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The Impact of Different Biotopes and Management Practices on the Burden of Parasites in Artificial Nests of *Osmia* spp. (Megachilidae) Bees

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Abstract: The decline in pollinator insect abundance and diversity is increasing on a global scale. Major threats are the byproducts of numerous negative environmental pressures acting individually or in combination. They vary throughout different geographical areas, affecting the solitary bees differently. One of the most important negative pressures are the many parasites, predators and pests representing a threat to the successful reproduction of solitary bees in artificial nests. Especially vulnerable are the managed *Osmia* spp. bee populations reared for commercialization and trade. The primary goals of our monitoring study were: (i) to examine the presence and the prevalence of brood parasites in the various types of bees' nesting material and in semi-field rearing conditions using the nest section analyses; (ii) to determine the presence of *Nosema* spp. in samples of feces and homogenized bee abdomens using a multiplex PCR method; (iii) the evaluation of the survival success level and emergence mass of healthy bees at each of the four studied bee rearing locations separately, depending on different environments and on the implementation of different managing practices. We determined the presence and prevalence of nest destructor parasites and accompanying fauna. Their presence was positively correlated with bee rearing failures. The results of this study may be used as a baseline for further solitary bee nest parasites monitoring schemes.

Keywords: *Osmia cornuta*; *Osmia rufa*; biotope; semi-field conditions; artificial nests; section analysis parasites; pathogens

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1. Introduction

In Europe, the most ubiquitous representatives of solitary bees are the mason bee species *Osmia bicornis* L. (syn. *Osmia rufa* L.) and *Osmia cornuta* L. These bee species live in similar environments but differ in phenology and time of emergence in the spring. These species are being increasingly used for pollination services in agricultural and natural environments [1,2]. *Osmia bicornis* is polylactic and collects pollen from a very broad spectrum of plants, while *O. cornuta* prefers Rosaceae, especially fruit trees. Both species are especially important in fruit tree pollination in orchards due to their specific foraging and nesting behavior [3]. They emerge early in spring and so they are important in pear plantations which bloom early [4]. Additionally, these bees forage readily among different trees and rows within the orchard, which is particularly important for self-incompatible fruit cultivars that require cross fertilization [2–4]. Because they are also common in non-agricultural environments, these bees are important for the preservation of natural

landscapes. Therefore, these bees have been introduced in urban areas as an environmental accompaniment [2,5].

Due to their importance for biodiversity and environmental health worldwide, losses in diversity and abundance of bees, including *Osmia* spp., are of great concern. The identity and status of wild bees in Europe are still unknown and incomplete [6]. However, losses of wild bees and diversity in northwestern Europe have been observed and Red Lists for bees under threat have been published [7]. These losses of populations are not limited to bees—alarming ongoing declines in the abundance and diversity of beneficial insects in general have been noted [8]. The main causes of the declines are the presence of various negative factors such as habitat unavailability, a lack of natural nest materials, new agricultural and agrochemical practices, climate changes, urbanization, the presence of non-native species and the spread of parasites and pathogens [3,9–12]. Those factors can act individually, in combinations or synergistically, varying in different geographical areas, affecting solitary bees differently [13]. For example, in urban environments, solitary bees have been found to change their behavior, including flight activity, and can have impaired development, a smaller body size, reduced immunity and longevity and lower biodiversity in general [14–19].

Many parasites, predators and pests found in the nests of *O. bicornis* and *O. cornuta* in areas of southeast Europe interfere with successful reproduction [20]. *Osmia* spp. are managed cavity-nesting bees. They nest in artificial nests in semi-field conditions and can be sold in the diapause stage of development, are transported and later hatched and developed into sexually mature adults for crop pollination [4]. The presence of nest parasites, predators or pests limits the number of solitary bees that can emerge from cocoons and can interfere with individual development [3]. Additionally, the limited access to pollen provisions within nests may affect the rate of reproductive success and even intensify the parasitism consequences. Bees reared in semi-field conditions tend to be kept in very high densities which are favorable conditions for the spread of parasites and pathogens. High densities of artificial nests promote increases in the density of predators which can also cause significant damage [3]. Accompanying fauna collected from *Osmia* spp. nests described by Krunić et al. (2005) included: cleptoparasites, parasitoids, predators, cleptobionts, nest destructors and accidental nest residents [20].

As part of strategies to improve the status of local insect pollinators, the *Osmia* spp. solitary bees are managed in semi-natural habitats or rearing conditions by beekeepers. Many different management practices aimed to reduce the effects of main drivers of decline, to contribute to biodiversity conservation and to improve yield. There are many protective short- and long-term measures that can be used to optimize the rearing system. For example, opening the artificial bees' nests in autumn months enables extraction of cocoons, and permits the mechanical removal of parasites, predators and pests. Nests can also be protected against natural enemies such as ants, mice, squirrels or birds through the use of sticky barriers or wire nets set in front of the aggregations of nest tube entries [3].

The primary goal of this study was to examine the presence and prevalence of brood parasites, predators and pests in various types of bees' nesting material and semi-field rearing conditions in Croatia.

