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The Nutritional Year-Cycle of Italian Honey Bees (*Apis mellifera ligustica*) in a Southern Temperate Climate †

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† This research work is the part of Ph.D. thesis of Evaluation of the nutritional status of worker honey bees (Apis mellifera ligustica S., 1806) across temporal patterns through morphological analysis.

Simple Summary: In this research, the nutritional status of Italian bees from Sassari was monitored over a yearly cycle leading to the first report of how honey bee nutrition varies over time and according to external factors in a warm temperate Mediterranean climate. During spring and summer, the nutritional status of sampled bees changed in parallel with the availability of feed resources: when flowering plants were plentiful bees were in a good nutritional state, and their nutritional state declined when flowers disappeared. During this period, rainfall was of great importance, with summer droughts representing a particularly challenging period for bees in the study area. In fall and winter, honey bee nutrition was in opposition to the availability of feed resources as deteriorating environmental factors and the disappearance of flowering plants caused honey bees to transform into their winter state (called winter bees) with increased individual nutrient storage. Nevertheless, winter bees were only present for a limited time, which was accredited to high winter temperatures and continuous (but limited) availability of flowering plants. These results provide valuable insights into the nutritional dynamics of Italian bees in the Mediterranean that could support management decisions to improve overwintering success and prevent unnecessary losses.

Abstract: Nutrition is a key aspect influencing honey bee health and overwintering. Since honey bee seasonality in southern temperate climates represents a significant research gap, this study conducted long-term monitoring of honey bees in the Mediterranean (Sassari, Italy). Specifically, individual weight, fat body, and size measurements (head, thorax, abdomen, and total body) were recorded monthly so to detect changes in the nutrient storage of worker bees during an annual cycle. Data were analysed according to sampling date, climate (temperature, precipitation, and daylength), and flower diversity and were conducted for nurse and forager bees separately. The nutritional honey bee year-cycle generally followed the nectar flow and showed two critical timepoints: summer and winter dearth. A short cessation of activities in late fall/early winter coupled with an increase in nutrient storage indicated the presence of winter bees. Precipitation was found to play an important role in honey bee nutrition in the study area through its impacts on colony demography and plants in particular illustrating how climate change could pose a threat to European honey bee populations in the future. These results provide valuable insights into the nutritional dynamics of *Apis mellifera ligustica* in the Mediterranean that could support management decisions to improve overwintering success and prevent unnecessary colony losses.

Keywords: winter bees; nutrition; morphometry; melliferous plants; climate change

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1. Introduction

Honey bee colony losses represent a grave and yet relatively poorly understood issue in modern apiculture [1–6]. Regardless of climate, most losses occur in winter, which is a particularly challenging period for these social insects as there is little to no natural forage available [7–9].

Historically, as honey bees (*Apis mellifera* Linnaeus, 1758) spread from tropical /subtropical regions to temperate climates of the northern hemisphere they evolved unique adaptations allowing colonies to bridge harsh winter conditions without entering a dormant state [10–13]. Specifically, honey bees synchronized their activities with plant phenology, greatly reduced brood rearing in winter, and assumed the formation of a thermoregulating cluster during the coldest months [7,8,14]. Furthermore, *A. mellifera* adopted significant seasonal changes in individual lifespan within its yearly cycle. This has led to the description of two temporally distinct worker bee types; while the honey bee workforce is made up of classical short-lived "summer bees" during most of the year, in winter, these bees are replaced by long-lived winter or *diutinus* bees [15].

Besides assuring colony survival through thermoregulation [7,16] winter bees effectively function as a "nutrient storage caste" [12,17,18]. These bees store large amounts of fat and protein within their bodies (through the accumulation of vitellogenin: Vg) which are conserved throughout winter and subsequently utilized to reinitiate brood rearing when the return of favourable environmental conditions is anticipated [8,19]. Moreover, it is this same Vg that grants winter bees their longevity [17,20–22], illustrating the fundamental role of nutrition for the survival of cold-adapted honey bees. Other typical features of *diutinus* bees (hypertrophied hypopharyngeal glands, enlarged fat bodies, and elevated hemolymph protein content) are also related to nutrition [7,8,14,17,23–25].

Extensive research has allowed for the description of an elegant system showing how honey bees in temperate zones have adapted mechanisms of age division of labour into a bimodal, biannual worker caste system governed by a multitude of internal and external factors with varying sensitivity (reviewed in [15]). In brief, deteriorating environmental conditions and the disappearance of nutrient resources (nectar and pollen) likely cause a drastic reduction in brood rearing, triggering the transition of newly emerging bees into *diutinus* bees. It is noteworthy that this seasonal shift is mainly linked to the dwindling pollen availability in fall rather than to fluctuations in meteorological factors offering temporal plasticity and adaptability in a changing climate [23].

Whereas overwintering of honey bees in northern regions have been well studied [7,8,15], much less is known regarding the seasonal dynamics of these insects in southern temperate climates. At these latitudes, warm summers and soft winters generally allow for a long foraging season and only a short cessation of activities in winter [26–29]. While this seems advantageous, relatively high winter temperatures can lead to unsustainable brood rearing causing exhaustion of worker bees towards spring [16,26]. Moreover, extended periods of foraging resource dearth (e.g., during summer droughts) can put nutritional stress on a colony, hampering preparations for winter [28,30–32].

Inadequate nutrition has been identified as a dominant factor in honey bee colony losses [33,34] and has been shown to have significant effects on individual and colony health and development, including colony size, lifespan, immunity, and overwintering success [27,33–44]. Moreover, poor nutrition increases the sensitivity of honey bees to biological stressors (e.g., pests and diseases) and anthropogenic stressors (e.g., agricultural intensification and climate change) contribute to malnutrition of honey bee colonies [33–51].

Lastly, research efforts in relation to seasonal adaptations of honey bees have mainly focused on northern subspecies (e.g., *Apis mellifera mellifera*) [13]. Since a higher survival rate of locally adapted subspecies [52] as well as adaptation to specific climatic conditions [53–55] has been shown, knowledge of southern honey bee populations is of increasing interest, especially in the face of accelerated climate change [30,56].

Against this background, this study aimed to conduct long-term monitoring of the nutritional status of locally adapted Italian honey bees (*Apis mellifera ligustica*, Spinola 1806) in a Mediterranean climate (Sassari, Italy). The goal of this research was to provide a better understanding of the activity and nutritional status of worker (both nurse and forager) bees in southern temperate climates and to generate new insights on the dynamics of the summer and winter bee transition in correlation with seasonal changes in environmental factors and feed resource availability. In addition, authors aimed to provide novel knowledge regarding the seasonal dynamics of Italian honey bees specifically, and the possible challenges these bees face in a changing climate.

2. Materials and Methods

2.1. Study Site and Apiary

Monitoring was conducted between February 2022 and January 2023 (12 months). A total of n. 5 colonies of Italian honey bees (*Apis mellifera ligustica* Spinola, 1806), located in a private apiary in the province of Sassari (Sardinia, Italy; 40°37′14.5″ N 8°20′43.1″ E), were studied. The southern temperate Mediterranean climate of the study area, with hot dry summers and mild wet winters, typically allows for a long foraging season and only a short cessation of activities in winter. The initiation of the study was planned according to the seasonal pattern of Italian honey bees in the region, coinciding with the start of the foraging season.

Meteorological data over the course of the study, including mean monthly temperature, precipitation, the number of days with precipitation, relative humidity, windspeed, and daylength (hours of daylight), as retrieved from the weather station of the meteorological services of the Military Airforce of ENAV (Ente Nazionale Assistenza al Volo) located approximately 15 km from the study area are summarized in Table 1.

Table 1. Summarizing meteorological data for the province of Sassari (Italy), February 2022–January 2023.

