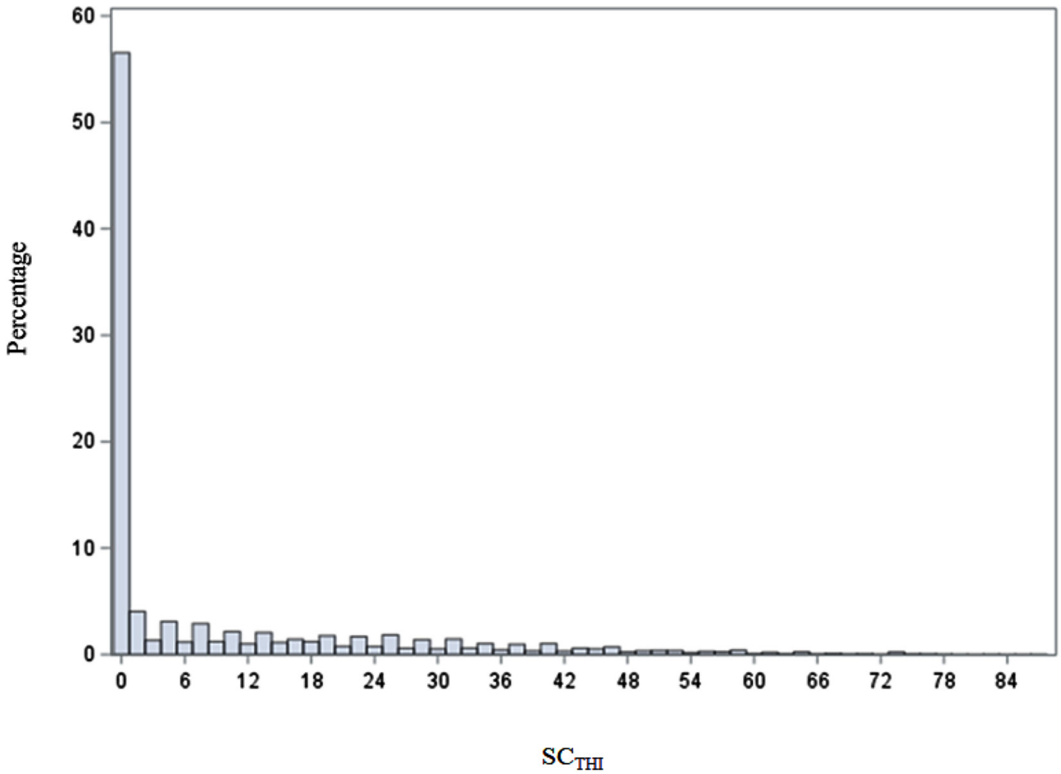

Figure 8. Distribution of THI score values (SC_{THI}; i.e., the number of days of the last third [100 d] of F_0 great-granddam pregnancy with a THI_{MAX} greater than 70).

Santana et al. (2025) that used mixed-model solutions of contemporary group effects as an indicator of the quality of the maternal environment approach for investigating TEI on growth and reproductive traits in Zebuine cattle. Moreover, a further challenge in estimating transgenerational effects on progeny phenotypes is represented by the fact that animals are likely to be exposed to high temperatures at multiple stages of their life. The power of the design due to the large dataset size (hundreds of thousands of records) and the use of phenotypes (EBV) that are already corrected for several environmental factors, should, at least partially, overcome these limitations. Due to the inclusion in the statistical model of corrections for the animal immediate environment, the F_3 birth month, and for the genetic trends, the effect of F_0 calving month on F_3 could likely be explained by the environment during F_0 gestation (Weller et al., 2021).

The existence of TEI has been demonstrated also by molecular evidence in plants, nematodes, and insects (Khatib, 2021), whereas for mammalians it is still controversial. Recently, molecular evidence of TEI has been reported for sperm DNA methylation patterns in rams, where modifications induced by a methionine supplementation on F_0 males where inherited by F_1 and F_2 generations (Braz et al., 2022). Due to difficulties in carrying out molecular based experiments on large scale, reports on inter- and transgenerational effects in livestock are mostly based on phenotypes or breeding values (Macciotta et al., 2023; Laporta et al., 2024). However, a recent development of an EPIchip (Costes et al., 2025), a technology able to target 44k methylation sites in the bovine genome in analogy to SNP beadchip, seems to provide an interesting tool for investigating the effect of methylome on phenotypic variation in cattle at population level.

In the present study, buffaloes whose ancestors had the last part of pregnancy in summer, and therefore presumably exposed to HS in that period, exhibit a reduction in their dairy performances. The evaluation of this effect could be confounded by different combinations of month of calving of F_0, F_1, and F_2 cows. Effects of different F_0 and F_1 generations on F_3 performances have been found to be additive in Israeli Holstein (Weller et al., 2021; Gershoni, 2023). In the considered dataset, all possible 1,728 combinations among the calving month of the 3 generations of ancestors were represented. This data structure should avoid the confounding between the effect of F_0 calving month with those of the following generations. Moreover, calving month of all the 3 ancestor generations were included in the statistical model. Results obtained

Table 3. Pearson correlations between the number of days with temperature-humidity index larger than 70 (SC_{THI}) during the last 100 d of the pregnancy of great-granddams (F_0) and the F_3 EBV of cows (F_3)

Trait	Correlation with SC_{THI}
Milk yield	−0.042*
Fat yield	−0.045*
Protein yield	−0.045*
Fat percentage	0.017*
Protein percentage	0.007

*Statistically different from 0 ($P < 0.001$).