2. Materials and Methods

2.1. Artificial Solitary Bee's Nests Sampling

Artificial nests were randomly chosen and taken in October 2019 on four semi-controlled rearing solitary bee stations (Location 1—L1, Location 2—L2, Location 3—L3, Location 4—L4) across the territory of Croatia (Figure 1). These locations were situated in the different biotopes of the continental part of the country. These selected locations were influenced by various factors such as environmental conditions, urbanization gradient and breeding management practices. Location 1 was settled in an urban environment close to the city center with a small percentage of greenery, mostly composed of balcony ornamental flowers and mini gardens, in an area approximately 200 m around the sam-

pling site (micro location). Location 2 represented the suburban environment in grassland surrounded by fields used for intensive agriculture and close to the city's built-up infrastructure. Its micro-location was under rapeseed and corn plantations earlier in the season. Location 3 was in a suburban area on the border with an industrial zone and intensive refinery activity, surrounded by meadows. Location 4 was in a pure rural environment surrounded with forest and hilly meadows. Therefore, the micro location was rich in different pollen sources. Sampled occupied artificial nests originated from different beekeepers with different management practices according to their pollination needs.

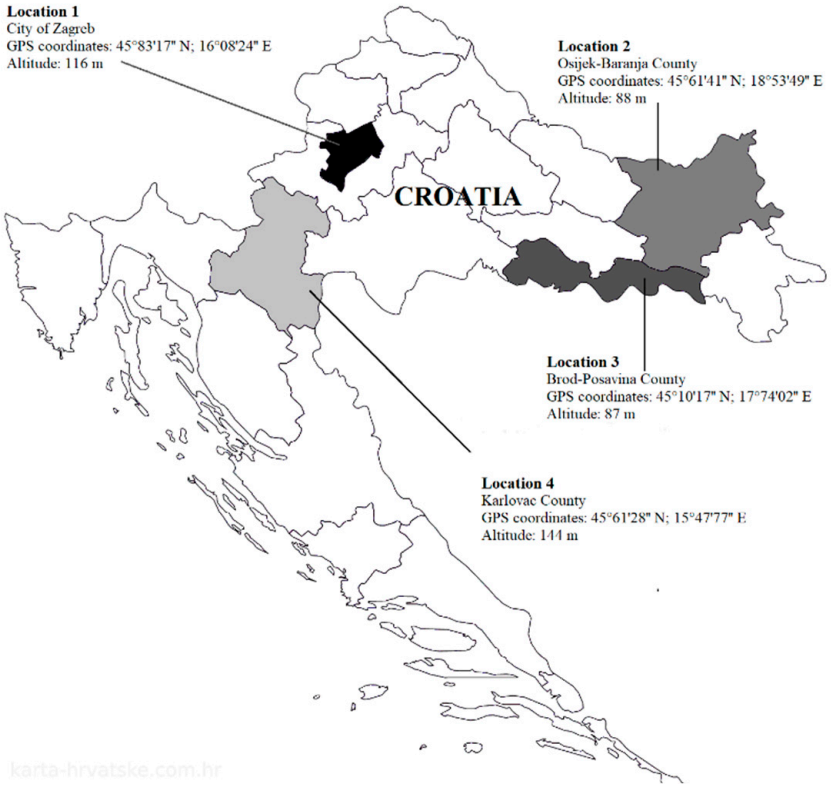

Figure 1. Sampling sites (Location 1, Location 2, Location 3 and Location 4) where few types of artificial nests of *Osmia* spp. bees were collected, from different environments in the continental part of Croatia.

At L1, beekeepers used different materials for artificial bee's nests. The length of the cane tubes used was between 12 and 16 cm and between 8 and 13 mm in inner diameter. They provide regular preventive disinfection during the winter months. The cane tube nests were opened and bees in cocoons proceeded with wintering in cardboard boxes at 2 °C and, during the darkness, to undergo diapause. At L2, only the long cane tubes were used (between 48 and 50 cm in length, and between 8 and 10 mm in width) bound in bigger bundles. At L3, the length of the used cane tubes was between 16 and 22 cm, with 8 to 11 mm in inner diameter combined with punched tree trunks of different sizes. At L4, beekeepers use cane tubes (between 10 and 14 cm long and between 9 and 12 mm in inner diameter) collected in thinner bundles (Figure 2). At all locations, one-year-old cane tubes were sampled and regularly used, except at L3 (combined with two- and three-year-old cane tubes and drilled tree trunks).

A total of 643 occupied dry cane tubes of different lengths and inner diameters (L1 = 221; L2 = 66; L3 = 286; L4 = 70) of marsh common reed (*Phragmites australis*), 192 plastic lamella (L1 = 192) and 80 individual bee cocoons (L1 = 80) were sampled. All collected nests and individual cocoons were placed into clean labelled cardboard boxes, transported

to the Laboratory for Honeybee Diseases APISlab at the Faculty of Veterinary Medicine University of Zagreb and stored in the refrigerator at 4 °C until the section analyses.

Figure 2. At semi-controlled rearing solitary bee stations (Location 1, Location 2, Location 3 and Location 4) different management practices were used.

2.2. Artificial Nests Section Analyses

The section of each nest was done by opening the reed tubes with horizontal cuts with a sharp scalpel to avoid disruption of the nest chambers. This has been done by the manual opening of the commercially available plastic lamella boxes for solitary bee rearing, and/or sections of individual cocoons using ophthalmological sterile scissors and tweezers. The visual inspection of the collected masoned nesting tubes and the lamellas with well-developed healthy cocoons (unchanged cocoon appearance), deceased larvae, leftovers of pollen provisions and soil chamber separators was done. The cocoons were classified into the groups of visually healthy, empty, destroyed or partially damaged cocoons as a consequence of infestations by different parasites and pests. Then, all the cocoons were pulled out from the nest brood chambers with sterile section tools; each was opened, and the content was taken out. For each vital solitary bee that emerged, the morphological identification (*O. bicornis* or *O. cornuta*) was done, as well as the weighting of the body mass (g) using digital scales (Sanitas, Hans Dinslage GmbH, Uttenweiler, Germany). Based on the sexual dimorphism, the gender of adult bees was determined.