	Temperature (°C)		Precipitation (mm)		Precipitation (Days)	Wind (Km/h)	Humidity (%)	Daylength (min)	
Month *		**Category *****		**Category *****					**Category *****
Jan **	10.4	Ta	83.4	Pb	17	11.0	82	575	Oa
range	−1.0–21.0		/		/	0–45.0	60–98	/	
Feb	10.3	Ta	19.3	Pa	4	11	77	641	Oa
range	−2.0–18.0		/		/	0–40.0	53–96	/	
Mar	10.8	Ta	33.7	Pa	12	11.2	71	728	Ob
range	−1.0–22.0		/		/	0–33.8	46–93	/	
Apr	14.2	Ta	61.5	Pb	8	14.0	70	813	Oc
range	3.0–25.0		/		/	0–45.0	44–95	/	
May	19.6	Tb	89.7	Pb	6	9.0	74	877	Od
Jun	25.0	Tc	3.8	Pa	4	9.9	66	903	Od
Jul	26.7	Td	0.3	Pa	0	8.9	66	893	Od
Aug	26.8	Td	12.9	Pa	3	10.0	72	822	Oc
Sep	23.3	Tc	108.6	Pc	9	7.0	75	730	Ob
Oct	19.5	Tb	46.6	Pa	6	7.5	81	654	Ob
range	9.0–29.0		/		/	0–29.0	50–100	/	
Nov	15.5	Tb	148.4	Pc	16	9.9	82	597	Oa
Dec	13.2	Ta	144.5	Pc	11	10.7	88	559	Oa

* Jan = January, Feb = February, Mar = March, Apr = April, Jun = June, Jul = July, Aug = August, Sep = September, Oct = October, Nov = November, and Dec = December. ** Data for the month of January were collected in 2023 while the rest of the months regard the year 2022. *** Categories of environmental factors for statistical analysis: (1) monthly average ambient temperature is divided into 4 levels; Ta, Tb, Tc, Td; range: 10–15, >15–20, >20–25, >25 °C. (2) mean monthly precipitation is divided into 3 levels; Pa, Pb, Pc; range: 0–50, >50–100, >100 mm. (3) monthly average daylength was divided into 4 levels; Oa, Ob, Oc, Od; range: 550–650, >650–750, >750–850, >950 h of daylight.

Hives were located in a semi-natural agricultural area, surrounded by managed and unmanaged fields, vineyards, olive groves, and small-scale mixed agriculture (vegetable gardens). The botanical composition of spontaneous flora and the phenological state of plants, with particular regard to pollen availability, in the direct vicinity of the apiary, was monitored throughout the sampling period. Specifically, in order to assess the diversity of flowering plants, 3 100 m × 2 m transects were defined prior to the initiation of the study. At each sampling date (concomitantly with bee monitoring, as described below), the

3 transects were walked by a single observer and the various species of flowering plants known to be visited by honey bees recorded.

Honey bee samples were collected from 5 individual hives selected by the responsible apiarist based on overall health and uniformity. Selected colonies were separated from the rest of the apiary by a distance of 25 m before the initiation of the study. Colonies received standardized care during the study period and were inspected weekly insuring good health. No clinical signs of disease were noted during the course of this study. Treatment against the ectoparasitic mite *Varroa destructor* was applied in March, August, and December using Amitraz and oxalic acid. Colonies were fed a homemade sucrose solution (3:2 sucrose/water) in spring and fall. No other nutritional supplements were provided.

Apis m. ligustica queens with a nucleus were acquired from a commercial queen breeder and introduced to each respective colony the year before. Queens remained during the whole duration of the study. Colonies were housed in wooden Dadant-type hives with 10 commercial brood frames with a cell diameter of 5.4 mm.

Brood rearing patterns were consistent with that of Italian bees in a southern temperate climate (exhibiting a "Mediterranean pattern" as has been described for bees on the neighbouring island of Corsica [57]); showing a steady increase from spring until peaking in June and subsequently decreasing during hot summer months. A second minor peak was seen in early fall. Three out of the five hives showed a cessation of brood rearing (for approximately 2 weeks) in early December and brood rearing remained relatively low until spring. No foraging stop was observed for any of the hives.

2.2. Sample Collection

Ten forager and ten nurse bees from each hive were collected separately on the last week of each month (100 individuals; 50 foragers/50 nurse bees per month). (1) Foragers: bees returning to the hive were captured from the flight deck using a horsehair brush. (2) Nurse bees: young bees from the centre of the brood nest were collected. Captured bees were stored in 250 cl glass containers with breathable fabric lids and transported to the laboratory of animal production and nutrition of the university of Sassari (UNISS) in a cooler box with icepacks. Individual bees were weighed using a digital scale (OHAUS Pioneer Corp. Las Vegas (Nevada) Pioneer USA corporation, LA, mod. PA512C; precision of 0.01 g) before being frozen (−18 °C) and stored in 1.5 mL microcentrifuge tubes until further analysis. Any pollen or visible attachments were removed manually prior to weighing.

2.3. Morphological Analysis

Sampled bees were analysed in their entirety and within a frozen state insuring correct proportional morphological retention. Using a digital calliper (precision 0.01 mm) under a stereomicroscope (Leica® EZ4 HD), six size measurements were taken for each individual bee; (1) Head width (HW), (2) thoracal width (TW), (3) thoracal length (TL), (4) abdominal width (AW), (5) abdominal length (AL), and (6) total body length (T). Width measurements of each respective body part were taken at the widest point. Length measurements of the thorax and abdomen were taken from the anterior end of the protergum to the caudal end of the first abdominal tergum (T_1-IT; T_1 includes the scutum and scutellum) and the anterior end of the second abdominal tergum to the caudal end of seventh abdominal tergum (IIT-VIIT) not including the stinger, respectively. All size measurements were taken in duplicate and averaged creating a single observation.

2.4. Fat Body Quantification

Ether extraction was performed to estimate the weight and relative size of the fat body of bees according to Wilson-Rich et al. [58]. Briefly, the abdomen of each bee was severed using surgical scissors and placed into separate holding cups to dry at 25 °C for 3 days. Next, abdomens were placed in individual 1.5 mL microcentrifuge tubes to which 500 µL of diethyl ether was added. Abdomens were removed after 24 h and dried again for 3 days (same conditions). A Binder ED 53 drying oven was used to insure continuity of drying conditions over the duration of the study. Dried abdomens were weighed before and after ether extraction using a ORMA BCA200 electric laboratory balance with a precision 0.0001 g. The fat body weight (FBW) was calculated as the difference between the weight of each abdomen before and after washing with diethyl ether. The relative size of the fat body (FB%) was calculated as the proportional weight of the fat body relative to the weight of the dried abdomens prior to ether extraction [58,59].

2.5. Data Analysis

All procedures were carried out using a software package (Minitab statistical software package, Minitab©, New York, NY, USA). Statistical significance was set at p-value < 0.05 and Tukey test was used for the *post hoc* pairwise comparison of means.

2.5.1. Worker Bee Type

Analysis of variance (ANOVA) was performed to detect significant differences in monitored metrics between the two types of sampled worker bees. A balanced linear model with interaction was used as follows:

$$y_{a,b,c,\ldots,k} = \boldsymbol{\mu} + W_{a,b} + H_{j,k} + W * H + \varepsilon$$

where y is the dependent variable (n = 9; Weight, HW, TW, TL, AW, AL, T, FBW, FB%), $\boldsymbol{\mu}$ is the overall mean, W is the fixed factor representing worker type (2 levels; Forager, Nurse), H is the fixed factor of hive (n = 5; *H1*, *H2*, *H3*, *H4*, *H5*), $W * H$ is the interaction term, and ε is the random error.

Further analysis for any dependent variable significantly affected by W was conducted for forager and nurse bees separately. Unaffected variables were analysed using the whole dataset.

2.5.2. Effect of Sampling Date

Analysis of variance (ANOVA) was performed to detect significant differences in monitored metrics between the sampling months. A balanced linear model with interaction was used as follows:

$$y_{a,b,c,\ldots,k} = \boldsymbol{\mu} + M_{a,b} + H_{j,k} + M * H + \varepsilon$$

where y is the dependent variable (n = 9; Weight, HW, TW, TL, AW, AL, T, FBW, FB%), $\boldsymbol{\mu}$ is the overall mean, M is the fixed factor of sampling month (n = 12; January-December), H is the fixed factor of hive (n = 5; *H1*, *H2*, *H3*, *H4*, *H5*), $M * H$ is the interaction term, and ε is the random error.

If a significant effect of H was found, the dataset was split accordingly, and the effect of M analysed separately.