Table 4. Estimates (SE) and level of significance of the covariate of the number of days with temperature-humidity index greater than 70 (SC_{THI}) during the last 100 d of the pregnancy of great-granddams (F_0) on the F_3 EBV of cows (F_3)

	SC_{THI}	
Trait	Estimate	*P*-value
Milk yield	−0.054 (0.022)	0.0126
Fat yield	−0.0064 (0.0013)	0.0373
Protein yield	−0.0024 (0.001)	0.0006
Fat percentage	0.00006 (0.00003)	0.0306
Protein percentage	0.0000 (0.00000)	0.7766

in the present study were also confirmed by substituting the calving month effect in the model with a meteorological variable representing the number of days where the THI_{MAX} exceeded the threshold of 70 during the last 100 d of pregnancy. The estimated regression coefficients of SC_{THI} were significant and always negative for yield traits highlighting a reduction of F_3 EBV as the SC_{THI} of their F_0 great-granddams increase.

In the present study, IEI and TEI showed a different magnitude (Figures 2, 3, 4, 5, and 6) especially for yield traits, with a progressive reduction of the effect of calving month on F_3 EBV passing from F_2 to F_0. This is a quite expected result because, if IEI and TEI are the consequence of the transmission of epigenetic marks, it should be considered that the epigenome is dynamic and therefore it can change throughout the life (Ibeagha-Awemu and Zhao, 2015), causing a progressive dilution along generations (David and Ricard, 2023; Baduel et al., 2024). Results of the present study are in agreement with those of Weller et al. (2021), that reported a stronger significance for effect the F_1 calving month on F_3 EBV compared with that of F_0.

Of interest is also the different pattern of IEI and TEI across the months of the years. In particular, as far as the effect of F_2 calving month on F_3 EBV for yield traits is concerned, largest values were observed in December and smallest in August, respectively (Figures 2, 3, and 4). These results partially disagree with what was observed on the effect of F_0 and F_1 calving months. Considering that the average pregnancy length in the Italian Mediterranean buffalo is of 308 d (ANASB, 2021), buffalo cows that had calvings in December conceive approximately in February, whereas those with August calvings had conceived in October, respectively. Thus, conceptions in winter had a positive effect on the offspring performances whereas those that occur in early fall had a negative effect. These findings suggest that for adjacent generations the effect of thermal conditions on offspring is larger in the periconception period rather than in late pregnancy. The opposite has been observed in generations far apart. This result has been confirmed also by testing with a mixed linear model similar to model [1], the effect of the calving month of F_1 or F_0 on milk yield EBV of F_2 and F_1 buffaloes, respectively (data not reported for brevity). Barash et al. (1996) found in Israeli cattle larger productions in cows born in fall and smallest for those born in spring. Several studies have underlined that HS experienced during the periconceptional period had negative effect on dairy performance of offspring (Brown et al., 2015; Pinedo and Devries, 2017; Rhoads, 2020). Recently, Vinet et al. (2024) in French Holstein and Montbéliarde found a negative effect of high THI experienced at the beginning of the gestation of the dam on the dairy performance of the daughters, whereas a slightly positive effect was reported for high THI at the end of pregnancy. Larger negative effects of HS occurring at the beginning of gestation were observed also in Italian Simmental cattle (Macciotta et al., 2024). It should be pointed out that epigenetic modifications occurring at the time of conception or during the early stages of embryo life can potentially affect a large proportion of cells of the organism (Burton and Metcalfe, 2014). Results of the present study seem therefore to indicate that HS experienced in different part of the gestation may affect differently offspring phenotypic expression (i.e., on adjacent generation the effects on conception month appears to be more relevant, whereas for generations far apart the late gestation seems to be the more critical period). It should be considered that the effect of F_2 calving month on F_3 EBV reflects also a direct effect of environmental conditions on the embryos in uterus. Moreover, also the match or mismatch between thermal stress experienced by the ancestor and that experienced by its offspring may have a role (Gershoni, 2023).