The presence, prevalence and morphological identification of parasites, parasitoids, predators and the other accompanying fauna species stuck on the mason bee cocoons was done according to the previously published methods [20].

Additionally, abdomens of adult offspring bee specimens extracted from the healthy cocoons and feces samples were separately collected into the sterile 2 mL Eppendorf tubes. Those subsequent samples were stored at −20 °C until the further diagnosis was done.

2.3. Estimation of the Osmia spp. Bee's Survival Level

To estimate the level of survival and health status at each of the studied solitary bee rearing locations, during the nest tubes section the following parameters were evaluated:

I. Failures included:
   1. The number of nest brood chambers containing the undeveloped bee's offspring, e.g., mummified and dry larvae and pupae specimens;
   2. The number of nest brood chambers containing the individual dead adult bees outside of their cocoons;
   3. The number of nest brood chambers containing parasites, predators or pests;
   4. The number of nest brood chambers containing unused pollen pellets.

II. The number of live and healthy adult bee specimens (non-symptomatic bees, bees free of parasites and predation, bees without visible characteristic clinical symptoms of diseases) which are fully developed in cocoons.

The survival level of solitary bees (SL) for each sampling location was determined using the following formula:

$$SL = II./II. + (1. + 2. + 3. + 4.) \times 100\% \quad (1)$$

2.4. Laboratory Microscopic and Molecular Diagnostic of Nosema spp.

The microscopic examination of the presence of microsporidia *Nosema* spp. spores and genetic analysis confirmation were carried out on the abdomens of 30 adult bees and 30 samples of feces collected from the brood nest chambers at each location. Firstly, separated abdomens were thoroughly crushed and homogenized in a plastic container with 1 mL of water per bee. Feces samples were dissolved in the same amount of water. The magnifications of 400× under a bright field microscope—model Olympus Bx41 (Olympus Europa SE & Co., Hamburg, Germany)—were used to check the presence of *Nosema* spp. spores in freshly prepared smears of bees' gut content dispersed in water, according to the Office International des Epizooties guidelines [21]. Each diagnostic procedure was replicated three times. The microscopic equipment was carefully washed after each sample to avoid contamination with previous samples.

The extraction of total DNA from the smashed bees' abdomens was done using the DNAeasy Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions. Further standard Polymerase Chain Reaction (PCR) molecular analysis was performed as was described elsewhere [5,21], through the literature within.

2.5. Statistical Analyses

The statistical analyses were performed using the statistical software package GraphPad Prism software version 7.00 for Windows (GraphPad Software, La Jolla, CA, USA). In order to assess and verify the differences between groups, the one-way analysis of variance (ANOVA) with Tukey's Post Hoc test was used. The results are presented as the mean values and standard deviations. The significance level of 0.05 was set to define the statistical differences (0.95 confidence interval).

3. Results

In this study, 4672 cocoons (6680 in total including the empty brood chambers) from artificial nests of *Osmia* spp. were examined and classified. Nests were located at four different bee rearing stations. Various nests per rearing station were analyzed, and various management practices were implemented at each studied location. The total number of healthy cocoons was 1379 (29.51%). Section analyses of artificial nests showed that

the studied localities differed in the number of determined healthy bees (L1 = 49.97%; L2 = 28.05%; L3 = 13.80%; L4 = 34.00%), number of brood parasites (L1 = 47.63%; L2 = 80%; L3 = 45.52%; L4 = 88.15%), mummified larvae (L1 = 24.70%; L2 = 10.98%; L3 = 2.73%; L4 = 2.66%) or dead adult bees (L1 = 10.77%; L2 = 5.57%; L3 = 2.48%; L4 = 0.87%), as well as chambers containing unused pollen pallets (L1 = 5.13%; L2 = 16.98%; L3 = 0.55%; L4 = 1.75%) (Table 1). Additionally, it is important to note that at L1 a different nest material was used (a, b, c).

Table 1. Comparison of the number of empty brood chambers with those which contain healthy *Osmia* spp. adult bees, and with rearing brood failures, in locations with different environment.

Number of Brood Chambers	Bee Rearing Station Location			
	L1 (a + b + c)	**L2**	**L3**	**L4**
Empty	0 + 403 + 592 Σ995	328	563	122
Brood failures	22 + 643 + 1166 Σ1831	218	1100	229
Healthy bee	58 + 453 + 404 Σ915	85	176	118

Note: a—individual cocoons; b—brood chambers in plastic lamella; c—brood chambers in reed tubes.

The weight of extracted healthy cocoons was significantly variable between the studied locations (F = 92.45, $p < 0.05$). In detail, the mean values of cocoon weight were as follows: L1 = 0.08 ± 0.02 g; L2 = 0.13 ± 0.04 g; L3 = 0.12 ± 0.05 g; and L4 = 0.12 ± 0.02 g. Results are presented in Figure 3.