2.5.3. Effect of Environmental Factors and Flower Diversity

Analysis of variance (ANOVA) was performed to detect any changes in dependent variables of sampled bees according to environmental factors and flower diversity. All data were analysed following a general linear model procedure with interaction as follows:

$$y_{a,b,c,\ldots,k} = \boldsymbol{\mu} + T_{a,b} + P_{c,d} + O_{e,f} + F_{g,h} + H_{i,j} + T * H + P * H + O * H + F * H + \varepsilon$$

where y is the dependent variable (n = 9; Weight, HW, TW, TL, AW, AL, T, FBW, FB%), $\boldsymbol{\mu}$ is the overall mean, T is the fixed factor of monthly average environmental temperature (monthly average ambient temperature was divided into 4 levels; *Ta*, *Tb*, *Tc*, *Td*; range: 10–15, >15–20, >20–25, >25 °C; Table 1), P is the fixed factor representing mean monthly precipitation (mean monthly precipitation was divided into 3 levels; *Pa*, *Pb*, *Pc*; range: 0–50, >50–100, >100 mm; see Table 1), O is the fixed factor representing monthly average daylength (monthly average daylength was divided into 4 levels; *Oa*, *Ob*, *Oc*, *Od*; range: 550–650, >650–750, >750–850, >950 h of daylight; see Table 1), F is the fixed factor effect of the monthly flower diversity (monthly flower diversity was dived into 3 levels; *Fa*, *Fb*, *Fc*; range: <5, 5–10, >10 species of flowering plants see Table 2), H is the fixed factor of hive ($n = 5$; *H1*, *H2*, *H3*, *H4*, *H5*), $T * H$ is the interaction term between temperature and hive, $P * H$ between precipitation and hive, $O * H$ between daylight and hive, $F * H$ between plant diversity and hive, and ε is the random error.

Table 2. Summarizing table of the monthly diversity of flowering plants in the honey bee flight area over the study period (February 2022–January 2023) in Sassari (Italy).

	Month *											
	Jan **	***Feb***	***Mar***	***Apr***	***May***	***Jun***	***Jul***	***Aug***	***Sep***	***Oct***	***Nov***	***Dec***
Species												
Acacia dealbata		X	X									
Anthemis arvensis			X	X	X							
Asphodelus ramosus		X	X									
Bellis perennis			X	X	X				X	X		
Borago officinalis				X	X	X						
Calendula arvensis		X	X	X	X	X			X	X	X	X
Centaurea					X							
Chrysanthemum croronarium				X	X							
Convolvulus arvensis							X					
Crepis vesicaria		X	X	X	X	X	X		X	X	X	
Cynara cardunculus						X	X					
Dittrichia viscosa									X	X	X	
Echium plantagineum			X	X	X	X						
Eucalyptus**sp.**			X	X	X	X	X	X	X			
Foeniculum vulgare					X	X	X	X	X	X		
Fumaria officinalis				X	X	X						
Galactites tomentosus				X	X	X						
Geranium molle			X	X								
Glebionis coronaria				X	X							
Helminthotheca echioides**sp.**							X	X	X			
Hypochaeris achyrophorus			X	X	X							
Malva sylvestris				X	X							
Onopordum horridum					X	X	X					
Oxalis pes-caprae	X	X	X	X	X						X	X
Prunus amygdalus		X	X									
Rafanus sativus				X								
Raphanus raphanistrum					X	X						
Reichardia picroides				X	X	X	X		X	X	X	X
Salvia rosmarinus	X	X	X	X	X	X			X	X	X	
Senecio vulgaris					X							
Sinapis alba			X	X	X	X						
Trifolium nigrescens			X	X	X							
Count	2	7	15	20	23	14	8	3	9	7	6	3
Category ***	***Fa***	***Fb***	***Fc***	***Fc***	***Fc***	***Fc***	***Fb***	***Fa***	***Fb***	***Fb***	***Fb***	***Fa***

* Jan = January, Feb = February, Mar = March, Apr = April, Jun = June, Ju l= July, Aug = August, Sep = September, Oct = October, Nov = November, and Dec = December. ** Data for the month of January were collected in 2023 while the rest of the months regard the year 2022. *** Categories of environmental factors for statistical analysis: monthly flower diversity is dived into 3 levels; *Fa*, *Fb*, *Fc*; range: <5, 5–10, >10 species of flowering plants.

If a significant effect of H was found, the dataset was split accordingly, and the effect of $T/P/F/O$ analysed separately.

2.5.4. Correlation Analysis

Pearson test for the assessment of correlation between measured metrics was used (Weight, HW, TW, TL, AW, AL, T, FBW, FB%) and was performed on both worker bee types separately. A statistically significant correlation was deemed (1) weak: $\rho < 0.300$, (2) mild: $0.300 < \rho < 0.600$, or (3) strong: $0.600 < \rho < 1.000$ [60]. The nature of the correlation was defined as follows: $+\rho$ or $-\rho$: positively or negatively correlated.

3. Results

The diversity of flowering plants steeply increased during spring before peaking in early summer. This peak was followed by a drastic decrease over the course of the summer, bottoming in August. Summer dearth was followed by a mild restoration in fall. Limited flower diversity was noted in early winter which increased in February marking the onset of the foraging season. The various species of flowering plants encountered during the study period are reported per sampling month in Table 2.

Mean values for all dependent variables for the whole database and per worker bee type are reported in Table 3.

Table 3. Mean morphological metrics of *Apis mellifera ligustica* nurse and forager bees recorded over a 12-month period (2022–2023).

	Overall *			*Nurse bee*			*Forager bee*		
	Mean	*SD* **	*Range*	*Mean*	*SD* **	*Range*	*Mean*	*SD* **	*Range*
Weight (g)	0.10	0.02	0.06–0.17	0.12	0.02	0.08–0.17	0.08	0.01	0.06–0.12
Head width (mm)	3.74	0.06	3.56–3.88	3.76	0.05	3.62–3.88	3.71	0.05	3.56–3.86
Thoracal width (mm)	3.77	0.05	3.56–3.93	3.77	0.05	3.56–3.92	3.77	0.05	3.58–3.93
Thoracal length (mm)	3.76	0.05	3.48–4.01	3.76	0.05	3.48–3.97	3.76	0.05	3.50–4.01
Abdominal width (mm)	4.27	0.15	3.82–4.79	4.35	0.14	4.00–4.79	4.18	0.11	3.82–4.50
Abdominal length (mm)	6.4	0.85	4.91–8.89	7.08	0.61	5.62–8.89	5.72	0.39	4.91–7.28
Total body length (mm)	11.84	0.79	10.21–14.43	12.42	0.66	10.41–14.43	11.27	0.38	10.21–12.78
Fat body weight (mg)	7.7	7.6	0–38.0	13.2	6.7	0–38.0	2.1	3.3	0.0–20.7
Fat body size (%)	29	20	0- 92	42	13	12–88	16	16	0- 92

* Overall mean values for the whole dataset (nurse and forager bees together). ** Standard Deviation.

A statistically significant difference ($p < 0.001$) was found between worker honey bee types for all analyzed metrics except for TW and TL ($F_{(1,1190)} = 2.11$, $p = 0.147$; $F_{(1,1190)} = 2.42$, $p = 0.120$). A significant effect of hive was found for TW, TL, and T ($F_{(1,1190)} = 13.30$, $p < 0.001$; $F_{(1,1190)} = 16.22$, $p < 0.001$; $F_{(1,1190)} = 2.44$, $p = 0.045$). No interaction effect was detected between hive and worker type for any of the variables.

Post hoc analysis showed H5 to be significantly different from other hives for TW and TL, while no decisive pattern was revealed for T. Mean TW and TL values for *H5* were lower than those of other hives (TW: H1 = 3.78, H2 = 3.78, H3 = 3.78, H4 = 3.78, H5 = 3.75; TL: H1 = 3.77, H2 = 3.76, H3 = 3.77, H4 = 3.77, H5 = 3.74).

No significant difference in TW and TL was found over the months for any of the hives.

Results of the analysis of variance on the effect of hive and sampling month for nurse and forager bees are reported in Table 4.

Table 4. Results of the analysis of variance for the effect of hive and sampling month on various metrics of *Apis mellifera ligustica* nurse and forager honey bees.