Data considered in the present work were EBV estimated in the routine genetic evaluation performed by the ANASB. The process of EBV estimation could have introduced some bias on the considered response variable, and the use of direct phenotypes, as happens in controlled studies (Laporta et al., 2020), might be preferred. If there is a transmissible nongenetic effect, the additive genetic values will include it, and therefore in large-scale observational studies the use of EBV is preferable (David and Ricard, 2023). Epigenetics is not currently accounted for

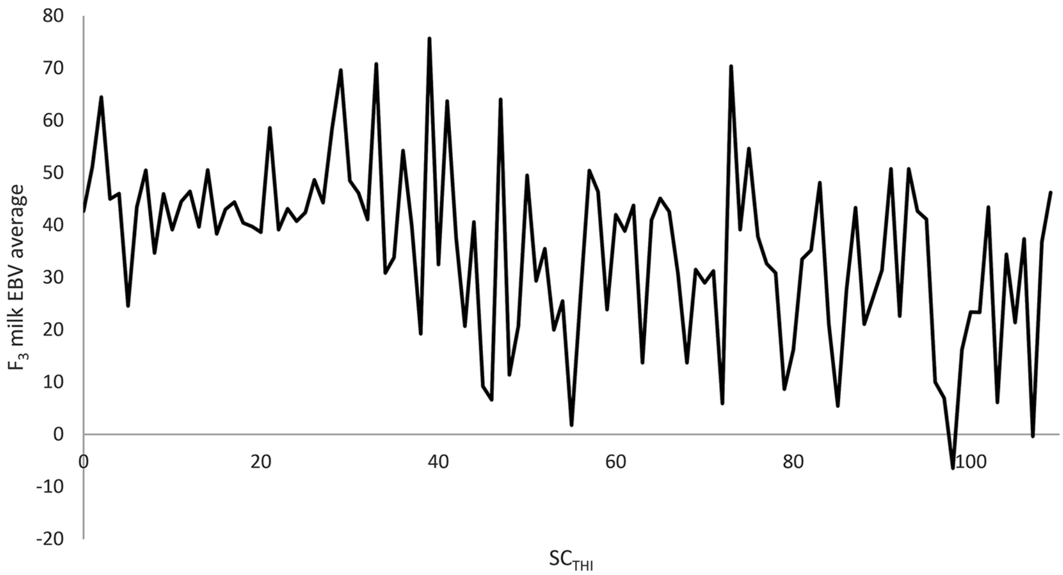

Figure 9. Pattern of F_3 buffaloes EBV for milk yield across THI score values (SC_{THI}; i.e., the number of days of the last third [100 d] of their F_0 great-granddam pregnancy with a THI_{MAX} greater than 70).

in breeding programs, particularly in statistical models used to estimate the genetic merit of selection candidates in livestock. Transgenerational epigenetic heritability (**TEh²**) has been estimated in different livestock species. The TEh^2 for BW has been estimated in cattle (0.04) by Varona et al. (2015), using a Bayesian approach developed on the theory proposed by Tal and al. (2010). Araujo et al. (2025) compared different models for the estimation of TEh^2 in meat and reproduction traits in pigs. They found moderate values for meat (about 0.33) and low (0.04) for reproduction traits, respectively.

Genomic selection has speed up breeding programs due to the remarkable reduction of generation intervals (Guinan et al., 2023). However, TEI is a mechanism involved in the transmission of phenotypic variation (Tal et al., 2010), and it should be accounted for. The inclusion of an epigenetic component in genetic models may lead to an improvement of EBV accuracy (Ibeagha-Awemu and Zhao, 2015). An interesting approach has been proposed by David and Ricard (2023), that developed a transmissibility model where, instead of the usual animal additive genetic and permanent environmental effects, a global transmissible potential (**gtp**) effect is included. In this approach, the gtp includes all possible transmissible values (genetic, epigenetic, microbiome, and culture) derived not only from the inherited genome but also by the unmatched ancestor's environment (Gershoni, 2023). A deeper knowledge of IEI and TEI in livestock could be beneficial also for the farm management, helping in the development of effective mitigation strategies (Laporta et al., 2024), especially in the periods where the effect of thermal stressors on epigenetic programming is more pronounced as the pregnancy or male puberty (Gershoni, 2023).

CONCLUSIONS

Transgenerational and intergenerational effects of the calving month of ancestors on the production performance of their female offspring progeny have been detected in Italian Mediterranean river buffaloes. The magnitude of the effect tended to decrease as the distance between the generations increases. Buffaloes whose great-granddams calved in summer showed the lowest LSM of EBV for dairy traits, whereas the largest values were exhibited by buffaloes whose F_0 had calvings in December. These results confirm previous reports in dairy cattle. Intergenerational effects showed a different pattern between adjacent and nonadjacent generations. Trans and intergenerational effects on phenotypes can be interpreted as evidence of the existence of mechanisms of EI also in this species. A deeper understanding of trans- and intergenerational epigenetic inheritance would provide useful knowledge for the management and breeding of river buffalo.

NOTES

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did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: ANASB = Italian Buffalo Association; EI = epigenetic inheritance; gtp = global transmissible potential; HS = heat stress; IEI = intergenerational EI; SC_{THI} = THI score; TEh^2 = transgenerational epigenetic heritability; TEI = transgenerational EI; THI = temperature-humidity index; THI_{MAX} = maximum THI.

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ORCIDS

Nicolò P. P. Macciotta, https://orcid.org/0000-0001-5504-9459
Roberta Cimmino, https://orcid.org/0000-0002-0146-1787
Gianluca Neglia, https://orcid.org/0000-0002-0989-6072
Alberto Cesarani https://orcid.org/0000-0003-4637-8669