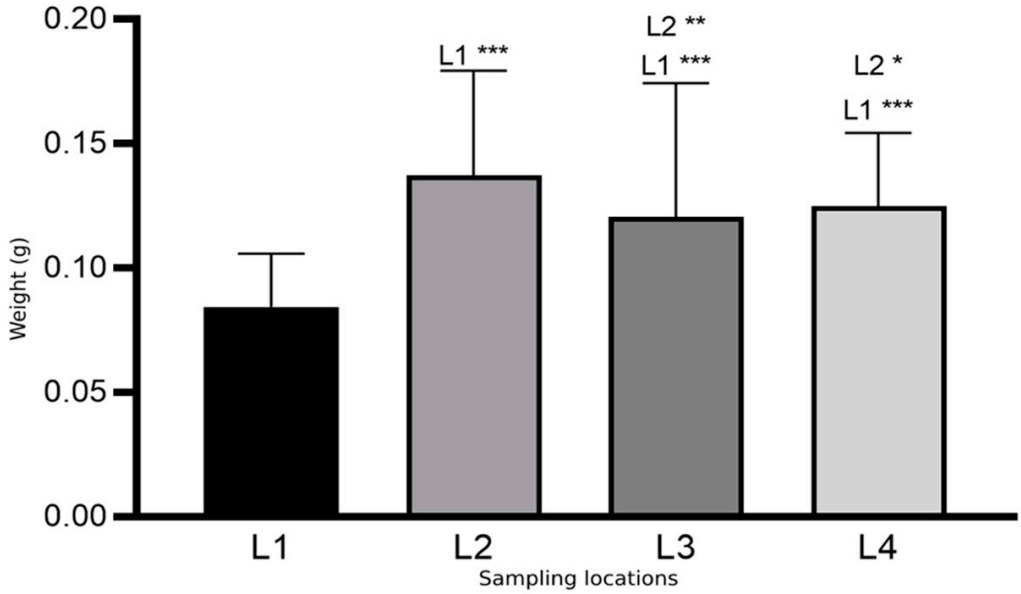

Figure 3. Weight means values of extracted cocoons from artificial nests situated at different locations. Asterisks indicates statistically significant differences: L1 vs. L2, L3, L4; L2 vs. L3, L4; * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$; mean ± SD.

Adult specimens of *O. bicornis* differ in weight at emergence (Figure 4), particularly those significantly lightweight originating from L4, in comparison with those from L1, L2 and L3 (F = 97.45; $p < 0.0001$). The mean weight values of cocoons extracted from live bees increased as follows: L4 = 0.053 ± 0.007 g, L1 = 0.075 ± 0.006 g, L2, L3 = 0.75 ± 0.010 g.

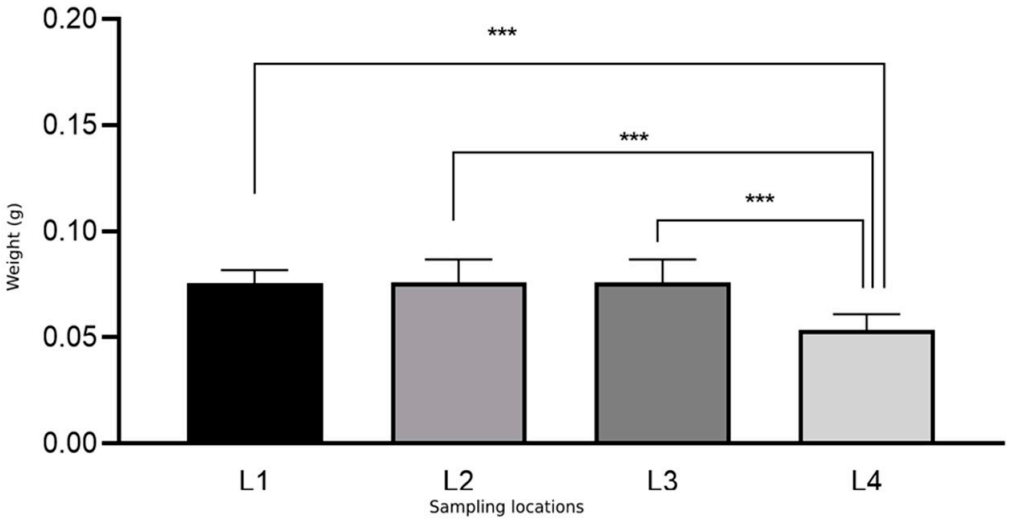

Figure 4. Weight means values of the emerged adult *Osmia bicornis* at different locations. Asterisks indicates statistically significant differences: L1, L2, L3 vs. L4, *** $p < 0.0001$; mean ± SD.

The weight means values of the emerged adult *O. cornuta* were highest at L2 (0.128 ± 0.028 g) and decreased as follows: >L4 (0.112 ± 0.028 g), >L1 (0.110 ± 0.029 g) and >L3 (0.070 ± 0.028) (Figure 5). Significant differences were observed between bees' weight between L2 and L1, L2, L4; between L3 and L1, L2, L4; and between L4 and L3 ($F = 168.7$; $p < 0.0001$).