	Effect of Hive			*Effect of Month*			*Interaction*		
	p-Value [a]	*F-Value*	*df* *	*p-Value* [a]	*F-Value*	*df* *	*p-Value* [a]	*F-Value*	*df* *
Nurse bees									
Weight	0.016	3.09	(4540)	<0.001	50.02	(11,540)	0.220	0.64	(44,540)
Head width	0.637	0.64	(4540)	<0.001	12.91	(11,540)	0.999	0.64	(44,540)
Abdominal width	0.299	1.23	(4540)	<0.001	23.99	(11,540)	0.795	0.82	(44,540)
Abdominal length	0.213	1.46	(4540)	<0.001	19.07	(11,540)	0.439	1.02	(44,540)
Total body length	0.009	3.42	(4540)	<0.001	26.47	(11,540)	0.338	1.08	(44,540)
Fat body weight	0.602	0.69	(4540)	<0.001	38.51	(11,540)	0.076	1.34	(44,540)
Proportional fat body size	0.744	0.49	(4540)	<0.001	31.22	(11,540)	0.100	1.3	(44,540)
Forager bees									
Weight	0.568	0.74	(4540)	<0.001	15.93	(11,540)	0.673	0.89	(44,540)
head width	0.483	0.87	(4540)	<0.001	17.57	(11,540)	0.999	0.45	(44,540)
Abdominal width	0.846	0.35	(4540)	<0.001	6.76	(11,540)	0.553	0.96	(44,540)
Abdominal length	0.023	2.85	(4540)	<0.001	15.83	(11,540)	0.773	0.83	(44,540)
Total body length	0.401	1.01	(4540)	<0.001	11.02	(11,540)	0.339	1.08	(44,540)
Fat body weight	0.709	0.54	(4540)	<0.001	10.77	(11,540)	0.999	0.45	(44,540)
Proportional fat body size	0.621	0.66	(4540)	<0.001	9.84	(11,540)	0.919	0.71	(44,540)

[a] Statistical significance set at $p < 0.005$. * Degrees of freedom.

Figures 1 and 2 show boxplots of the different variables significantly affected by month for nurse and forager bees respectively. The effect of month on the Weight and T of nurse bees according to hives is depicted in Figures 3 and 4. The effect of month on the AL of forager bees for the different hives is shown in Figure 5. Significantly different months are indicated by a red "*". No significant interaction effect was found.

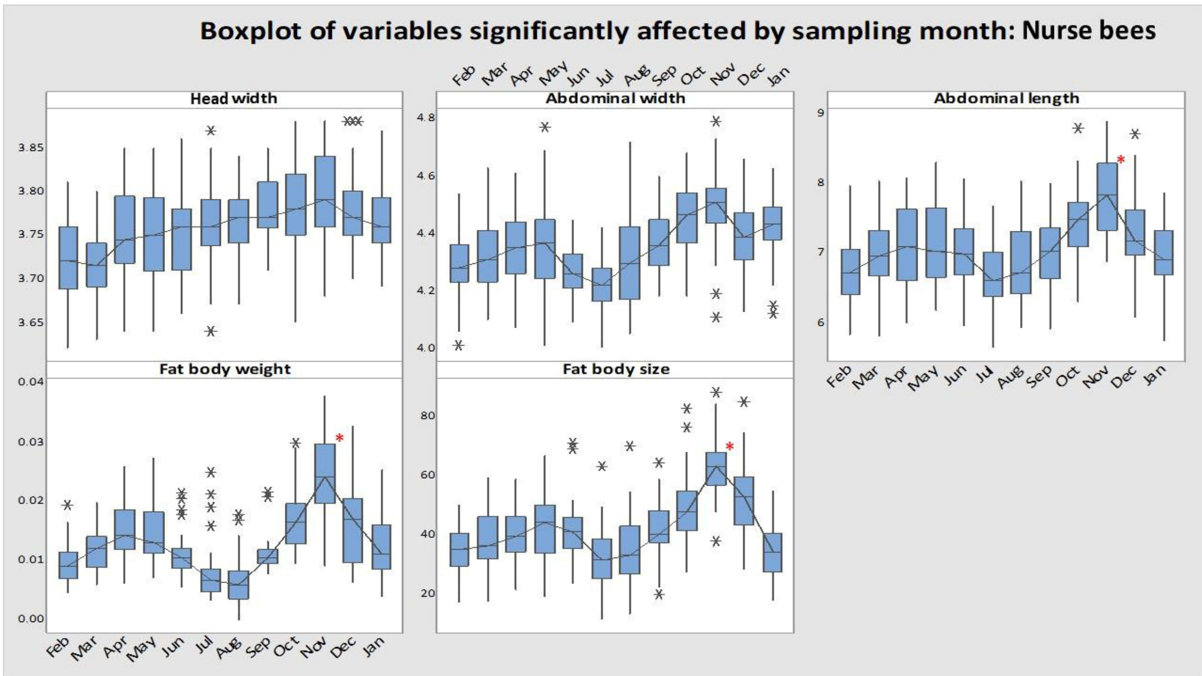

Figure 1. Box plots of Head width (mm), Abdominal width (mm), Abdominal length (mm), Fat body weight (mg), and Fat body size (%) of *Apis mellifera ligustica* nurse bees according to sampling months. The boxplot represents the interquartile range (IQR = Q3 − Q1) and bars represent first (Q1, top) and third quartiles (Q3, bottom) of metric values. Black "*" represent outliers and the black line represents the mean connect line. Red "*" indicates months significantly different from unmarked months. Feb = February, Mar = March, Apr = April, Jun = June, Jul = July, Aug = August, Sep = September, Oct = October, Nov = November, Dec = December, and Jan = January.

Analysis of variance revealed no significant effect of environmental factors nor flower availability on TW for any of the hives. A significant effect of temperature ($F_{(3,229)} = 2.66$, $p = 0.049$) and flower diversity ($F_{(3,229)} = 3.44$, $p = 0.034$) on TL was found for H3, and a significant effect of temperature ($F_{(3,229)} = 2.66$, $p = 0.049$) for H2. However, post hoc analysis showed no difference in TL between the groupings of various factors.

Results of the analysis of variance and post hoc analysis on the effect of environmental factors (mean monthly temperature, precipitation, daylength) and flower diversity for the dependent variables of nurse and forager bees are reported in Table 5. A significant effect of hive was found for Weight and T in nurse bees and are therefore reported here. Specifically, a significant effect of temperature was found on Weight and T for all hives (Weight: H1: $F_{(3,109)} = 23.47$, $p < 0.001$; H2: $F_{(3,109)} = 18.18$, $p < 0.001$; H3: $F_{(3,109)} = 13.61$, $p < 0.001$; H4: $F_{(3,109)} = 11.79$, $p < 0.001$; H5: $F_{(3,109)} = 11.79$, $p < 0.001$; T: H1: $F_{(3,109)} = 10.62$, $p < 0.001$; H2: $F_{(3,109)} = 10.28$, $p < 0.001$; H3: $F_{(3,109)} = 12.52$, $p < 0.001$; H4: $F_{(3,109)} = 3.93$, $p < 0.001$; H5: $F_{(3,109)} = 4.58$, $p = 0.005$). Weight was significantly affected by mean monthly precipitation for H2 ($F_{(2,109)} = 5.11$, $p = 0.004$), H3 ($F_{(2,109)} = 4.43$, $p = 0.014$), H4 ($F_{(2,109)} = 6.93$, $p = 0.001$), and H5 ($F_{(2,109)} = 5.11$, $p = 0.026$). Lastly, precipitation had an effect on T for H1 and H2 ($F_{(2,109)} = 3.98$, $p = 0.022$; $F_{(2,109)} = 3.98$, $p = 0.008$) and plant diversity on Weight for H1 ($F_{(2,109)} = 3.52$, $p = 0.033$). No interaction effect was found for any of the factors for both nurse and forager bees.