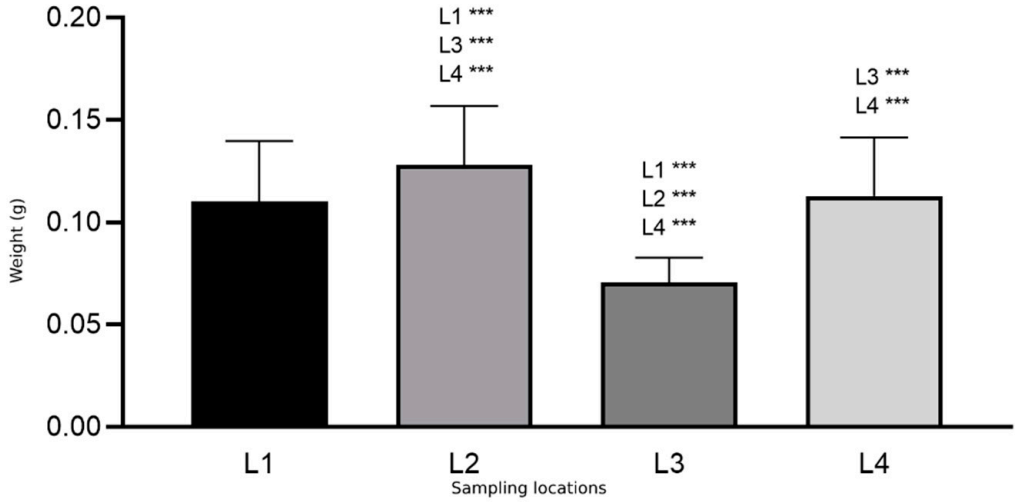

Figure 5. Weight means values of the emerged adult *Osmia cornuta* at different locations. Asterisks indicates statistically significant differences: L2 vs. L1, L3, L4; L3 vs. L1, L2, L4; L4 vs. L3; *** $p < 0.0001$; mean ± SD.

The overall bee emergence weight statistic based on the number of healthy bees and their species differentiation is shown in Table 2.

Table 2. Bee emergence weight means values based on the number of healthy bees and their species differentiation.

Species	Location	Sample Size (n)	Mean (g)	S.D.
Osmia bicornis	L1 (a + b + c)	613 (45 + 341 + 227)	0.075	0.006
	L2	38	0.076	0.010
	L3	122	0.076	0.010
	L4	66	0.053	0.007
Osmia cornuta	L1 (a + b + c)	302 (13 + 112 + 177)	0.110	0.029
	L2	47	0.128	0.028
	L3	53	0.075	0.012
	L4	52	0.112	0.028

Emergence success of solitary bees *Osmia* spp. at different rearing locations increased in following order: L1a > L1b > L1c > L4 > L2 > L3. Results are presented in Table 3.

Table 3. Summarized data of survival level and health status of solitary bees for different sampling location and management practices, based on number of healthy bees in comparison with determined individual rearing failures.

Location	Brood Chambers Contain					SL (%)
	Mummified Larvae and Pupae	Dead Adult Bees	Parasites, Predators or Pests	Unused Pollen Pellets	Live and Healthy Adult Bees	
L1a	2	7	13	-	58	72.5
L1b	256	152	216	19	453	41.33
L1c	286	108	657	115	404	34.64
	Σ613	Σ267	Σ886	Σ134	Σ915	
L2	30	16	128	44	85	28.05
L3	34	31	1028	7	176	13.80
L4	9	3	211	6	118	34.00

During the visual inspection of 80 cocoons at L1, 9.00% of the cocoons containing cleptoparasite *Cacoxenus indagator* and 8.00% containing the mite *Chaetodactylus osmiae* were found. In the nests of plastic lamella at L1, we determined that 6.00% of brood chambers were invaded by *C. indagator*, 7.00% by *C. osmiae* mites, 4.00% by parasitoid *Monodontomerus obscurus*, 0.27% containing predator larva *Trichodes apiarius*, 2.00% invaded by *Trogoderma glabrum* and 0.36% by *Ptinus fur* nest destroyers. Additionally, we determined characteristic clinical signs for chalkbrood in 0.55% of examined brood chambers. In reed tubes at the same rearing station, as in natural nest materials, we found higher parasitization rates. In detail, parasitization included: 12% of brood chambers invaded by *C. indagator*, 10.00% by *C. osmiae* mites, 12% by *T. glabrum*, 0.36% by *P. fur*, 4.00% containing *M. obscurus* and 3.00% with adult Eumenidae wasps.

In nests sampled at L2 during section analyses, we determined *C. osmiae* mites in 22.00% of examined brood chambers, 9.00% contained *T. glabrum* and 5.00% were invaded by Eumenidae wasp larvae. Additionally, in 6.00% of brood chambers there were mummies characteristic for chalkbrood disease.

In cane tubes from L3, 5% of *C. indagator*, 20% of *C. osmiae* mites, 20.00% of *T. glabrum*, 30.00% of Eumenidae wasp, and *T. apiarius* and Chalkbrood disease, each at 3.00%, were found.

After the section of cane nests originated from L4, it was determined that 12.00% of brood chambers were invaded by *C. indagator*, 20.00% by *C. osmiae* mites and 9.00% by parasitoid *M. obscurus*. Then, 20.00% of the chambers contained *T. glabrum*, 3.00% contained *T. apiaries*, 30.00% contained Eumenidae wasp larvae and 3.00% contained mummies characteristic of chalkbrood.

A detailed presentation of the determined occurrence and prevalence of brood parasites and clinically visible chalkbrood is shown in Figure 6. The most common parasites are shown in Figure 7.