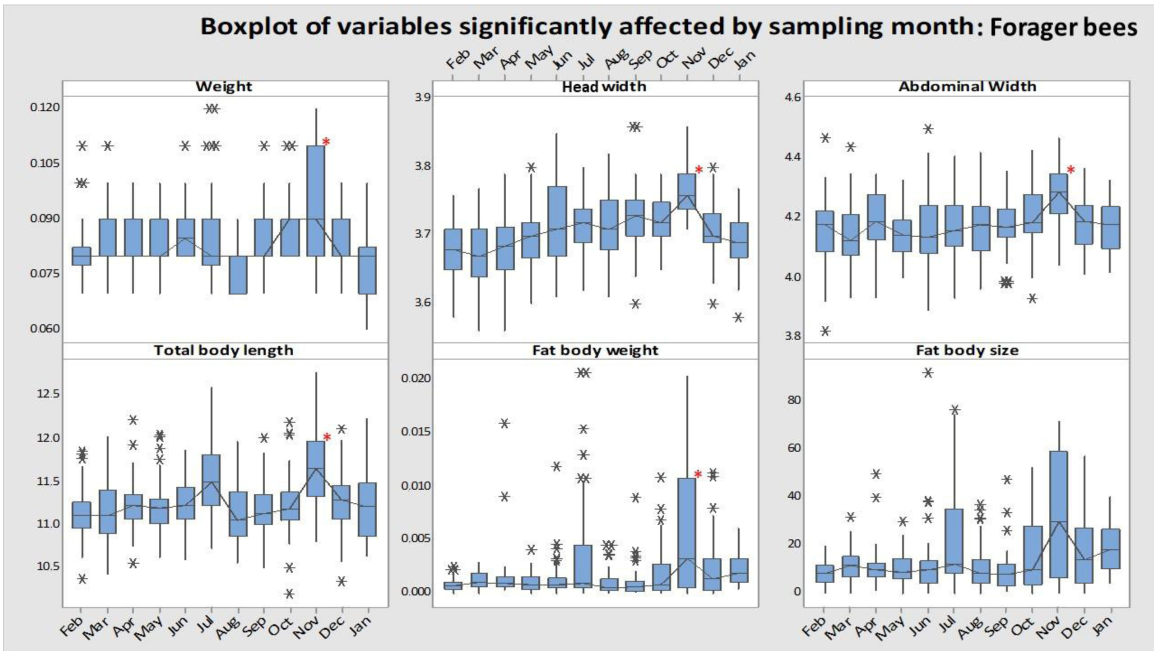

Figure 2. Box plots of Weight (g), Head width (mm), Abdominal width (mm), Total body length (mm), Fat body weight (mg), and fat body size (%) of *Apis mellifera ligustica* forager bees according to sampling months. The boxplot represents the interquartile range (IQR = Q3 − Q1) and bars represent first (Q1, top) and third quartiles (Q3, bottom) of metric values. Black "*" represents outliers and the black line represents the mean connect line. Red "*" indicates months significantly different from unmarked months. Feb = February, Mar = March, Apr = April, Jun = June, Jul = July, Aug = August, Sep = September, Oct = October, Nov = November, Dec = December, and Jan = January.

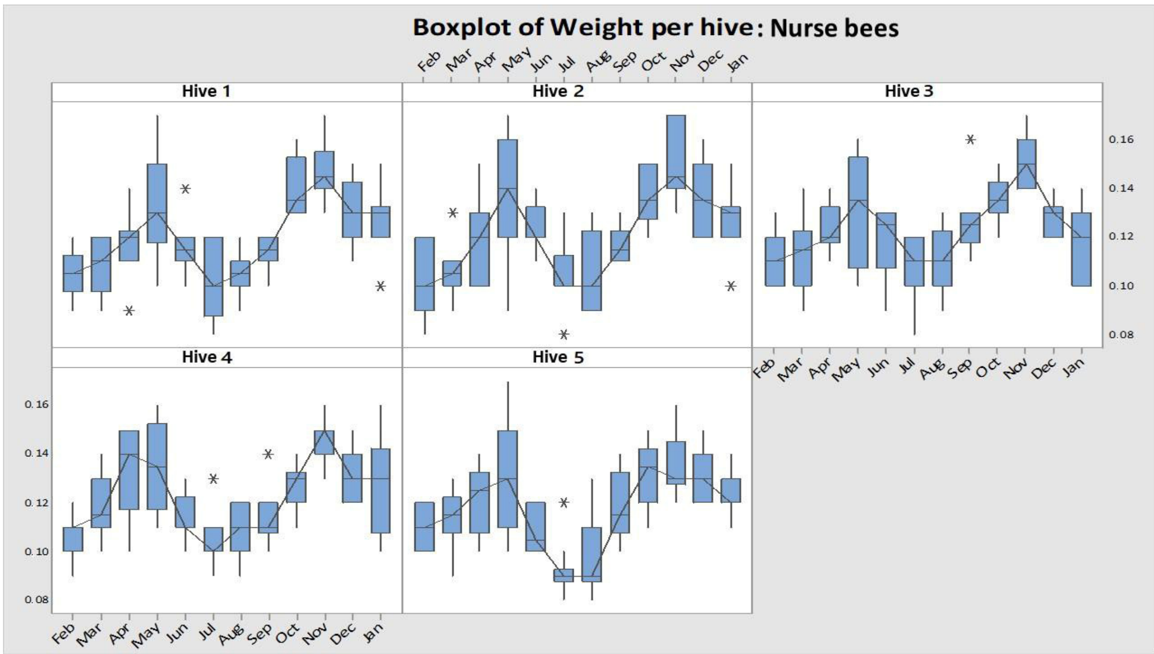

Figure 3. Box plots of the Weight (g) of *Apis mellifera ligustica* nurse bees according to hive and sampling months. The boxplot represents the interquartile range (IQR = Q3 − Q1) and bars represent first (Q1, top) and third quartiles (Q3, bottom) of metric values. Black "*" represent outliers and the black line represents the mean connect line. Feb = February, Mar = March, Apr = April, Jun = June, Jul = July, Aug = August, Sep = September, Oct = October, Nov = November, Dec = December, and Jan = January.

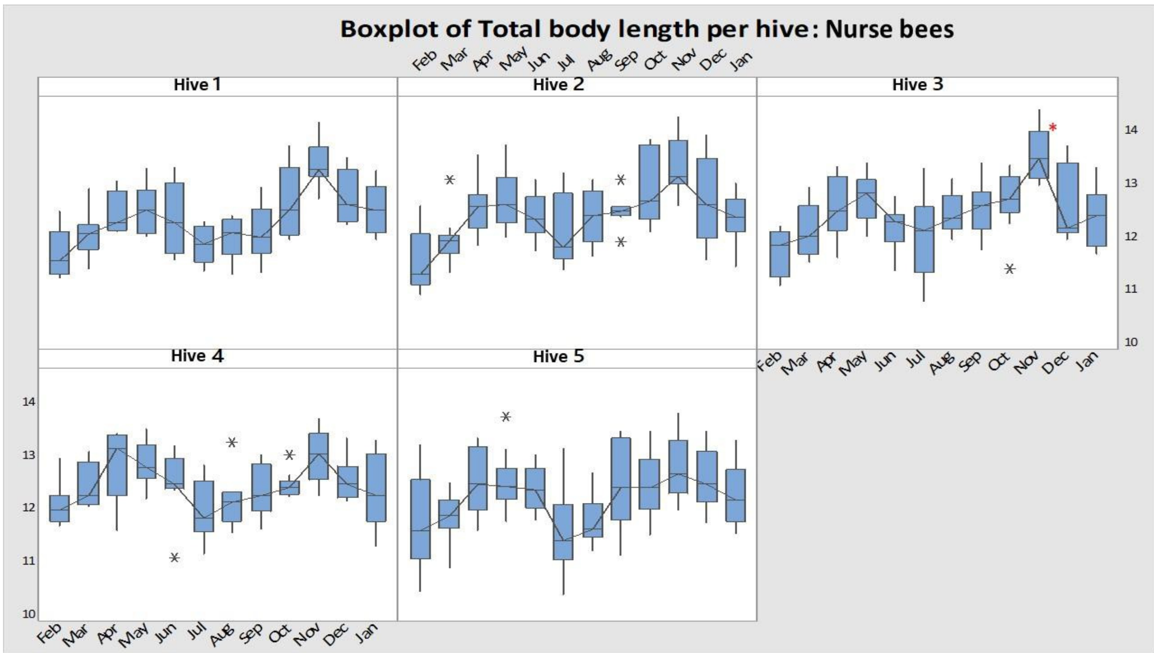

Figure 4. Box plots of the Total body length (mm) of *Apis mellifera ligustica* nurse bees according to hive and sampling months. Red "*" indicates months significantly different from unmarked months. The boxplot represents the interquartile range (IQR = Q3 − Q1) and bars represent first (Q1, top) and third quartiles (Q3, bottom) of metric values. Black "*" represents outliers and the black line represents the mean connect line. Red "*" indicates months significantly different from unmarked months. Feb = February, Mar = March, Apr = April, Jun = June, Jul = July, Aug = August, Sep = September, Oct = October, Nov = November, Dec = December, and Jan = January.

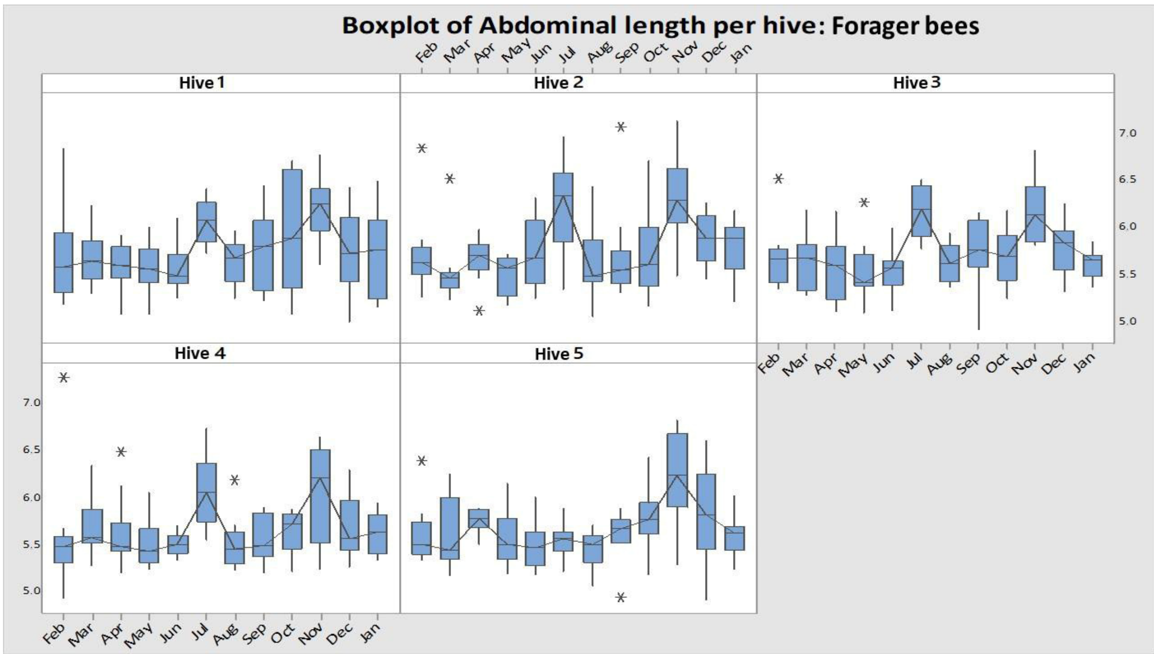

Figure 5. Box plots of the Abdominal length (mm) of *Apis mellifera ligustica* forager bees according to hive and sampling months. The boxplot represents the interquartile range (IQR = Q3 − Q1) and bars represent first (Q1, top) and third quartiles (Q3, bottom) of metric values. Black "*" represents outliers and the black line represents the mean connect line. Feb = February, Mar = March, Apr = April, Jun = June, Jul = July, Aug = August, Sep = September, Oct = October, Nov, =; November, Dec = December, and Jan = January.

Table 5. Results of the analysis of variance for the effects of environmental factors (mean monthly temperature, precipitation, daylength, and flower diversity) on various metrics of *Apis mellifera ligustica* nurse and forager honey bees.

	Temperature				*Precipitation*				*Hours of Daylight*				*Diversity of Flowering Plants*			
	F-Value	*df* *	*p-Value* [a]	*Post Hoc* **	*F-Value*	*df* *	*p-Value* [a]	*Post Hoc* **	*F-Value*	*df* *	*p-Value* [a]	*Post Hoc* **	*F-Value*	*df* *	*p-Value* [a]	*Post Hoc* **
Nurse bee																
Head width	8.25	(3589)	<0.001	*Tb,Tc,Td > Ta*	7.17	(2589)	0.001	*Pc > Pb,Pa*	2.20	(3589)	0.087	*X*	7.10	(2589)	0.001	*Fa,Fb > Fc*
Abdominal width	28.41	(3589)	<0.001	*Tb > Ta,Tc > Td*	5.20	(2589)	0.006	*Pc > Pb > Pa*	8.83	(3589)	<0.001	*Oa,Ob > Oc,Od*	3.01	(2589)	0.05	*Fa,Fb > Fc*
abdominal length	29.45	(3589)	<0.001	*Tb > Ta,Tc > Td*	10.22	(2589)	<0.001	*Pc > Pb,Pa*	3.41	(3589)	0.017	*Oa,Ob,Oc,Od*	1.70	(2589)	0.183	X
Fat body weight	39.14	(3589)	<0.001	*Tb > Ta > Tc > Td*	18.82	(2589)	<0.001	*Pc > Pb > Pa*	3.29	(3589)	0.021	*Oa > Ob,Oc,Od*	2.21	(2589)	0.111	*Fa,Fb,Fc*
Fat body size	40.36	(3589)	<0.001	*Tb > Ta,Tc > Td*	39.31	(2589)	<0.001	*Pc > Pb,Pa*	3.67	(3589)	0.012	*Oa,Ob > Oc,Od*	2.26	(2589)	0.105	*Fa,Fb,Fc*
Forager bee																
Weight	9.14	(3589)	<0.001	*Tb > Tc > Ta > Td*	7.8	(2589)	<0.001	*Pc > Pb,Pa*	1.3	(3589)	0.272	*X*	6.9	(2589)	0.001	Fb > Fc > Fa
Head width	18.2	(3589)	<0.001	X	5.84	(2589)	0.003	*Pc > Pa > Pb*	3.56	(3589)	0.014	*Oa,Ob,Oc,Od*	0.65	(2589)	0.520	X
Abdominal width	5.84	(3589)	0.001	X	1.44	(2589)	0.239	X	4.1	(3589)	0.007	*Oa,Ob,Oc,Od*	1.93	(2589)	0.146	X
abdominal length	10.57	(3589)	<0.001	*Tb,Td > Ta,Tc*	9.83	(2589)	<0.001	*Pc > Pa > Pb*	5.48	(3589)	0.001	*Oa > Ob,Oc,Od*	14.16	(2589)	< 0.001	*Fb > Fa,Fc*
total body length	8.09	(3589)	<0.001	*Tb,Td,Ta,Tc*	7.82	(2589)	<0.001	*Pc > Pb,Pa*	7.36	(3589)	<0.001	*Oa,Od > Ob,Oc*	9.69	(2589)	< 0.001	*Fb > Fa,Fc*
Fat body weight	9.07	(3589)	<0.001	*Tb,Td,Ta,Tc*	6.34	(2589)	0.002	*Pc > Pb,Pa*	6.56	(3589)	<0.001	*Oa,Ob,Oc,Od*	6.44	(2589)	0.002	*Fb > Fa,Fc*
Fat body size	8.28	(3589)	<0.001	*Tb,Td,Ta,Tc*	4.01	(2589)	0.019	*Pc > Pb,Pa*	5.85	(3589)	0.001	*Oa,Ob,Oc,Od*	2.75	(2589)	0.065	X

[a] Statistical significance set at $p < 0.005$. * Degrees of freedom. ** Categories that were shown to be different through *post hoc* analysis (Tukey test) are separated by “>”, while groupings that are not different from each other are separated by “,”.

Results of the correlation analysis between various metrics are reported for nurse and forager bees in Table 6.

Table 6. Results of Pearson correlation analysis between morphologic metrics of nurse and forager bees.