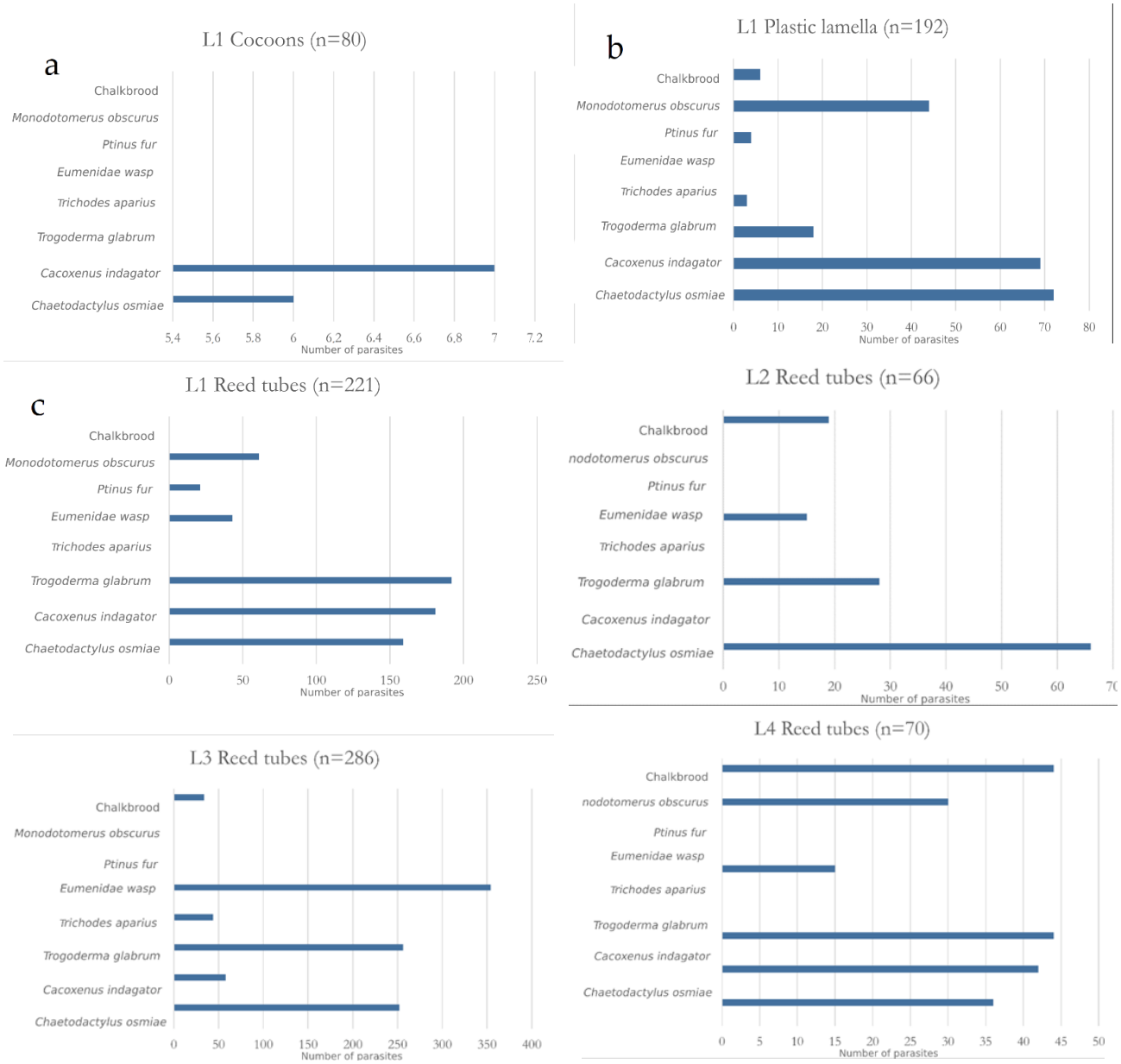

Figure 6. Occurrence and prevalence of brood parasites and clinically visible chalkbrood in various types of bees' nesting material and rearing conditions at four locations (L1—different nesting material: extracted cocoons—(**a**), plastic lamella—(**b**), reed tubes—(**c**), L2, L3, L4).

(**a**) (**b**)