	Weight	Head Width	Thoracal Width	Thoracal Length	Abdominal Width	Abdominal Length	Total Body Length	Fat Body Weight
Nurse bees								
Head width	0.578 **	/	/	/	/	/	/	/
p-value	<0.001	/	/	/	/	/	/	/
Thoracal width	0.241 *	0.224 *	/	/	/	/	/	/
p-value	<0.001	<0.001	/	/	/	/	/	/
Thoracal length	0.272 *	0.216 *	0.557 **	/	/	/	/	/
p-value	<0.001	<0.001	<0.001	/	/	/	/	/
Abdominal width	0.638 ***	0.457 **	0.323 **	0.330 **	/	/	/	/
p-value	<0.001	<0.001	<0.001	<0.001	/	/	/	/
Abdominal length	0.796 ***	0.768 ***	0.205 *	0.244 *	0.572 **	/	/	/
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	/	/	/
Total body length	0.770 ***	0.636 ***	0.213 *	0.259 *	0.529 **	0.794 ***	/	/
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	/	/
Fat body weight	0.753 ***	0.424 **	0.104 *	0.144 *	0.493 **	0.675 ***	0.666 ***	/
p-value	<0.001	<0.001	0.024	0.002	<0.001	<0.001	<0.001	/
Fat body size	0.310 **	0.133 *	0.045	0.069	0.183 *	0.257 *	0.293 *	0.498 **
p-value	<0.001	0.004	0.324	0.131	<0.001	<0.001	<0.001	<0.001
Forager bees								
Head width	0.214 *	/	/	/	/	/	/	/
p-value	<0.001	/	/	/	/	/	/	/
Thoracal width	0.117 *	0.780 ***	/	/	/	/	/	/
p-value	<0.001	<0.001	/	/	/	/	/	/
Thoracal length	0.072	0.692 ***	0.830 ***	/	/	/	/	/
p-value	0.079	<0.001	<0.001	/	/	/	/	/
Abdominal width	0.614 ***	0.317 **	0.286 *	0.219 *	/	/	/	/
p-value	<0.001	<0.001	<0.001	<0.001	/	/	/	/
Abdominal length	0.731 ***	0.212 *	0.095 *	0.035	0.639 ***	/	/	/
p-value	<0.001	<0.001	0.020	0.396	< 0.001	/	/	/
Total body length	0.735 ***	0.218 *	0.102 *	0.063	0.636 ***	0.871 ***	/	/
p-value	<0.001	<0.001	0.012	0.125	<0.001	<0.001	/	/
Fat body weight	0.669 ***	0.204 *	0.088	0.064	0.471 **	0.649 ***	0.643 ***	/
p-value	<0.001	<0.001	0.053	0.160	<0.001	<0.001	<0.001	/
Fat body size	0.662 ***	0.212 *	0.097 *	0.071	0.458 **	0.645 ***	0.652 ***	0.907 ***
p-value	<0.001	<0.001	0.034	0.121	<0.001	<0.001	<0.001	<0.001

* indicates a weak correlation. ** indicates a mild correlation. *** indicates a strong correlation.

4. Discussion

Nutrition is a key aspect influencing honey bee health and overwintering success [38,49,61,62]. Nevertheless, there are relatively few studies that explore honey bee seasonal activity in southern temperate climates [28,29]. In this research, the nutritional status of the Italian bee (*A. m. ligustica*), a subspecies well adapted to the warm temperate climate of the Mediterranean, was studied [26,55,57]. Specifically, individual weight, fat body, and size measurements (head, thorax, abdomen, and total body) were recorded on a monthly basis in order to detect temporal changes in the nutrient storage of worker bees during a complete annual cycle (2022–2023). Recorded parameters were analysed according to climatological factors and the availability of feed recourses (flower diversity) in order to get a better understanding of the annual bimodal dynamics of the honey bee workforce in a southern temperate Mediterranean climate.

Besides following seasonal variations in honey bee nutrition, novel data regarding two distinct worker bee types with varying biological age; in-hive (nurse bees) Vs. out-hive (forager bees) is presented. Given the consistent and fundamental behavioural, physiological, and nutritional differences between these two worker bee types [17,63–69] authors hypothesised nutrition-related size metrics to vary significantly between them. Furthermore, as nurse and forager bees have different responses to similar conditions [64], the analysis of fixed factors (sampling time, environmental factors, and feed resource availability) was conducted separately for both cohorts.

While body size is a known indicator of nutritional stress reflecting the quantity and quality of food available during development in honey bees [44,47,70–73], to the best of our knowledge, no empirical evidence has so far been produced showing size variations between worker honey bees to be related to age division of labour. In fact, body size variations of worker bees within a single *A. mellifera* colony are believed to be negligible [74–76]. Here we show significant differences in nutrition-related size measurements between individual forager and nurse bees. With the exception of thoracal dimensions, all measured metrics differed between both worker bee types. Correspondingly, individual size measurements were strongly or mildly positively correlated to known biological markers of honey bee nutrition (body and fat body weight [3,25,44,77]) (Table 6). These findings are in accordance with worker physiology, showing nurse bees to have substantially larger nutrient stores as compared to foragers [17,62–65,67–69]. The weak correlation between Weight and FBW Vs. HW in foragers is explained by the fact that these bees have hypotrophied hypopharyngeal glands [7,17]. In contrast, these glands, which serve for the production of brood food, are well-developed in nurse bees [7,17,78–81]. Since brood food is produced from Vg [18], it is logical HW to be correlated to nutritional markers in this cohort.

The long-term monitoring of selected metrics allowed us to paint a detailed picture of the annual cycle of Italian bees in the study area from a nutritional point of view. In accordance with the seasonal adaptations of worker honey bees (summer Vs. winter bees) [7,8,15,25,29,82], a functional bimodal division of the honey bee cycle is followed.

The “summer-bee portion” of the nutritional cycle, running from mid-winter (end of December) to early fall (September) in this study, closely followed the nectar flow. When feed resources were abundant, individual honey bee nutrition increased and the opposite was seen during resource dearth [28,70,83–85]. Contrarily, a general increase in HW and TW was seen over the course of the foraging season. This corresponds to previous findings describing an increase in worker bee size within a yearly cycle [26,86].

Present data reflects a controversial increase in W, AL, T, FBW, and FB% of forager bees during the summer dearth period (peaking in July; Figures 2 and 5) which has been accredited to an explicit sampling error. Specifically, ambient temperatures at the time of sampling were so high that a large portion of bees had exited their hives and were found clustered around their respective hive entrances. This common strategy to prevent overheating [87,88] likely resulted in the sampling of a mixed population of worker bees rather than bees of a single biological age. Our deduction of this finding to be a sampling error is supported by the overlapping ranges of measured metrics between both worker bee types for the month of July, as well as the increased variability seen for that sampling date.

Consistent with our present understanding of honey bee physiology in southern temperate climates of the northern hemisphere [26,28,29,57], the “winter-bee portion” of the nutritional cycle in this study was short and restricted to late fall/early winter (November-December). October can be considered a transition month as the shift from summer to winter bee-state is known to occur gradually within a colony and thus a balanced number of both castes is most likely present at this time [7,8,23]. This portion of the honey bee year-cycle was characterized by a steep increase in nutrient storage in opposition to the overall diversity of feed resources (Table 2) with a subsequent decrease over the course of the winter period [17]. This correlates well with current knowledge of honey bee seasonality with the arrival of winter bees primarily related to the disappearance of flowering plants in fall [7,8,15]. As indicated by the significant difference in average monthly FBW, FB%, and AL (Figure 1), nutrient storage of nurse bees peaked in November, showing the presence of winter bees [13,25,70,81]. Whereas the overwintering state of honey bees in warmer climates differs from northern regions (e.g., sustained foraging and brood rearing activities), accumulation of fat and protein (Vg) is believed to be universal for overwintering honey bees in temperate zones [28]. Recent research monitoring Vg levels in the fat body of worker bees over a yearly cycle in the Czech Republic revealed a strikingly similar pattern even though nutrient storage in said research peaked in December [25]. Nevertheless, because sampling in the present study was conducted at the end of each month, nutrient storage of nurse bees could have peaked early to mid-December (as the noted brood rearing patterns would suggest) rather than in November. It is also necessary to stress on the fact that homemade sucrose solution was offered in negligible amounts, out of the long-term monitoring, because strictly necessary to colony survival and for a very limited period of days (like reported above).