Figure 7. *Cont.*

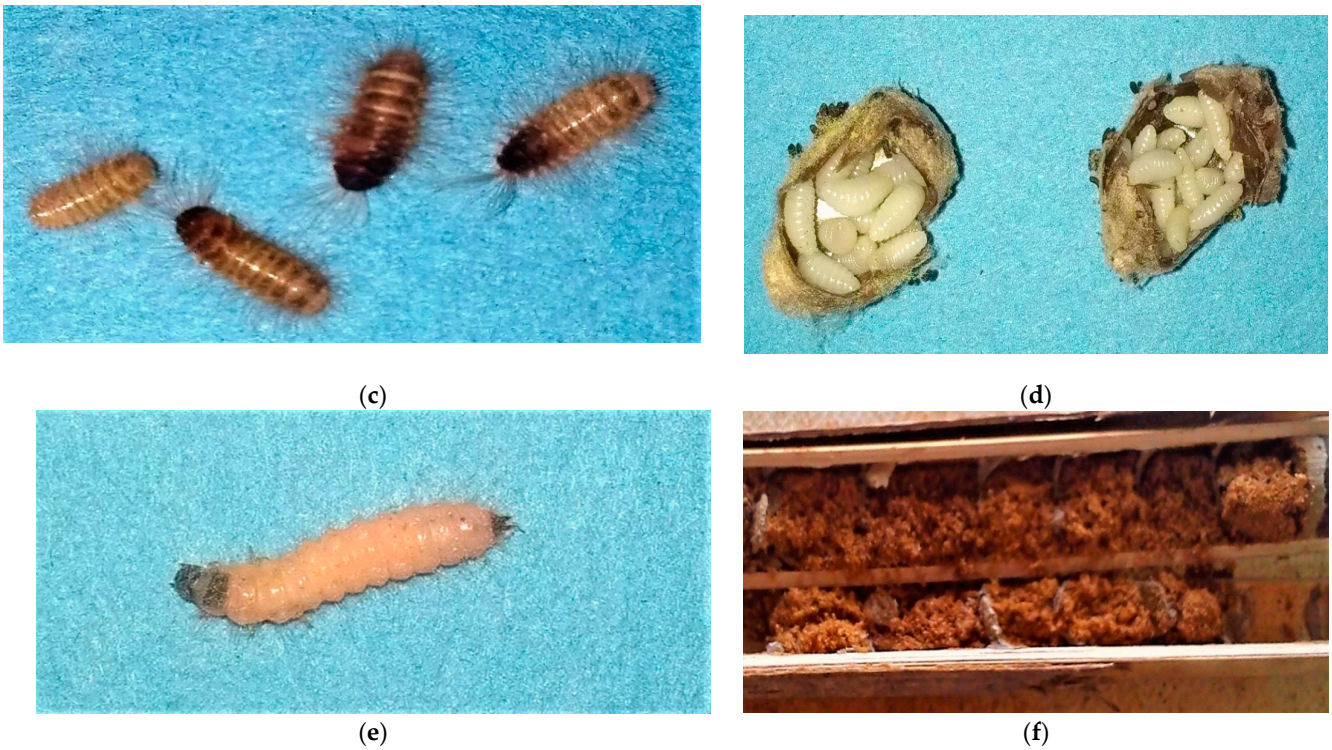

Figure 7. Common parasites of *Osmia* spp. bees' nests: (**a**)—*Cacoxenus indagator* young larvae; (**b**)—*C. indagator* adults; (**c**)—*Monodontomerus obscurus* overwintering prepupa inside cocoons; (**d**)—*Trichodes apiarius* larvae; (**e**)—*Trogoderma glabrum*; (**f**)—destroyed cocoons invaded by *Chaetodactylus osmiae* mites.

The results of the microscopic examination on the *Nosema* spp. spores' presence in homogenized abdomens and feces of natural watery smears were negative. Additionally, the results of multiplex PCR show that all analyzed samples were negative for the genetic material of both *Nosema apis* and *Nosema ceranae* when amplified with their specific primer pairs.

4. Discussion

In this monitoring-designed study, we firstly examined the occurrence and the prevalence of parasites in *Osmia* spp. bees artificial nests, settled at different locations in the continental part of Croatia. We found significant differences in the bees SL for different sampling locations, as well as between nesting materials used in implementing managing practices. Insect pollinators can be secondary carriers of the parasites in their nest surroundings [22,23]. However, it was recently reported that solitary *Osmia* spp. bees do not transmit or introduce the pathogen microorganisms in their nests from the environment [5]. Findings such as unused pollen provisions or brood failures in the early bees' developmental stages could be caused by other environmental or anthropogenic factors, such as pollution or urbanization. Additionally, the part of urban gardens and horticultural nurseries as solitary bees' food sources have just recently received attention and the consequent impacts are still unknown [24]. The highest percentage of unused pollen and dead younger brood was found at the urban environment in the reed tube nests (L1c). This can possibly be explained by the fact that the larval bacterial microbiome is linked with the available pollen [25], and an unusual environmental bacterial community might be harmful to the bee larvae [26]. Due to the limited sources of pure pollen from balconies, mini garden flowers and ornamental plants in the urban site micro location and the high possibility of its contamination with various xenobiotics or protein diet content of the changed microbiome, adult bees are not able for efficient detoxification. Additionally, they avoid laying the eggs on collected provisions and sealed young brood die in a short period. Generally, diversity

and overlapping of the bacterial communities between pollen and bee larvae is significantly lower in disintegrated than in the healthy solitary bee larvae [27]. In the protection of park plants and ornamental flowers, systemic insecticides are used at higher levels and various formulations than in the production of food crops [28,29]. Additionally, pollution mechanisms of their nectar and pollen are poorly understood.

Previously published results showed that in urban areas there is a lower level of parasite invasion in artificial bees nests than in the suburban and rural sites [5]. In our study, we included the results linked with parasitization rates in different nesting materials of the same rearing location and management practice. As was anticipated, in cocoons mechanically cleaned and extracted from reed nests before wintering, the highest bees SL was calculated, and we found only two parasites, *C. indagator* and mites *C. osmiae*. *C. indagator* females lay their eggs on the pollen provisions and larvae actively feed until the next pupal stage [1]. Here, we found it in a few noticeable smaller deformed neighboring cocoons without other content. Similarly, *C. osmiae* was found in partially damaged small cocoons with tiny walls inside which were dead but developed bees surrounded with hypopi. Such a finding was also previously described [30,31]. Despite prewinter disinfection being applied per recommendation [32], for 8% of the cocoons it was not successful. Comparison with other artificial nests is interesting due to a higher percentage of healthy bees in plastic lamella (41.33%) than in the natural reed tubes (34.64%). In both kinds of nests, we mostly found the same six parasites (*M. obscurus*, *P. fur*, Eumenidae wasp (just L1c), *T. apiarius* (just L1 b), *T. glabrum*, *C. indagator* and mites *C. osmiae*). Furthermore, in the plastic lamella in which moisture was higher, the characteristic signs of chalkbrood caused by fungi were also present. According to the results, we determined that in the urban environment (L1 a, b, c) there was a lower occurrence and prevalence of nest parasites, which agrees with previously published results for experimentally settled initial bee populations [5].

At L2 and L3, the manifestation of the nest parasites was higher than at the L1 and L4. This observation was especially visible at L3 where 80.56% of brood chambers contained parasites. Although the nests of solitary bees are not the reservoir of infectious pathogenic microorganisms [5], they can be the source of different parasites and nest destructors. At L3, management practice involved the combination of a few different nest materials. In detail, except two- and three-year-old cane tubes, there are drilled tree trunk nests. In this study, we sampled only one-year-old cane tubes, but the presence of older multiple-used nests could be a reason for the easier spread of the parasites. Earlier studies showed that the mason bee artificial nests should be used only once, because repeated use of tubes (or other kinds of nest material) increases the level of parasite infestations [33]. The same authors determined that in comparison with one-year-old tubes, in the two-year-old cane tubes there are more than ten times less healthy cocoons and three times more injured or destroyed cocoons, with a wider diversity of parasites [33]. Additionally, it was previously reported that continuous breeding in the same place for more than ten years means significantly higher numbers and diversity of brood parasites in nests [34,35]. Our results showed the lowest determined bees SL was at L3, which was 13.80% in total, while in a similar environment of L2, it was 28.05%. The bees SL was determined as almost equal at the L1c (34.64%) and L4 (34.00%) sites.

In the samples of *O. bicornis* adults caught near honeybee apiaries, Ravoet et al. (2014) confirmed the presence of *N. ceranae* genetic material [36]. Our finding, that adult bees collected from nests situated on different biotopes, as well as samples of their feces, were not invaded by microsporidia *Nosema* spp., is in agreement with other studies [5,37].

It is very interesting that a high percentage of brood chambers with unused pollen provisions were found during the section analyses of reed tube nests samples from L2 (17% of examined nest brood chambers; empty chambers were excluded from the calculation). It was a significantly higher percentage in comparison with the situation at L3 (0.55%). As around this bee rearing station there are fields for intensive agricultural production, there is also the possibility for chemical contamination of different nest materials, pollen and nectar, as well as mud, water and leaf nest material. Additionally, residues of agrochemicals or fer-

tilizers used in plant cultivar production, natural vegetation and soil can be detected [38,39]. Again, as at L1, these results indicate the possibility that bee females avoid laying eggs on contaminated or changed bacterial community composition of pollen provisions. Recently, the moderate impact of horticultural management practices including the imidacloprid application, which strongly reduced solitary bee reproduction success, was described [40–42]. Additionally, the water availability in artificial nest environments is strongly linked with insect pollinator's food quality and consequently to all bees' developmental stages, fitness and survival [43].

At L4, we found a small incidence of nest chambers with unused pollen provisions and early bee developmental stage failures in comparison with L1c, L2 and L3. Parasites' occurrence and their diversity were similar to the other rearing station locations.

The overall emergence success of *O. bicornis* and *O. cornuta* at different rearing locations cannot be determined because this study is not an experimental study with meaningful control of the independent variables. The number and diversity of parasites which act as nest destructors increased in the following order: urban, rural and suburban environments. Additionally, those parameters were positively correlated with the presence of solitary bee rearing failures. Those results are in accordance with the reports of Los et al. (2019) and Fliszkiewicz et al. (2012), who noticed lower parasite infestations and diversity in rural and urban sites than in suburban areas in experimental initial bee populations [5,44]. The mortality of *Osmia lignaria* offspring caused by brood parasites is also higher at natural and rural sites [45].

The differences in cocoon mass and emergence mass of healthy adult bees were determined between the observed locations. Interestingly, cocoon weight increased as follows: L1, L3, L4 and L2. Additionally, the weights of adult emerged bees were different depending on the rearing location and bee species (Figures 3–5; Table 2). However, due to morphological differences and the differences in metabolic rates between sexes, as well as the water content, body weight can differ between males and females [46]. The body weight of *O. bicornis* was significantly lower at L4, and all other rearing sites' mean values of bees' weight were similar. At L2, adults of *O. cornuta* were significantly weightier in comparison with the emerged bees originating from other nests at other locations. That may relate to the management practice of using longer reed tubes as artificial nests. Adults from L3 were significantly smaller, which may be linked with the high parasitization rate but also environmental factors, such as the lack of quality natural food. Here, it must be noted that we opened the nests during November and December, and until then the material was kept at fridge temperature. Schenk et al. (2018) reported that environmental temperature influences the emergence term and body weight [47]. The same authors concluded that the timing of emergence also depends on the individual's body condition, due to the variability in natural emergence terms among specimens which survive the winter period at the same or similar environmental temperatures [47]. As we did not measure weather conditions, it is hard to make any conclusions about the body weight variability among the bees from the different experimental rearing stations.

5. Conclusions

The number and diversity of solitary bees are declining in many landscapes. Therefore, pollination by these insects is vulnerable to ecosystem services. The results of this study include the presence and prevalence of brood parasites, predators and pests in various types of *Osmia* spp. bees' nesting material and the semi-field rearing conditions in Croatia for the first time. Managed rearing practices of *Osmia* spp. bees can be useful for agricultural and horticultural sustainable development in different biotopes. Therefore, this study may be used as a baseline for further solitary bee nest parasite monitoring schemes.

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