The enlarged Weight, FBW, HW, AW, and T of forager bees in November, indicate that monitored hives exited their winter state somewhere between November and December. Indeed, increased morphological dimensions of forager bees during the winter dearth are indicative of a winter-bee-like state and can be considered remnants of the nutrient accumulation that occurred during in-hive activities [89]. Analogously, previous research has identified forager bees with increased morphological dimensions in early spring likely to be winter bees hatched the year before [86]. Authors expected to see a delay in the detection of winter-bee-like foragers as compared to nurse bees. Nevertheless, the cessation of activities of Italian bees in this research was shorter than the sampling frequency likely resulting in the absence of a notable temporal divergence in seasonal transition between both worker bee types.

Overall changes in recorded metrics corresponded to the variation in environmental factors observed within the study period known to influence seasonal honey bee colony activity [7,8,15,16,25].

For both nurse and forager bees, monthly average ambient temperatures between 15–20 °C were correlated to the highest degree of nutrient storage (Table 5). These temperatures coincide with peak honey bee activity during the nectar flow in spring as well as the appearance of winter bees in fall. The fact that honey bees show two distinct physiological states within similar temperature ranges illustrates it is unlikely mean temperature alone influences the seasonal transition of honey bee colonies. Alternatively, interaction of temperature with other factors (e.g., photoperiod and feed resource availability), or the direction of temperature change in combination with reaching a threshold value could serve as a possible seasonal trigger [7,14,16,23,81].

Current understanding of honey bee behaviour in temperate climates describes the formation of a thermoregulating cluster when ambient temperatures drop below 10 °C [7,90]. While average temperatures were well above this mark in November in the present research (and remained so for the whole duration of the study), minimum ambient temperatures did dip below 10 °C in November. More significantly, temperatures below this threshold were first recorded the month before (October). Given winter bees start appearing during this transition month, the first cold nights in fall could signal colonies to prepare for winter. The physiological mechanisms of how dropping ambient temperatures allow worker bees to accumulate Vg has previously been described [22,25,91,92].

In accordance with previous research efforts [25,62,63,93], an association between decreasing daylength and the accumulation of nutrients in the fat body of in-hive bees was noted. These results strengthen the hypothesis that decreasing photoperiod is involved in the seasonal appearance of winter bees [7,15,16] although present morphometrical results did not reveal further insights into the possible influence of daylength on the nutritional cycle of Italian honey bees.

Lastly, high average monthly precipitation (>100 mm) was consistently associated with an elevated nutrient status in both nurse and forager bees and coincided with the presence of winter bees. With the exception of AW in foragers, all measured metrics were significantly higher during months with high precipitation (Table 5). This finding is intriguing and could point towards weather conditions to be of particular importance in the seasonal dynamics of honey bees in southern temperate climates. Indeed, impaired meteorological conditions (“bad weather”) are known to influence honey bee demography by affecting the pheromone balance of a colony resulting in the active suppression of the biological maturation of young bees and the appearance of winter bees in fall [7,15,92,94–96].

The flower diversity surveys conducted in the honey bee flight area provided a significant contribution to the study. We detected substantial variations in forage diversity (with specific regard to pollen availability) over the course of the monitoring period with an explicit pattern matching that of brood rearing. Despite honey bee colonies do not generally prefer to store large amounts of pollen [97] (and when they do, they is mix it with nectar and seal the compounds with wax) the availability of this resource (the main nutrient supply for brood rearing) is chiefly correlated to the brood rearing activity [23,27,31,38,76,98].

This pattern together with rest of the present data allowed us to identify two critical periods for honey bee health and nutrition in southern temperate climates, i.e., summer and winter dearth. While seasonal fluctuation in pollen availability showing one or two distinct peaks is not unusual [26,27], large temporal variations in feed resource availability (nutritional irregularity) are known to affect honey bee health and longevity [84,99]. Indeed, poor foraging conditions and related malnutrition are believed to be key factors in global colony losses [36,38,49,85,100], especially in warm temperate climates [29]. Sugars from nectar, on one side, and amino-acids and sterols from pollen, on the other side, are differently involved in metabolic patterns of honeybees, in which energy storage is limited in foragers and while being physiologically higher in nurse bees (depending on the period of the year and according to feeding source availability, like we observed).

High winter temperatures [16] together with the prolonged availability of pollen [23] offer a viable explanation for the late appearance of *diutinus* bees in this research as well as the sustained brood rearing observed for two out of the five hives [29,101]. Although this might seem beneficial, continuous brood rearing during periods of limited pollen availability can cause premature exhaustion of fat and protein nutrient stores leaving colonies in a vulnerable state [16,28,29,31,67]. In effect, in-hive colony reserves and reserves within bees themselves are rapidly depleted in times of pollen dearth [38]. Besides, flower diversity has been shown to be an important factor in honey bee nutrition since different pollen and nectar sources vary significantly in their nutritive value, e.g., protein and mineral contents [35,41,85,99,102]. Hence, even though nutritional resources would be available during winter months, the limited variety of flowering plants during this time might not provide adequate nutrition in order to support brood rearing or honey bee colonies in general [28,38,40,41,60,102,103].

A prominent finding of the present study is that the nutritional state of *A. m. ligustica* workers was significantly negatively affected during periods of high ambient temperatures (>25 °C) and low precipitation (0–50 mm) (Table 5). With the exception of T and HW, all nurse metrics were lowest during the summer drought period (June–August) (Figures 1, 3 and 4).

The precipitation pattern during the study period coincided with that of plant diversity during the "summer-bee portion" of the year which can be considered an illustration of the bottleneck effect of precipitation on plant growth in warm and dry Mediterranean climates [104,105]. The noted influence of weather on honey bee nutrition in summer therefore likely stems from an indirect effect on plants resulting in an overall resource dearth [8,30,90,106–108]. Moreover, hot and dry conditions have been shown to reduce nectar and pollen production and the overall nutritional quality of these resources as well [30,90,105]. For these reasons, in addition to the winter dearth, summer food shortages could be of serious concern for honey bee colonies in southern temperate climates. This could be especially true in the face of accelerated climate change [5,30,31,45,109–111] as conditions in the Mediterranean head towards a similar scenario seen in particularly arid climates such as in the Middle East [111,112] where summer droughts are a key factor in colony losses since many plants suffer from heat stress leading to feed shortage for honey bees [32].

Temporal mismatches with possible nutritional consequences were pointed out at Mediterranean latitudes [16,104,105,113,114]. Indeed, a particularly early initiation of the foraging season, well before the start of the nectar flow, was noted followed by a sharp decrease in nutrient storage over the course of winter (Figure 1).

5. Conclusions

The present research contributes to our understanding of the seasonal dynamics of honey bees in a southern temperate climate showing a short cessation of activities in late fall/early winter coupled with an increase in nutrient storage of in-hive bees. While the fall decrease in feed resources appears to be the main factor governing honey bee seasonality, a combination of changing environmental factors seems to be required for the arrival of winter bees. The continuous but limited availability of flowering plants and forgiving ambient temperatures during winter likely allowed for the observed brood rearing pattern and consequential sharp decrease in nutrient storage over the winter dearth period. In addition, a first description of the annual nutritional honey bee cycle in a southern temperate climate is presented showing two critical timepoints. Overall, our results contradict the common assumption that warm climates are more suited for honey bees as besides winter, the Mediterranean summer, which is characterised by droughts and high temperatures, was identified as a second critical timepoint. It seems precipitation plays a particularly important role in southern latitudes, influencing nutrition in both the summer- and winter-bee portion of the honey bee year-cycle. Finally, present data seem to support the notion that the shift of environmental conditions have significant effects on honey bees in temperate Europe through a pronounced impact on melliferous plants, indirectly affecting health and nutrition of honeybees. Our results provide valuable insights into the seasonal and nutritional dynamics of locally adapted *A. m. ligustica* populations that could aid beekeepers to make management decisions in relation to environmental factors and availability of flowering plants with the ultimate goal of improving overwintering success and preventing undesirable colony losses.

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